TRANSLATING THE IMPACTS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE EXACERBATIONS: FROM POPULATIONAL BURDENS TO INFLAMMATION

Laura Elisabeth Labonté

Division of Experimental Medicine, Faculty of Medicine

McGill University, Montreal

30 June 2015

A Thesis submitted to McGill University

in partial fulfillment of the requirements of the degree of PhD

TABLE OF CONTENTS

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is one of the most under-diagnosed chronic diseases. Its burden is mostly attributable to exacerbations, acute events of worsened symptoms that lead to disease progression as well as significant morbidity and mortality. As many individuals remain undiagnosed, exacerbations are simply treated as acute events by healthcare providers. Through lack of proper disease management, these individuals will continue to experience exacerbations, and as disease progresses, will become susceptible to many comorbid conditions. Cardiovascular (CV) comorbidity is the second cause of death among COPD patients. However, some determinants of increased CV risk could be mediated using specific interventions. An important common link between CV disease and COPD is enhanced inflammation. As such, inflammatory mediators are attractive targets for modulating CV risk in COPD.

The purpose of this thesis was to evaluate the wide range of impacts of COPD exacerbations, from population burdens, to CV risk, to inflammation in subjects representing different stages of disease using a translational research approach.

Two research projects were carried out to address this objective. The first consisted of an analysis of exacerbation-like respiratory events among subjects with undiagnosed and previously diagnosed COPD, embedded within an existing population based cohort study called Canadian Cohort Obstructive Lung Disease (CanCOLD). Exacerbation-like respiratory events were measured in these subjects using a questionnaire administered every three months. Differences in the characteristics of subjects with undiagnosed and diagnosed COPD were assessed, as well as those associated with reporting exacerbation-like respiratory events. The proportion of subjects

reporting events and odds of using health services were also compared. The second was a local study of diagnosed COPD patients hospitalized for exacerbations, who have advanced disease and elevated CV risk. A measure of arterial stiffness, carotid-femoral pulse wave velocity (cf-PWV), and powerful predictor of CV events, was measured sequentially in these patients, as well as several inflammatory mediators and other clinical variables. The associations between these were determined over time.

In the CanCOLD cohort, subjects with undiagnosed COPD had milder symptoms, better lung function and less functional impairment compared to diagnosed subjects. However, lower health-related quality of life was associated with reporting exacerbation-like respiratory events in both groups of subjects. Although the proportion of subjects reporting these events was lower among undiagnosed subjects, health service utilization was similar to that of diagnosed subjects. Within the local study, several important predictors of increased cf-PWV over time were identified, including decline in lung function and subsequent exacerbations. Additionally, club cell (CC)-16, a lung-specific inflammatory mediator was identified as a determinant of changes in cf-PWV and exacerbation frequency over time. Moreover, RelB, a nuclear factor (NF)-κB family member and mediator of pulmonary inflammation, was identified as a potential novel mediator of CV function in COPD patients.

This thesis demonstrates that, at the populational level, many subjects are not diagnosed for their COPD, but experience exacerbation-like respiratory events that result in a much greater utilization of health services than previously thought. With regards to CV morbidity, several novel predictors of arterial stiffening were identified; some of these representing potentially modifiable targets. CC-16 was identified as an important novel inflammatory link between

pulmonary function and CV function in COPD patients. Consequently, the results of this thesis describe the wide range of impacts of exacerbations from populational burdens to inflammation.

RÉSUMÉ

La maladie obstructive pulmonaire chronique (MPOC) est une des maladies chroniques les plus sous-diagnostiquées. Son impact est surtout attribuable aux exacerbations, des épisodes aigus de symptômes aggravés, qui font progresser la MPOC et contribuent à sa morbidité et mortalité. Plusieurs individus atteints ne sont pas diagnostiqués et leurs exacerbations traitées simplement comme épisodes aigus. Ainsi, celles-ci continuent, et à mesure que la MPOC progresse, ces individus deviennent susceptibles à d'autres comorbidités. Les maladies cardiovasculaires (CV) sont la deuxième cause de décès chez les patients MPOC, mais certains déterminants du risque CV élevé seraient contrôlables par des interventions appropriées. Un lien important entre les maladies CV et la MPOC est l'inflammation. Les médiateurs de celle-ci représentent donc des cibles attrayantes pour la modulation du risque CV.

L'objectif de cette thèse fût d'évaluer les impacts des exacerbations de la MPOC; des fardeaux populationnels, au risque CV, à l'inflammation, chez les sujets représentant différents stages de la MPOC, en utilisant une approche de recherche translationnelle.

Deux projets de recherche furent entrepris dans ce but. Le premier consistait en une analyse des épisodes respiratoires similaires aux exacerbations chez les sujets ayant une MPOC non-diagnostiquée ou diagnostiquée, utilisant l'étude à base d'échantillonnage populationnelle existante "*Canadian Cohort Obstructive Lung Disease (CanCOLD)"*. Ces épisodes furent mesurés tous les trois mois avec un questionnaire. Les différences entre les caractéristiques des sujets non-diagnostiqués et diagnostiqués furent évaluées, ainsi que celles associées avec le signalement ou non d'épisodes respiratoires. La prévalence des épisodes ainsi que la probabilité d'utiliser des soins de santé furent comparées. Le deuxième projet était une étude locale chez les

patients diagnostiqués et hospitalisés pour une exacerbation, qui avaient une maladie avancée et un risque CV élevé. La rigidité artérielle, correspondant à la vitesse de l'onde de pouls carotide-fémorale (VOP-cf), un indicateur puissant d'épisodes CV, fût mesurée séquentiellement chez ces patients, avec certains marqueurs inflammatoires et variables cliniques. Les associations temporelles entre ces facteurs furent évaluées.

Les sujets non-diagnostiqués de CanCOLD avaient des symptômes moins graves, une meilleure fonction pulmonaire et une moindre déficience fonctionnelle que les sujets diagnostiqués. Par contre, une diminution de qualité de vie due à la santé était associée au signalement d'épisodes respiratoires chez tous les sujets. Malgré que leur prévalence fût plus basse chez les sujets non-diagnostiqués, l'utilisation des services de santé était similaire entre les groupes. Dans l'étude locale, des prédicteurs d'augmentation temporelle de la VOP-cf furent identifiés, dont la perte de fonction pulmonaire et l'occurrence ultérieure d'exacerbations. De plus, club cell (CC)-16, un médiateur d'inflammation pulmonaire fût identifié comme déterminant des changements au niveau de la VOP-cf et de la fréquence d'exacerbations. RelB, un membre de la famille NF-κB et médiateur d'inflammation pulmonaire, fût identifié comme nouveau médiateur potentiel de fonction CV chez les patients MPOC.

Cette thèse démontre qu'au niveau populationnel, plusieurs sujets ne sont pas diagnostiqués avec la MPOC, mais éprouvent des épisodes respiratoires qui résultent en l'utilisation de plus de services de santé que l'on pensait auparavant. En ce qui concerne la morbidité CV, des nouveaux prédicteurs d'augmentation de la rigidité artérielle furent identifiés; dont certains pourraient être contrôlés par des interventions. Le médiateur CC-16 fût identifié comme un nouveau lien inflammatoire entre les fonctions pulmonaire et CV. En conclusion, les résultats présentés dans

cette thèse décrivent la gamme étendue des impacts de l'exacerbation du fardeau populationnel jusqu'à l'inflammation.

LIST OF ABBREVIATIONS

Abs	Absolute
AECOPD	Acute exacerbation of COPD
AIx	Augmentation index
AP	Augmentation pressure
BMI	Body mass index
BOLD	Burden of Obstructive Lung Disease
BMP	Beats per minute
BP	Blood pressure
CanCOLD	Canadian Cohort Obstructive Lung Disease
Ca++	Calcium
CAD	Coronary artery disease
CAT	COPD Assessment Test
CC	Club cell
CCQ	Clinical COPD Questionnaire
Cf	Carotid-femoral
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
Cox-2	Cyclooxygenase-2
Cr	Carotid-radial pulse wave velocity
CRP	C reactive protein
CS	Cigarette smoke
CT	Computerized tomography
Ct	Cycle threshold
CV	Cardiovascular
CVD	Cardiovascular disease
ECLIPSE	Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints
EXACT-Pro	EXAcerbation of Chronic pulmonary disease Tool- Patient Reported Outcome
FACIT	Functional Assessment of Chronic Illness Therapy
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GERD	Gastroesophageal Reflux Disease
GOLD	Global Initiative for Chronic Obstructive Lung Disease
Hct	Hematocrit
HDL	High-density lipoprotein
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
K+	Potassium
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
MCI	Montreal Chest Institute
MI	Myocardial infarction
MMP	Matrix metalloproteinases

MNC	Monocyte
mRNA	Messenger ribonucleic acid
Na+	Sodium
NE	Neutrophil elastase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHANES	National Health And Nutrition Examination Survey
NO	Nitric oxide
OR	Odds ratio
O_2	Oxygen
PCO_2	Partial pressure of carbon dioxide
PCR	Polymerase chain reaction
PLATINO	Latin American Project for the Investigation of Obstructive Lung Disease
PO_2	Partial pressure of oxygen
PP	Pulse pressure
PWA	Pulse wave analysis
PWV	Pulse wave velocity
qRT-PCR	Quantitative real-time polymerase chain reaction
RAAS	Renin angiotensin aldosterone system
RBC	Red blood cell
RelB	V-rel avian reticuloendotheliosis viral oncogene homolog B
RR	Risk ratio
SD	Standard deviation
SGRQ	St-Georges Respiratory Questionnaire
SPD	Surfactant protein D
TEXAS	Telephone Exacerbation Assessment Systems
TNF	Tumor necrosis factor
TORCH	TOwards a Revolution in COPD health
Vol	Volume
WBC	White blood cell
WHO	World Health Organization

LIST OF TABLES AND FIGURES

TABLES

FIGURES

ACKNOWELDGEMENTS

As I reflect on the experience that has been my PhD, many thoughts come to mind. At the beginning of this journey Dr. Bourbeau, my supervisor and mentor, told me that a PhD was not about the final destination but about the path you take to get there. Today these words resonate with me. These last four and a half years have been the most intellectually stimulating, transformative, and enriching years of my life. Along the way I have met some truly inspirational people, and made some lifelong friends while having the continued support of old friends and family. Importantly, I realized my vision of "bench to bedside" not only through the research I carried out for my doctoral studies, but also in my career, whereby I started my PhD as a cellular biologist, and am now ending it as a newly admitted candidate to Medicine.

I owe many thanks for the guidance and support that Dr. Bourbeau has given me over the years. His trust and confidence in me allowed me to grow as a researcher and find my voice in Science. From day one, his unwavering support of my desire to pursue a PhD in translational research truly allowed me to accomplish this goal. He also taught me that no dream is too big, and pushed me to better myself while always aiming higher. Dr. Bourbeau showed me the importance of perseverance in research, as well as the importance of forming a strong and collaborative research team. Many of the accomplishments, awards and honours I received throughout my PhD would not have been possible without his constant encouragement and incitement. His eternal optimism was a true inspiration both in my daily life and in my studies. I also thank him sincerely for having let me come to his clinics and while he was on service at the hospital; this, along with the gratitude and appreciation I saw in his patients towards him, allowed me to confirm my desire to pursue medicine.

I have much gratitude to Dr. Carolyn Baglole. Although she was not my supervisor, she always took the time to meet with me, provide critical and valuable feedback on my work, while letting me explore my ideas in research. Without her help and support, I would not have been able to complete the inflammatory analyses that were part of my thesis. I also have tremendous regards for her professionalism and knowledge, and was inspired by her drive and motivation in research.

I also thank Dr. Dennis Jensen- who was essentially my doctoral big brother. He inspired confidence in my abilities as a presenter and researcher. His insights and encouragement were a driving force for me over the years. His passion for research inspired mine as well. I thank him for taking the time to provide valuable and critical feedback on my work, while supporting me and listening to my concerns and stresses with regards to life in general.

I have much gratitude towards my entire RECRU family. Firstly I thank Katrina Metz, the person with whom I spent the most time throughout my PhD, who became one of my closest friends. Many times she has been a shoulder to lean on. We have also shared many adventures in research, laughs and chocolate/protein bar-filled lunches. I leave with many stories and fond memories with her. Most importantly, Katrina showed me the importance of professionalism when interacting with patients and other members of the research team. Moreover, she showed me how to implement a research project and keep everything organized. I look forward to our continued friendship for many years to come.

I thank Palmina Mancino, who was without a doubt an invaluable support throughout my PhD. Without her help I would not have been able to develop the CanCOLD exacerbation questionnaire, and implemented it throughout the nine CanCOLD sites. I thank her for all the

times she listened to my stresses, drove me to conferences, and provided general support both in my personal and professional life. I wish her much happiness in the years to come.

I would like to express my gratitude to Louise Auclair, who provided me with considerable support through my applications for funding, scholarships, awards and other work. I will not forget hearing her laughter resonate in the hallways of the MCI, a greeting that welcomed me each time I arrived, and warmed even the rainiest and coldest of days.

I thank Pei Z Li for her help with statistical analyses for all my projects. Her patience and knowledge helped me enormously. I also thank Meena Patel, who helped me with my research project at the Chest, and who quickly became a good friend. I owe much gratitude to Maria Sedeno, for being a true role model of professionalism. She helped me understand the importance of structure and organization in research, and was a great support with the CanCOLD study. Dr. Andrea Benedetti guided me through some statistical analyses, and for that I am grateful. I must also thank Dr. Hélène Perreault, who challenged me and provided important feedback on the early stages of my PhD. She pushed me to aim higher and not be intimidated by questions and different opinions and interpretations of data. I am grateful to Riany Sena, who helped me many a times during my PhD. Being able to complete this journey together was a pleasure. Having someone who fully understands my concerns, joys and stresses was invaluable. I wish her much success and happiness in her career in research. I also thank my summer students, Patrick, Katie, Michele, and Stefania who were instrumental in helping with my study's patient recruitment, data collection and analysis. They also showed me how to be a good team leader. I wish them all the best of luck in their future endeavours. Finally I thank the RECRU for having provided me with

workspace, and the facilities to carry out my research project, as well as the many nurses from the MCI who helped me with patient recruitment.

I owe many thanks to Dr. Wan Tan for her support throughout my work on exacerbations in CanCOLD.

I am eternally grateful to Dr. David Allan, my Master's supervisor who first introduced me to the concept of translational research. His initial confidence in my research abilities nine years ago while I was still in my undergraduate studies is what started my journey. I will not forget the many lessons he taught me in life and research.

I thank the Baglole lab for their support. I would like to recognize Michela in particular, for teaching me about PCR and the importance of being meticulous. Moreover, her constant smile and positive attitude helped me get through 10+ hour days at the lab. I also thank Dr. Angela Rico de Souza for her help with ELISAs, and for showing me how to be precise and consistent in my laboratory work.

I also thank Dr. Stella Daskalopoulou and the vascular lab for their help with arterial stiffness measurements. It was with them that I had my first exposure to working with patients. This experience allowed me to appreciate my own capabilities as well as fortifying my desire to pursue Medicine. I thank Yessica and Andrew in particular, for their patience and generosity towards me and the time they spent showing me how to precisely assess arterial stiffness on patients, while teaching me related physiological principles.

I also thank my entire thesis committee for helping me stay on track with my doctoral studies.

I am grateful to the Fonds de Recherche du Québec-Santé and Canadian Institutes of Health Research for providing funding for my doctoral studies, which made my PhD progress smoothly, while allowing me to also attend many international conferences.

I owe many thanks to all my friends who were here before I started this doctoral journey, and who are still here today, as well as the new ones I made along the way. They were often a much-appreciated outlet for my stress.

Finalement, je remercie ma famille. Il est difficile d'essayer de résumer toutes les choses pour lesquelles je suis reconnaissante envers eux en quelques phrases. Mes parents Elisabeth Engelhardt et Gilles Labonté- je vous dois énormément de gratitude. Votre support depuis toujours, et amour inconditionnel, ont été des forces motivantes dans ma vie. Vous m'avez montré qu'il faut toujours viser vers le haut, mais vous m'avez aussi montré qu'il faut travailler fort et être déterminée pour atteindre celui-ci. Je vous remercie pour toutes les fois où vous avez lu et révisé mes textes, demandes de bourses, et écouté mes présentations lorsque je les pratiquais, non seulement durant mon doctorat, mais toutes les fois auparavant aussi. Un des buts motivateurs au cours de ma vie a été de vous rendre fiers de moi, et j'espère que cette thèse et ce doctorat seront un testament envers ceci. Je vous aime énormément et ne vous souhaite que du bonheur.

Et finalement, à mon mari David, je dois dire que ce trajet aurait été incroyablement plus seul sans toi. Je suis reconnaissante du fait que nous avons franchi cette importante étape ensemble et en même temps. Que tu comprennes les stresses, inquiétudes et joies associés aux études doctorales, m'ont permis de me sentir supportée et réconfortée. Je te remercie pour ta patience aussi envers moi, ainsi que l'amour et la compassion que tu as eu et continues d'avoir pour moi. Je t'aime, et suis excitée de continuer notre vie ensemble, alors que nous commençons le prochain chapitre de celle-ci.

PREFACE

It can be said that the ultimate purpose of biomedical and clinical research is to apply novel scientific discoveries to the development of new approaches for the prevention, diagnosis and treatment of disease [1]. Having adopted a "bench to bedside" approach has led to many such advancements in medicine, and has resulted from a process known as translation [2]. Translation describes a conversion of knowledge through different fields of research spanning basic or "fundamental" science all the way to public health and vice versa [2]. Although the concept of translation is relatively longstanding [3], it has only recently become a central focus of much health research, where it may serve as a bridge for knowledge gaps.

One of the major goals of the projects constituting this doctoral thesis was the adoption of a translational approach to research. In this attempt, I may not have developed as deep an understanding on a single subject matter as others during their PhD. However in contrast, I had the unique opportunity and privilege of gaining a profound understanding of the totality of chronic obstructive pulmonary disease (COPD) as a disease and of studying its impacts from inflammation to population-wide burdens.

This thesis was prepared according to the McGill University rules for a manuscript-based thesis. It also satisfies the requirements of translational research, whereby its content range from the broad healthcare burdens of COPD on the population at large in Canada, to the systemic consequences of COPD as they pertain to cardiovascular and inflammatory outcomes in subjects with severe disease. It consists of four manuscripts that address important translational research topics related to exacerbations of COPD: 1) impacts of exacerbations on subjects with undiagnosed COPD selected from the general population, 2) predictors of increased arterial

stiffness, a predictor of cardiovascular (CV) risk, during and subsequent to exacerbations in subjects with advanced COPD, 3) discovery of a novel inflammatory marker of CV outcome at exacerbation, and 4) evaluating the relationship between lung-specific inflammatory mediators and of a novel marker of CV outcome, with arterial stiffness during and subsequent to exacerbations in subjects with advanced COPD.

Thesis composition and manuscripts overview

Chapter 1 is a brief introduction of the research background and research questions that are addressed in my thesis. Chapter 2 provides a comprehensive literature review, discussing the problems associated with COPD exacerbations, as well as their inflammatory and CV consequences. Chapter 3 summarizes the rationale, objectives and hypotheses of my thesis. Chapter 4 describes the populations and designs of the two studies from which the following manuscripts were written.

Chapters 5 to 8 include the manuscripts, which constitute my thesis. They are organized as follows: title page, abstract, introduction, methods, results, discussion, acknowledgements, tables and figures.

Chapter 5 constitutes an analysis of data from the existing population-based Canadian Cohort Obstructive Lung Disease (CanCOLD). It consists of the manuscript entitled "Exacerbations in undiagnosed chronic obstructive pulmonary disease greatly impact use of health services: The CanCOLD Study". This is the first study to assess the proportion of undiagnosed subjects reporting exacerbation-like events in a population-based cohort, the factors associated with reporting these, and medication and/or health service utilization, and to compare them to those of

subjects who received a previous physician diagnosis of COPD. As such, this reflects the impacts of exacerbation-like events in milder, asymptomatic and untreated subjects. This article is in submission to the European Respiratory Journal.

Chapters 6-8 are based on a local study on exacerbations and the course of change in inflammatory and CV outcomes over time. It focuses on subjects with previously diagnosed, advanced COPD, who experience severe exacerbations that require hospitalization. This reflects the detrimental impacts of exacerbations once disease has progressed and subjects have become severely impaired, very symptomatic and are being treated for their disease.

Chapter 6 consists of the manuscript entitled "Longitudinal predictors of increased arterial stiffness during COPD exacerbations and recovery". This is the first study to examine the longitudinal predictors of increases in carotid-femoral pulse wave velocity (cf-PWV), a measurement of arterial stiffness in COPD patients during and subsequent to an exacerbation. This article is in submission.

Chapter 7 consists of the manuscript entitled "Alterations in the Expression of the NF-κB Family Member RelB as a Novel Marker of Cardiovascular Outcomes During Acute Exacerbations of Chronic Obstructive Pulmonary Disease". This is the first study to examine the role of RelB in COPD. This article has been published by *PLoS One;* 2014; 9(11): e112965.

Chapter 8 consists of the manuscript entitled "Club cell-16 and RelB: predictors of arterial stiffness in chronic obstructive pulmonary disease". This is the first study to examine the course of change in 2 lung-specific inflammatory mediators, club cell-16 (CC-16) and surfactant protein

D (SPD), as well as in RelB, a novel marker of CV outcomes over the time course of an exacerbation and evaluate their relationship to changes in cf-PWV and exacerbation frequency over time. This article is in submission to the American Journal of Respiratory and Critical Care Medicine.

Chapter 9 summarizes the findings of my manuscripts and includes a general discussion and overall conclusion, followed by the Bibliography and Appendices.

Table 1: Thesis composition and manuscript overview

Thesis chapter: title/manuscript title	Objectives	Originality	Publication
Chapter 1: Introduction **Chapter 2: Literature review** **Chapter 3: Rationale, objectives and hypotheses** **Chapter 4: Setting, population and design**			
Chapter 5: manuscript 1 Exacerbations in undiagnosed chronic obstructive pulmonary disease greatly impact use of health services: The CanCOLD Study	To assess in a population-based cohort 1) whether there are differences in the characteristics of subjects with undiagnosed and diagnosed COPD, as well as whether some of these characteristics are associated with reporting exacerbation-like respiratory events; 2) to determine the proportion of subjects reporting exacerbation-like respiratory events and whether there are differences in the odds of using medication and/or health services	1) The first study to look at differences in characteristics of subjects with undiagnosed vs. diagnosed COPD, and characteristics associated with reporting exacerbation-like events in undiagnosed subjects in a population-based cohort 2) The first study on the prevalence of exacerbation-like events in subjects with undiagnosed COPD from a population-based cohort in Canada 3) The first study to report that health service utilization is much greater than previously thought among undiagnosed subjects	In submission to the European Respiratory Journal
Chapter 6: manuscript 2 Longitudinal predictors of increased arterial stiffness during COPD exacerbations and recovery	To assess cf-PWV during exacerbations requiring hospitalization and subsequent recovery, and to identify determinants of increases in this over time	1) The first study on arterial stiffness during severe exacerbations requiring hospital admission 2) The first study on longitudinal predictors of increases in arterial stiffness in COPD	In submission
Chapter 7: manuscript 3 Alterations in the Expression of the NF-κB Family Member RelB as a Novel Marker of Cardiovascular Outcomes During	To assess RelB expression relative to markers of inflammation as well as its association with cardiovascular and pulmonary features of COPD patients at	The first study to examine the role of RelB in COPD	Published in *PLoS One;* 2014; 9(11): e112965.

Acute Exacerbations of Chronic Obstructive Pulmonary Disease	stable-state and during exacerbation		
Chapter 8: manuscript 4 Club cell-16 and RelB: predictors of arterial stiffness in chronic obstructive pulmonary disease	To measure the course of change in lung-specific inflammatory mediators and RelB during and after exacerbation, and their relation to arterial stiffness and exacerbation frequency	1) The first study to measure lung-specific inflammatory mediators, and RelB during and subsequent to severe exacerbations 2) The first study to assess their relationship with arterial stiffness	In submission to the American Journal of Respiratory and Critical Care Medicine
Chapter 9: Summary of findings, general discussion and overall conclusions			

Contribution of co-authors

One of the main challenges with regards to implementing translational research is the need to involve a research team of experts with different training backgrounds. As a result, the research projects that constitute this thesis have involved collaborations among several different researchers across Canada. With regards to the local study on exacerbations, I, Laura Labonté, the PhD candidate, generated the original research questions and hypotheses, designed the studies and developed the research protocols. I also obtained ethical approvals, developed all bilingual study consent forms, as well as case report forms and manuals of procedures. I coordinated data collection, data management, participated in statistical analyses with the help of a biostatistician, and wrote all the manuscripts with the feedback of my supervisor and co-authors. With regards to the analysis embedded within the CanCOLD study, I generated original research questions and hypotheses, wrote the research substudy application, generated the questionnaire used for data collection, helped with questionnaire implementation across Canada, participated in quality control exercises, and was responsible for data analyses with the help of a biostatistician. I wrote the resulting manuscript with the feedback of my supervisor and co-authors.

Dr. Jean Bourbeau was my thesis supervisor and is a principal investigator for both the CanCOLD study and the local study. He supervised and oversaw all aspects of this thesis and the studies that constitute it. More specifically, he provided significant and critical input on research questions and hypotheses, and on study design and protocol development. Dr. Bourbeau was responsible for funding acquisition, initiation of collaborations, and helped with questionnaire development (for the CanCOLD study), study implementation, data analyses and interpretation. He also provided significant feedback on the organization and content of all manuscripts.

Dr. Baglole was a member of my doctoral thesis committee and co-investigator for the local study. She participated in study design and protocol development, study implementation and result interpretation. More specifically, Dr. Baglole provided substantial and critical feedback for the generation of research questions and hypotheses pertaining to inflammation, as well as providing the means for me to acquire the necessary molecular and cellular research techniques to carry out my project. She also provided significant feedback and input on the organization and content of the 3 manuscripts relevant to the local study.

Dr. Stella Daskalopoulou was a member of my doctoral thesis committee and co-investigator for the local study. She participated in study design and protocol development, and study implementation. More specifically, Dr. Daskalopoulou provided input for the generation of research questions and hypotheses pertaining to arterial stiffness, as well as providing the means for me to acquire the necessary training to assess arterial stiffness in patients. She provided feedback on 2 of the manuscripts relevant to the local study.

Dr. Wan Tan is a co-principal investigator for the CanCOLD study. Dr. Tan was involved in the original conception of the CanCOLD study, including development of research questions, study design, protocol development, and study implementation. She also provided to me critical input in the development of research questions and hypotheses as well as in the substudy application; in addition to providing feedback on the content and organization of the resulting manuscript.

Drs. Aaron, Benedetti, Chapman, Cowie, Fitzgerald, Hernandez, Maltais, Marciniuk, O'Donnell and Sin are co-investigators of the CanCOLD study. They were not directly involved in the analysis carried out for my thesis, however they were involved in the original conception of this

cohort study including development of research questions, study design, protocol development and study implementation. They also provided feedback on my manuscript.

Pei Z Li is a biostatistician at the Montreal Chest Institute who was involved in both the CanCOLD study and the local study. She helped me with the statistical analyses for all 4 manuscripts included in this thesis.

Palmina Mancino is the national project director for the CanCOLD study. She helped me with the generation of the questionnaire used for data collection, implementation of the questionnaire across Canada, and participated in quality control exercises with me.

Michele Zhang, Patrick Coulombe, and Katie Garland were summer students that I supervised who participated in the local study. They helped with study implementation and data collection. More specifically, they were involved in screening and recruiting patients, and assessing patients, where Michele Zhang and Patrick Coulombe were also involved in carrying out molecular and cellular laboratory work.

Katrina Metz and Meena Patel were research assistants who helped with patient assessment in the local study.

Statement of originality

This thesis and the manuscripts within represent my original work. The major project that constituted my PhD was a local observational study on the impacts of COPD exacerbations on inflammation and CV outcomes, which was funded by an investigator-initiated grant from GSK

Canada. My own contributions to this study included its development that consisted of generating original research questions and hypotheses, designing the study and writing its protocol, discussing with GKS for grant development, and obtaining ethical approval. I also contributed to study implementation, by conceiving the study's bilingual consent forms, as well as case report forms and manuals of procedures. I learned the specific techniques required such as genetic analysis and methods of collecting clinical and physiological measurements on patients. I trained research staff and supervised four summer students. I participated in data entry, as well as organized meetings with co-investigators. I also participated in all data analyses together with a biostatistician who performed statistical analyses. Finally, I wrote the final manuscripts that report the results of my research projects. The other project in my PhD consisted of an analysis embedded in an existing population-based cohort study. I was responsible for generating original research questions and hypotheses for this analysis, writing the research substudy application, designing the questionnaire that was used for data collection, helping with the implementation of this questionnaire across nine different sites in Canada, participating in quality control exercises, performing data analyses with the help of a biostatistician, and finally, for writing the final manuscripts that reported the results.

Several elements of my doctoral work constitute original contributions to different fields of research on COPD exacerbations. In terms of the epidemiological aspects of this disease, a fundamental concern among researchers has been the widespread under-diagnosis of COPD and the resulting economic and healthcare consequences. My work shows that many individuals within the population at large are not diagnosed with COPD. Moreover, despite having milder and less symptomatic disease, these undiagnosed subjects use health services to a much greater extent than previously thought, and thereby contribute significantly to the burden of

exacerbations. This is the first study to report this. With the growing global prevalence of this disease, there is urgency in addressing this public health issue.

In terms of the clinical and physiological aspects of COPD, cardiovascular disease (CVD) and acute CV events have been shown to represent a significant cause of morbidity and mortality for COPD patients, especially with advanced disease. As a result, much research has been dedicated to identifying potentially modifiable targets that could impact this susceptibility. Yet there is a lack of data on the longitudinal predictors of CV outcomes and how they relate to exacerbations. In my work I identified several potential clinical variables that may predict adverse CV outcomes over time, certain of which may be modifiable. As such, my findings can provide an important stepping-stone towards better understanding the determinants of CV susceptibility in COPD as well as laying the groundwork for future research addressing their potential therapeutic modulation.

Finally, in terms of the cellular and molecular aspects of COPD, a key research question has been to identify biomarkers of disease or disease-specific outcomes in COPD. In my doctoral work I identified a novel inflammatory mediator of vascular function in COPD patients. Moreover, my study was the first one to examine the contribution of lung-specific inflammatory mediators to adverse CV outcomes over time. Given the impact of CV morbidity on COPD, these potential biomarkers of CV outcome are highly clinically relevant, since they are all easily measured and have been shown to be modifiable. Thus my results suggest that further research is warranted to better understand the molecular and cellular pathways involved and to determine whether they represent viable therapeutic targets.

A final novel aspect of my doctoral work that I feel must be mentioned is its translational nature. The concept of translational research was conceived several decades ago. Yet despite its promise, it has been a challenge to design and carry out training protocols for individuals such as graduate students who aim to become translational researchers. I believe that my doctoral work serves as a testament that translational research training is possible at the graduate level. This involves much collaboration among individuals with different fields of expertise, but as a result of this effort, my doctoral work truly reflects the wide spectrum of impacts that exacerbations have on COPD.

CHAPTER 1: INTRODUCTION

COPD affects many people globally and is a significant cause of morbidity and mortality [4]. It is predicted to be the third leading cause of death by 2020 [5]. This disease is characterized by progressive airflow obstruction that is not fully reversible [4]. Its symptoms such as chronic dyspnea, sputum production and cough are disabling and significantly impact the quality of life of patients and the health care system [6-9].

The course of COPD is aggravated by multiple morbidities that manifest in various systems from the lung e.g. COPD exacerbations, to extra-pulmonary e.g. CVD, diabetes mellitus, osteoporosis and psycho-behavioural disorders. It is thus necessary to take into consideration the possible interplay between these morbidities, their amplified effects on disease, and their common mechanisms such as inflammation. As such, it becomes necessary to adopt an "integrated body" approach to COPD, rather than continued focus on the lungs in isolation of other body systems, and this is best done by approaching research questions from a translational perspective. In doing so, one can look at the relationship between COPD and morbidities starting at the molecular and cellular level, to the physiology and then progressing to the community and populational level.

COPD exacerbations are the most important cause of the increasing global burden of this disease [10]. These acute events of worsened symptoms lead to increased hospitalization [10], declined overall patient health status [11, 12] and increased mortality [13]. As a result, they represent the most important adverse event in COPD. Most of the information available to date on exacerbations in COPD has come from clinical populations of subjects diagnosed and treated for their disease,

who reflect convenience samples and more symptomatic, severe disease. There is a lack of data on exacerbations or exacerbation-like respiratory events that may occur in the early phases of disease, when subjects are more asymptomatic, less functionally impaired, and not likely to have been diagnosed with COPD; what characteristics are associated with susceptibility to these events and what their medication and health service utilization impacts may be.

Among the many extra-pulmonary morbidities associated with COPD, CV events are the 2nd most frequent cause of mortality in COPD patients after pulmonary causes [14, 15]. Furthermore, patients with airflow limitation are at increased risk of CVD independently of age, gender and smoking history [16]. Chronic inflammation is a predominant characteristic of COPD and is associated with the development and rupture of atherosclerotic plaque in CVD [17]. There is increasing evidence suggesting a link between systemic inflammation, atherogenesis, vascular dysfunction and risk of CV events in COPD [18-21].

It is likely that exacerbations, which are associated with acute increases in inflammation, directly influence susceptibility to CV events, and subjects who experience acute events such as myocardial infarction (MI) or stroke have greater annual exacerbation frequency than those who do not [22, 23]. New methods of assessing CV risk such as measures of arterial stiffening, which are validated, non-invasive and predictive of CV events in many different disease populations and healthy controls offer the possibility of evaluating CV risk in COPD populations [24-31]. Interestingly, although one study examined the course of change in arterial stiffness at exacerbation [31], and one assessed cross-sectional determinants of arterial stiffness in stable state [32], there is no data on the longitudinal determinants of increases in arterial stiffness during and subsequent to exacerbations, nor is there data on the relationship between lung-specific

inflammatory mediators and arterial stiffness.

Therefore, to address these questions using translational research, 2 research projects were carried out. The first was an analysis of the existing population-based CanCOLD cohort study, and the second was a local study of severe COPD patients who were hospitalized for acute exacerbations and followed for 6 months. Studying these two populations allowed for the assessment of the impacts of exacerbations from inflammation, to CV outcomes, to populational healthcare impacts; thus fulfilling the “bench to bedside” requirements of translational research.

CHAPTER 2: LITERATURE REVIEW

This chapter presents a review of the existing literature pertaining to the research questions addressed in this thesis. Due to the broad scope and translational nature of this thesis however, at times, only the most important aspects of relevant concepts are covered so as to limit the length of this chapter. Section 2.1 presents an overview of the burden of COPD including its definition (2.1.1), global burden (2.1.2), prevalence (2.1.2.1), morbidity (2.1.2.2), mortality (2.1.2.3) and health care and economic burden (2.1.2.4). Section 2.2 reviews the different methods that have been used to capture exacerbations including retrospectively (2.2.1) and prospectively (2.2.2). Section 2.3 reviews the concept of COPD exacerbations including their specific definitions (2.3.1), ascertainment of their severity (2.3.2), and frequency (2.3.3). Section 2.4 is a general overview of the aetiology of exacerbations including respiratory tract infections (2.4.1), and environmental pollution (2.4.2). Section 2.5 is a summary of the inflammatory response at exacerbations including a very brief summary of stable state inflammation, as well as an introduction to the concept of biomarkers (2.5.1). Section 2.6 discusses the various health impacts of exacerbations, including that on mechanical effects and decline in lung function (2.6.1), physical activity (2.6.2), health-related quality of life (2.6.3), and hospital admission and mortality (2.6.4). Section 2.7 reviews non-CV comorbidities in relation to COPD and exacerbations including neuropsychiatric conditions (2.7.1), metabolic syndrome and diabetes (2.7.2), skeletal muscle dysfunction (2.7.3), osteoporosis (2.7.4), gastroesophageal reflux disease (2.7.5) and lung cancer (2.7.6). Finally, section 2.8 is an in-depth review of CV comorbidity in relation to COPD and exacerbations, including an overview of shared risk factors of CVD and COPD (2.8.1) with a discussion on the role of aging and senescence (2.8.1.1), lung function (2.8.1.2), smoking (2.8.1.3), physical inactivity (2.8.1.4), hypertension (2.8.1.5), and environmental factors (2.8.1.6); a discussion of increased CV susceptibility in COPD beyond

traditional risk factors (2.8.2), with an overview of inflammatory mechanisms (2.8.2.1), an overview of oxidative stress mechanisms (2.8.2.2) and of other physiologic and metabolic factors (2.8.2.3); and finally an overview of markers of CV risk in COPD (2.8.3), including carotid intima-media thickness (2.8.3.1), endothelial function (2.8.3.2), arterial stiffness (2.8.3.3) with an overview of measurement of arterial stiffness (2.8.3.3.1).

2.1 Overview of the burden of COPD

2.1.1 Definition of COPD

According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) COPD is defined as *"a common preventable and treatable disease, (that) is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients"* [4]. COPD is generally regarded as an "umbrella" term that includes chronic lung diseases such as emphysema and chronic bronchitis that lead to airflow obstruction. Inhaled cigarette smoke (CS) and other noxious particles such as smoke from biomass fuels induce lung inflammation [4]. This normal response of the lungs becomes abnormal in persons with COPD. As a result, parenchymal destruction leading to emphysema, in conjunction with impaired repair and defense mechanisms leading to small airway fibrosis and narrowing, may occur. Moreover, destruction of the lung parenchyma may lead to loss of alveolar attachments and decreased elastic recoil. Together, these changes can cause air to become trapped in the small airways and impair the airways' ability to remain open during expiration. Consequently this results in progressive airflow limitation, that is best measured using spirometry [4]. Spirometrically defined COPD is based on a forced

expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) ratio of less than 70% [4]. The predominant symptom associated with COPD is breathlessness, which can be accompanied by other symptoms such as sputum production, cough and wheeze. However, there is much heterogeneity in the symptoms experienced by people with COPD, and as a result, it may be difficult to accurately diagnose.

2.1.2 Global burden of COPD

It is estimated that there are at least 210 million people living in the world with COPD, and that this disease is responsible for 3 million deaths per year [33]. COPD is expected to be the 3rd leading cause of death globally by 2020 according to the World Health Organization (WHO) [5], and its burden is predicted to increase significantly in the coming years [4]. The prevalence, morbidity and mortality of COPD across different countries and even among different groups within a country are highly variable [4]. The development of COPD depends on the cumulative exposure to risk factors such as CS and outdoor or indoor air pollution. It is expected that the prevalence and burden of COPD will increase substantially due to ongoing exposure of people to COPD risk factors particularly CS and the ageing of the world's population [4, 5, 34].

2.1.2.1 Prevalence

Estimating the prevalence of COPD by the WHO has been difficult due to limited data availability [35]. Moreover, available data on COPD have been collected using various inconsistent methods, and there has been a general under-recognition and under-diagnosis of this disease [36, 37]. Despite that spirometry is simple, inexpensive and suitable to be used in clinical practice, and that lung function has been shown to be a strong predictor of all-cause mortality

independent of smoking, COPD remains a seriously overlooked disease by healthcare providers [38]. It is likely that the burden of this disease is not appreciated, nor is the fact that it is preventable and treatable. The international Burden of Obstructive Lung Diseases (BOLD) initiative [39] was developed to provide standardized methods for measuring the global prevalence of COPD among the general population and by collecting information on risk factors that could be used in countries at all levels of development. The BOLD initiative was developed alongside the Latin American Project for the Investigation of Obstructive Lung Disease (PLATINO), which was carried out in 5 Latin American countries [40]. The data from the BOLD initiative revealed that there were significant variations in COPD prevalence across countries throughout the world, but that overall, the global prevalence for GOLD stage 2+ COPD (moderate to very severe disease) was approximately 10.1%, with a prevalence of 11.8% in men and 8.5% in women [35]. Moreover data from a systematic review and meta-analysis of studies from 28 countries dating between 1990 and 2004, suggest that the prevalence of COPD is significantly higher in smokers and ex-smokers than in never smokers, and that it is highest in subjects at least 40 years of age, and greater in men than in women [37]. Using the BOLD methodology, a Canadian analysis of 3042 subjects selected from the general population, found that overall, 16.7% of subjects met the criteria for GOLD stage 1 or higher COPD [9]. This was approximately four-fold higher than previous estimates based on annual community surveys, and importantly, highlighted that COPD is a greater health concern in Canada than previously thought.

2.1.2.2 Morbidity

Assessment of morbidity in subjects with COPD is typically based on doctor visits, emergency room visits, and hospitalizations [4, 38]. Like with prevalence, there is a lack of data on these outcomes, and most data come from health databases. Additionally, these may be influenced by many factors including availability of hospital beds, variation in terminology and coding [38]. Available data suggest that morbidity increases with age, and may be affected by comorbid conditions such as CVD, diabetes etc. that are likely to impact a patient's health status and interfere with disease management [40, 41]. A detailed discussion of comorbid conditions associated with COPD is given in sections 2.6 and 2.7.

2.1.2.3 Mortality

There are several complications in establishing the burden of mortality due to COPD. The most important include the issue of under-recognition and under-diagnosis of COPD, which restricts the reliability of mortality data [42, 43], and that COPD is often listed as a contributing cause of death or may even be completely omitted from death certificates [44, 45]. The WHO's data on mortality attributable to COPD is based on the use of an International Classification of Diseases category, which in its tenth revision, categorized it as "COPD and allied conditions". It is clear that COPD is a significant contributor to global mortality, as previously stated, COPD is expected to be the third leading cause of death worldwide by 2020 [5]. The sustained increase in mortality rates due to COPD over the last decades (estimated to have increased by 163% between 1965-1998) have been a stark contrast to those of CVD and most other common chronic diseases, which have seen a significant drop [38, 46]. This increase is largely due to the persistent smoking

epidemic, ageing of the world's population, as well as a general decrease in mortality from other causes such as infectious diseases [4].

2.1.2.4 Healthcare and economic burden

Recent data from the Canadian Institute for Health Information show that COPD is responsible for the highest rate of hospital admission and readmissions among the "major" chronic diseases in Canada [47]. This report also revealed that 18% of COPD patients were readmitted once within a year and 14% were readmitted twice; figures much greater than those for heart failure, angina, hypertension or diabetes [47].

COPD exacerbations account for the greatest proportion of the total healthcare burden of COPD in Canada and beyond (this has been observed across the European Union, the United States, etc.) [48]. These acute events often lead to emergency room or hospital admission as well as unplanned physician visits. Patients admitted for COPD exacerbation have an average hospital length-of-stay of ten days, with a cost of $10,000 per stay, which in Canada, has been estimated to represent $1.5 billion a year [49].

In a more recent study of 285 patients from 23 sites across Canada, average annual COPD-related costs were found to be $4,147 per patient [50]. Maintenance costs accounted for $2,475 per patient, whereby medications accounted for 71% of this. Subjects who exacerbated were treated with medication, outpatient care, emergency room visits and hospitalizations, which amounted to a mean cost per exacerbation of $3,036. In 2010 in the United States, the estimated direct cost of COPD was $29.5 billion, including $13.2 billion in hospital care costs, $5.5 billion in physician services and $5.8 billion in prescription medications, as well as $20.4 billion in indirect costs

(morbidity and mortality) [51]. Similarly in the European Union, among respiratory diseases, COPD represents 56% of total cost adding up to 38.6 billion euros per year [52].

In developing countries direct medical costs may be less informative than measuring the workplace and productivity impacts of COPD. This is because the healthcare systems in these countries do not all provide long-term support care for patients [4]. As a result, individuals affected by COPD, as well as a family member or close friend who must care for them, may be forced to miss work when COPD becomes too debilitating. Consequently, this indirect cost, may severely impact the economies of these countries [4].

As the prevalence of COPD is on the rise, and the fact that with increasing age and disease severity exacerbations become more frequent, the associated healthcare and economic costs will likewise continue to increase. As a result, the only way to reduce this burden is to prevent exacerbation by diagnosing and treating COPD appropriately.

2.1.3 Summary

Despite increasing efforts, there is still a lack of reliable data on the prevalence, morbidity, mortality and economic costs associated with COPD. The majority of the healthcare and economic burden of this disease is attributable to its exacerbations. With the ageing of the world's population and continued exposure to risk factors, obtaining accurate estimates of the global prevalence and burden of COPD will be essential to guide public-health initiatives and increase public awareness of this disease.

2.2 Measuring COPD exacerbations

Exacerbations can be measured retrospectively via patient interview (e.g. questionnaire) or from patient treatment records (e.g. health administrative databases) [53]. Prospectively, their ascertainment can be done using patient diary cards or using the more recent EXACT-Pro tool (EXAcerbation of Chronic pulmonary disease Tool- Patient Reported Outcome). A main limitation to exacerbation measurement is that tools and approaches vary widely and often lack validity testing, and as a result, these may affect exacerbation rates.

2.2.1 Measuring exacerbations retrospectively

Ascertainment of exacerbations can be based on patient recall of events, which can be done either during healthcare visits, by telephone interview [53] or using an online questionnaire. Using the East London COPD Cohort, it was shown that patients can reliably recall the number of exacerbations they had during a previous year, and this estimate is comparable to that measured using daily diary cards that record symptom change [54]. There is also sufficient accuracy when relying on patient recall to allow for stratification of patients as frequent (≥3/year) or infrequent exacerbators. Despite this, patient recall can lead to potential bias and omission of events. Patient-reported exacerbation rates tend to be greater than those reported in clinical trials, as very sick and unstable COPD patients tend not to be recruited into clinical trials and differences in the definitions of exacerbations may impact exacerbation rates. As a result, the "real experiences" of COPD patients may significantly vary from that of the research setting [55]. Underreporting of COPD exacerbations is another problem with relying on patient recall, as well as being a fundamental issue of COPD. Up to 70% of exacerbations go unreported [56, 57]. It seems that experiencing more symptoms at exacerbation onset is an important predictor of exacerbation

report [56], and that unreported exacerbations are associated with patients fearing hospitalization, being housebound or bedridden [58]. Consequently, caution must be taken when relying on patient recall to ascertain exacerbations.

COPD exacerbations can also be detected using information found within patient health records or health administrative databases [53]. These sources can be used to determine when and if medication was prescribed (antibiotic and/or corticosteroid) to a COPD patient or whether they were admitted to hospital or if other healthcare services were sought (e.g. doctor visit) [59]. The limitations of this approach are that it can only be used to determine exacerbations based on events (healthcare utilization), and as a result, milder exacerbations that do not require treatment may not be measured; there is also uncertainty regarding how to differentiate between two separate episodes based on healthcare use, and this approach will not take into account unreported exacerbations.

2.2.2 Measuring exacerbations prospectively

Prospectively, exacerbations have mostly been ascertained using patient-kept daily diary cards [11, 12, 53]. Diaries for COPD were initially developed based on those used in asthma studies, but with greater emphasis on cough and sputum [60]. Daily diary cards are a means by which patients record their daily symptoms, which allows for the detection of symptom change that goes beyond day-to-day variations and to assess their impacts [61, 62]. As a result, this provides a precise way to detect symptom-based exacerbations. Data on the use of daily diary cards with daily measurement of peak expiratory flow rate in COPD patients have largely come from the East London Cohort [11, 12, 54, 63, 64]. The use of daily diary cards has provided important

information on symptoms associated with exacerbations, the "prodromal" phase and how symptoms recover with treatment [61]. This approach has also shown that many patients do not report exacerbations to healthcare professionals or research staff despite encouragement, and as a result many go undetected [11, 56].

An issue with daily diary cards relates to their widespread use and study in the East London Cohort. This group of patients is well trained to fill these out and react to changes in symptoms, prompting patients to seek out medical attention earlier in their exacerbations than "typical" COPD patients resulting in earlier treatment [65]. As such, data emerging about this cohort may not be generalizable and reflective of the "typical" COPD patient. Moreover, there is no standardized diary card, and as a result, this may affect reported results, and exacerbation ascertainment.

The EXACT-Pro instrument was also developed to assess exacerbations [66]. The basis for this tool was to reliably detect exacerbations in clinical trials and to be able to accurately measure rates, severity and duration. The EXACT-Pro is a personal digital assistant device consisting of a 14-item scored questionnaire recording changes in predefined symptoms. The change in EXACT-Pro score from stable state at exacerbation has been shown to be useful in detecting exacerbations [67]. However this change was also found to be smaller in severe COPD patients than in milder patients, suggesting that it is difficult to use change in scores that remain valid across the COPD disease spectrum [67]. Moreover, further limitations to the EXACT-Pro arise because 1) its relies on a personal digital assistant device, which reduces the number of subjects who can use it (as some may have difficulty with this), 2) it is based on symptoms and its output may be unspecific (e.g. a significant changes in score could result from worsening of conditions different from

COPD exacerbations), and 3) to date there have been issues in terms of accurately defining the time of exacerbation resolution [68]. Automated telephone exacerbation assessment systems (TEXAS), have also been evaluated as means to detect exacerbations [69]. Although they proved to be useful in ascertaining exacerbations, the implementation of automated systems may restrict their use, in addition to patient compliance with using this system. Finally, there have been attempts to use other measurement tools that have been primarily developed to measure health-related quality of life in COPD patients to assess exacerbations. The Clinical COPD Questionnaire (CCQ) has been used to evaluate disease severity and treatment response [70, 71] in daily clinical practice. It has been found that weekly CCQ assessment may provide a useful tool to detect unreported exacerbations [72]. However, limitations to these approaches include suitability for long-term use as many subjects are unwilling to perform this for ≥12 months, the number of subjects who are capable of using these, as a certain level of cognitive and visual skills are required, as well as variability in the content and structure of diaries [53]. The COPD Assessment Test (CAT) questionnaire has been used to detect exacerbations, whereby a worse score would correlate with health impairment due to exacerbation. However, the CAT was developed primarily as a health-related quality of life assessment tool and was not validated for use at exacerbation. Consequently it is thought that it lacks the specificity to detect exacerbations in the day-to-day life of patients, and as a result, its effectiveness is limited [73]. As the CCQ and CAT were not specifically developed and validated to ascertain exacerbations, there are important limitations in their use and reliability.

2.2.3 Summary

Exacerbations can be measured retrospectively or prospectively. Limitations in terms of ascertaining exacerbations are mainly due to patient underreporting, as well as the lack of a prognostic and objective measurement tool.

2.3 Definition, severity and frequency of COPD exacerbations

The natural course of COPD is often perturbed by acute events of worsened respiratory symptoms known as exacerbations [74]. These events greatly influence the progression of COPD and are key determinants of health and functional status in patients [11, 12, 64, 73, 75]. Despite their importance in COPD management, finding an appropriate consistent definition of these events and their severity remains a core challenge of COPD research. Moreover frequency of exacerbations has serious implications with regards to their overall impacts.

2.3.1 Definitions of COPD exacerbations

Yet, despite their importance in COPD management, finding an appropriate consistent definition and method of evaluating COPD exacerbations remains one of the core challenges of COPD research. Operational definitions have been used in the literature in order to measure their frequency and impact on disease. These fall into two broad categories [76]: symptom-based (requiring the change in/worsening of respiratory symptoms) [60] or event-based (requiring symptom change in addition to the increase use of maintenance medication and/or treatment with antibiotics and/or corticosteroid and/or hospitalization) [77]. Both of these usually require that symptom change persist for a minimum of two consecutive days [53], the first day of which is used to determine exacerbation onset.

However both symptom- and event-based definitions have failed to reflect the range of symptom manifestation and inconsistent access to or seeking of healthcare associated with treating COPD exacerbations. Patients may experience a multitude of symptoms at exacerbation, however, it is generally agreed that most experience at least 1 of three "major" symptoms including: increased dyspnea, increased sputum purulence and/or increased sputum volume, which are most debilitating to patients [60]. Other symptoms, so-called "minor" symptoms, include: increased cough, increased wheezing, sore throat or nasal discharge, fever or chills without other cause, chest tightness, fatigue, difficulty with expectoration and night-time awakening [53].

Both symptom-based and event-based definitions have advantages and limitations [76]. Symptom-based definitions have been shown to be more sensitive in detecting exacerbations [78]. This is likely because symptom worsening and subsequent functional impairment is a major concern for patients and will prompt them to contact their Physician. Moreover, symptom-based definitions can capture exacerbations that are not severe enough to warrant a healthcare visit [53]. Their limitations can be attributed to several factors. There is much variation in symptoms experienced by patients at exacerbation, and it may be difficult to distinguish those related to exacerbation from those due to another health condition or comorbidity [53]. There is also a lack of objective and diagnostic measurement tools to record symptom change. Finally, the reliance on patients to come forward to a healthcare professional to report symptom change and for that healthcare professional to recognize an exacerbation can severely limit the usefulness of symptom-based definitions [53].

When considering event-based definitions, the main advantage is that they provide an objective measure of exacerbations by defining standard parameters for medication and healthcare use. Moreover, they provide an easy way to distinguish between the severities of events (which will

be discussed later) [73, 79, 80]. However, their use is limited by introducing a potential bias related to regional differences in access to healthcare (whereby regions with "liberal admission policies" report more frequent and severe exacerbations) [66]. Event-based definitions may also fail to take into account events unreported by patients and fail to take into account a change in symptom that goes beyond normal day-to-day variation that may negatively impact a patient's health and quality of life without necessarily prompting the patient to require medication or healthcare.

Currently the GOLD guidelines [4] define an exacerbation as: "*an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication* [53, 77, 81]".

2.3.2 Severity of exacerbations

Ascertaining the severity of COPD exacerbations is an important part of COPD management as it will dictate treatment choice and be a critical factor in assessing the effectiveness of new therapy [82]. From a clinician perspective, severity of exacerbation will be determined based on symptoms, physical signs and response to therapy. This will be greatly influenced by patients' reporting and access to healthcare. Furthermore, reporting is in part determined by the severity of the underlying COPD (severity of the airflow obstruction) and the characteristic of exacerbation, in particular the average number of symptoms [56]. Exacerbations as defined from the operational event-based definitions are typically considered to be "mild" when they require treatment with an increase in regular medication, "moderate" when they require the prescription of antibiotics and/or corticosteroids, and "severe" when they require hospital admission [53]. An important caveat to this is that all exacerbation-like respiratory events which are not treated

pharmaceutically or with hospital admissions are not considered. This is a major limitation considering that underreporting is a widespread phenomenon [83, 84]. Unreported exacerbations have been shown to have significant negative impacts on the health status of patients [56, 84] [83]. Up to 43% of patients with unreported exacerbations can experience clinically important declines in health status compared to 52% of patients who report exacerbations [56]. As a result, there has been ongoing effort in developing tools that may aid in measuring exacerbation severity that take into account the patient's perspective [66].

2.3.3 Frequency of exacerbations

Exacerbation frequency tends to increase with disease severity [4]. Frequent exacerbations have typically been defined as 2 or more exacerbations per year [11, 12, 80]. However, the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study data showed that there is an intrinsic frequent exacerbator-susceptibility phenotype that exists among patients that remains stable over time [85]. Moreover, having a history of frequent exacerbations is the major determinant of future risk of frequent exacerbations among COPD patients across GOLD stages [85]. Studies have shown that this group of patients also has worse health status and functional impairment with an accelerated decline in lung function and worse quality of life [11, 12], increased risk of hospitalization and increased mortality [13, 74, 86]. Consequently it is important to identify these at risk subjects and target them for exacerbation prevention, management and therapy.

2.3.4 Summary

Exacerbations perturb the natural course of COPD, and become more frequent as disease progresses. Due to the heterogeneous nature of these acute events, different operational definitions have been use to characterize them. This has rendered it difficult to compare outcomes across studies. Moreover, as up to 70% of exacerbations may be undetected [56, 57], this likely contributes to the problem of underestimation of the total burden of COPD. Currently, the GOLD definition of exacerbations is event-based, and severity of exacerbations is also measured based on healthcare utilization. Patients who frequently exacerbate are at particularly high-risk for functional impairment and increased mortality.

2.4 Aetiology of COPD exacerbations

Exacerbations are most often triggered by respiratory tract infections, but have also been caused by air pollution [6, 79, 81].

2.4.1 Respiratory tract infections

COPD exacerbations are often caused by upper respiratory tract infections, which occur frequently in the winter months as infections are common in the community [79]. It is thought that approximately 50% of exacerbations are associated with viral infections [81]. These exacerbations tend to be more severe and associated with longer recovery times than those caused by other factors [87, 88]. Rhinoviruses are most frequently detected at exacerbation, however other viruses may include: coronavirus, respiratory syncytial virus, influenza, parainfluenza and adenovirus [79]. Polymerase chain reaction (PCR) techniques allow for the detection of viral origin. Rhinoviruses can be detected in induced sputum and nasal aspirates of COPD patients at

exacerbation [88], and it has been shown that low-dose experimental rhinovirus infections in patients with mild COPD can produce symptoms similar to exacerbation [89]. The importance of influenza in causing exacerbations has decreased with the administration of the flu vaccine to COPD patients. Respiratory syncytial virus has been observed at exacerbation [90], however it is unclear whether it is the sole cause of exacerbations as this virus has been detected in the airways of steady-state COPD patients and associated with increased airway inflammation in stable COPD [91]. Latent adenoviral protein E1A expression in alveolar epithelial cells has been found to enhance the consequences of CS-induced lung inflammation [92].

COPD exacerbations are also caused by bacterial infections. The specific role of these during exacerbations has been difficult to assess as airway colonization in stable state is associated with the same types of bacteria as those seen at exacerbation including: Haemophilus Influenzae, Streptococcus Pneumoniae, Moraxella catarrhalis, Staphylococcus aureus and Pseudomonas aeruginosa [93]. Some of the first evidence indicating a role for bacteria in exacerbations came from Anthonisen *et al.* [60], who demonstrated that patients experiencing increased dyspnea, sputum volume and sputum purulence may experience significant benefits from treatment with antibiotics. Moreover, positive bacterial cultures have been associated with purulent sputum and increased sputum neutrophilia [94].

Bacteria in the lower airways may disrupt host dense leading to increased epithelial injury, defective mucociliary clearance, chronic mucous hypersecretion, and inflammatory cell infiltration, which in turn result in further host defense impairment [95]. This could explain why stable state colonization has been described and associated with exacerbation frequency [96]. There remains significant work to be done in order to fully understand the role of bacteria in

exacerbations. Molecular typing techniques are helping in the detection of changes in bacterial strains at exacerbation, rather than focusing on bacterial species [79].

An enhanced systemic inflammatory response and greater decline in FEV_1 have been observed in exacerbating COPD patients who have evidence of concomitant infection with H. influenzae and rhinovirus when compared to those with only H. influenzae infection [97]. Moreover, greater decline in lung function and increased hospital length-of-stay has been reported in patients with co-infection [98]. It is generally thought that approximately 25% of COPD patients admitted to hospital for exacerbation have evidence of co-infection [81, 99].

It is clear that viral and bacterial infections play an important role in triggering exacerbations. Yet there remains much uncertainty with regards to the specific contributions of each and their possible interplay [79].

2.4.2 Environmental pollution

Increased environmental pollution has been shown to be able to trigger COPD exacerbations [100, 101]. Ambien concentrations of air pollutants (including sulphur dioxide, nitrogen dioxides, ozone and particulate matter) have been associated with increased hospitalization for COPD patients [102]. While diesel exhaust has been shown to induce airway inflammation including increased sputum neutrophilia, interleukin (IL)-6 and methylhistamine in healthy subjects [103], and may reduce T-cell activation while enhancing alveolar macrophage migration into airspaces [104]. Consequently it seems that environmental pollutants can trigger an inflammatory response in the airways, which may in turn trigger an exacerbation.

2.4.3 Summary

The majority of exacerbations are caused by upper respiratory tract infections, where viruses and bacteria have been shown to play an important role in the aetiology of exacerbations. Although there remains considerable uncertainty with regards to what this may be. Air pollution can also induce an inflammatory response that may lead to an exacerbation.

2.5 Overview of inflammatory response during exacerbation

The inflammation associated with COPD and its exacerbations is extensive. To understand the importance of changes at exacerbation, it is important to understand the chronic inflammation that occurs in stable disease, and how this contributes to disease progression.

In stable state, neutrophil elastase (NE) plays an important role in COPD pathogenesis, including a role in the progression of emphysema, mucus gland hyperplasia and mucus hypersecretion [105]. NE levels have been found to be elevated in COPD patients when compared to smokers [106]. Other inflammatory mediators have also been found to be elevated in subjects with stable COPD when compared to smokers including tumor necrosis factor (TNF)-α, IL-1β, leukotriene B4 (a chemoattractant), IL-8 (CXCL8), and growth-related oncogene-α [107-109]. These factors all play important roles in the inflammatory cascade that occurs in COPD by attracting and activating other inflammatory cells and contributing to tissue damage [93]. Irritants such as CS or particulate matter activate respiratory epithelial cells and phagocytes, which will then release several pro-inflammatory mediators such as IL-8, which will activate other cells such as leukocytes and endothelial cells [93]. Mediators including leukotriene B4 and IL-8 will chemoattract neutrophils towards the site of epithelial injury [110]. Neutrophils can then release proteases that will degrade components of the extracellular matrix, thereby allowing them to pass through the matrix and enter the airways, where they will cause further tissue damage and

activate other inflammatory cells to release cytokines and chemokines such as IL-6 and IL-1β [93].

Existing airway and systemic inflammation increases significantly during COPD exacerbations [75, 99, 111, 112]. Briefly, pathogenic insult or environmental irritants activate the transcription factor nuclear factor (NF)-kB in airway epithelial cells and macrophages, which triggers the production of pro-inflammatory cytokines including IL-8 (CXCL8) [81]. These will then be released, and in turn, attract neutrophils and TNF-α and IL-6, which will further amplify the inflammatory cascade, including for example, triggering the release of fibrinogen into circulation and increasing C reactive protein (CRP) production [113]. These additional neutrophils will release more NE in the airways, which will cause further lung injury, and they will generate oxidative stress, which will likewise damage the airways [98, 114]. During severe COPD exacerbations, glutathione (an anti-oxidant) levels have been shown to decrease significantly compared to stable state levels [115]. Furthermore, free radicals such as nitric oxide (NO) are found in greater quantity in the exhaled breath of patients during exacerbations [116].
Although airway neutrophilia is predominant in exacerbations, the expression of many inflammatory cell types in the bronchial mucosa also increases depending on the type of insult that instigates the exacerbation [117]. Viruses tend to stimulate an eosinophillic inflammatory response compared to bacteria that that tend to trigger neutrophilia [98, 118].
Overall, the airways of frequent exacerbators tend to be more inflamed. There is evidence that these patients have higher levels of sputum IL-6 and IL-8, even when stable [111]. Moreover, their inflammatory response appears to persistently increase over time. Sputum IL-6 and plasma fibrinogen have been found to continue to increase more rapidly over time than that of infrequent

exacerbators [119]. During exacerbation recovery, subjects who frequently exacerbate have greater sputum IL-6 and serum CRP than infrequent exacerbators [120].

Taken together, this suggests that subjects who frequently exacerbate may have an innate persistent and increased tendency to produce heightened inflammatory responses. However, it is still unclear whether this is causative or a result of susceptibility to the insults that will trigger an exacerbation [117].

2.5.1 "Biomarkers" of exacerbation

The Natural Institutes of Health define biomarkers as a "*characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*" [121]. Given the predominant role of inflammation in COPD and its exacerbations, finding an inflammatory mediator that could be used as a biomarker of disease activity or risk stratification has been a key research objective. More specifically, having biomarkers of COPD exacerbations could allow for prompt diagnosis, and help guide treatment choice and thereby improve patient outcomes [122]. However, to date, there have been no conclusive markers that satisfy this need.

Lung-specific inflammatory mediators are garnering more attention since the findings of the ECLIPSE study showed that CC-16 and SPD could predict several health outcomes of COPD [123-125]. It is thoughts that inflammation from the lungs may "overspill" into the circulation and contribute to systemic inflammation, and as a result, the systemic consequences of COPD [113]. Continued research and development of new detection technologies will help advance this search.

2.5.2 Summary

Inflammation becomes significantly enhanced with exacerbations, and as a result, represents a key contributor to the accelerated progression of disease associated with exacerbations in COPD. Discovering a useful inflammatory biomarker of relevant health outcomes in COPD remains a fundamental research goal. It is thought that an inflammatory mediator produced locally in the lungs may be the answer, however, the advent of new biomarker detection platforms may also provide much needed guidance in this pursuit.

2.6 Health impacts of exacerbations

COPD exacerbations have significant impacts on the overall health status and functional capacity of patients. They contribute considerably to disease progression by aggravating several pathophysiological features of COPD.

2.6.1 Mechanical effects and decline in lung function

The injury associated with exacerbation is probably most apparent to patients through greater decline and impairment in lung function. This typically manifests as increased dyspnea, which functionally limits patients. The symptom of dyspnea in COPD patients is largely attributable to dynamic lung hyperinflation [126].

At exacerbation, airway inflammation increases and patients tend to have greater expiratory flow limitation. This results in worsened dynamic hyperinflation and greater respiratory effort, cardiovascular strain, and inspiratory muscle overload, which manifests as increased dyspnea [127]. Some patients who have severely impaired airflow obstruction may have severe lung

hyperinflation in stable state, and as a result, also have a reduced ability to withstand further impairment [117]. It is thought that exacerbations may be more easily triggered in these patients [117].

Treatment with bronchodilator has been shown to improve small airway obstruction and decrease lung hyperinflation in some subjects in stable state [128]. Consequently, this may "reset" the baseline of subjects, and lower their susceptibility to exacerbations.

Patients who have moderate to severe COPD have been shown to have accelerated decline in lung function over time with repeated exacerbations [12, 129]. Initially, Donaldson *et al.* [12] showed that subjects who frequently exacerbated had a 25% greater loss of lung function over time (-32.1 mL/year vs. -40.1 mL/year), and Kanner *et al.* [129] showed that smoking patients with mild COPD who experienced 1.5 lower respiratory tract illnesses per year had a decline in FEV_1 of -69.4 mL/year vs. -55.9 mL/year in subjects with fewer illnesses. Many larger studies have since supported the notion that frequent exacerbations accelerate lung function decline and thus contribute significantly to disease progression [130-133].

2.6.2 Physical activity

Exacerbations have been shown to be associated with peripheral muscle weakness and lower muscle force (as measured using quadriceps peak torque) [134]. Moreover, exacerbations tend to decrease the amount of time patients spend on outdoor activity [63]. It is possible that exacerbations can permanently impair physical activity, whereby patients who frequently exacerbate (>2.47 exacerbations/year) have a faster decline in daily time spent outdoors equalling -0.17 hours/year, and even infrequent exacerbators were shown to have a decline of -0.10

hours/year [63]. Several factors may contribute to this including: lack of physical activity, increased bed rest, inflammatory processes, nutritional factors, metabolic status and treatment with corticosteroids [135].

2.6.3 Health-related quality of life

Exacerbations are one of the most important factors that determine the health status of patients with COPD. Patients with frequent exacerbations (including both treated and untreated events), have been shown to have poorer health-related quality of life in general as assessed using the St-Georges Respiratory Questionnaire (SGRQ), which is a disease specific measurement tool [11, 136]. Frequent exacerbators have also been found to have lower health-related quality of life as measured using the CAT [137]. Moreover, even patients with unreported exacerbations have lower health status [56]. The impact of exacerbations on health-related quality of life goes beyond the duration of symptom worsening experienced by subjects in association with the event. It has been shown that SGRQ scores may take up to 6 months post exacerbation to return to baseline levels [138]. In frequent exacerbators, health-related quality of life is slower to recover following an exacerbation, and it is thought that with continued relapses and recurring events, patients will not return to their baseline condition and will continue to experience further deterioration over time [139].

Fatigue is an important consequence of exacerbations on health-related quality of life and has been measured using the Functional Assessment of Chronic Illness Therapy-fatigue (FACIT-fatigue) scale [140]. Patients tend to have worsened fatigue at exacerbation with worsening FACIT-fatigue scores as exacerbation frequency increases [140].

2.6.4 Hospital admissions and mortality

Frequent exacerbators are those subjects who are most likely to be admitted to hospital when they exacerbate. The ECLIPSE study, showed that patients with GOLD stage 2 COPD had a 7% hospitalization rate for COPD exacerbation-related causes during the first twelve months of follow-up. This increased to 33% in patients with GOLD stage 4 disease [85]. The most likely cause for hospital admission is delayed treatment and exacerbation management. These processes tend to lead to more severe and longer exacerbations [117].

Risk factors for inpatient mortality include cor pulmonale, congestive heart failure, low arterial pH, leg oedema, age, an oxygen saturation less than 86%, assisted ventilation and low body mass index [141, 142]. Frequent exacerbations are themselves an independent risk factor for all-cause mortality in COPD, and hospitalization for each exacerbation is associated with disease progression [13]. Over time, risk of mortality increases, and at the 10^{th} admission, risk of death is 5-fold greater compared to that at first admission [143].

2.6.5 Summary

Exacerbations lead to significant impairment in the functional status of patients by accelerating the decline in lung function and reducing physical activity. The more frequent subjects will exacerbate, the lower their health-related quality of life will be. Moreover, frequent exacerbators will be at greater risk of hospital admission and mortality.

2.7 Non-cardiovascular comorbidities, COPD and exacerbation

A comorbidity is generally defined as a disease that coexists with a primary disease of interest [144]. Several comorbidities have been well described in patients with COPD. The majority of which arise due to exposure to common risk factors such as CS [4]. The detrimental health impacts of exacerbations contribute to the development of comorbid conditions, and likewise comorbid conditions can increase susceptibility of patients to exacerbations.

COPD is associated with several major non-CV comorbidities including neuropsychiatric disorders, metabolic syndrome and diabetes, skeletal muscle dysfunction, osteoporosis, gastroesophageal disease, and lung cancer.

2.7.1 Neuropsychiatric conditions

One of the predominant neuropsychiatric conditions among COPD patients is depression. Findings from the ECLIPSE study showed that 26% of patients reported symptom-defined depression compared to only 12% of controls, and that prevalence increased with disease severity [145]. A clear relationship has been established between exacerbation frequency and depression. Subjects who frequently exacerbate tend to be more depressed even in stable state, and depressed patients tend to have worse breathlessness and quality of life, be socially isolated and spend less time doing outdoors activities [146]. In COPD, depression has been associated with worse outcomes including hospital readmission and all-cause mortality [147]. Moreover, depression is a risk factor for poor medication adherence and non-compliance in pulmonary rehabilitation programs [148, 149], which may impact the overall treatment of depressed COPD patients and contribute to poor patient outcomes.

Cognitive function impairment has also been described in COPD, particularly at exacerbation [117]. Evidence suggests that COPD patients have significantly poorer cognitive function than non-COPD control subjects, and that those patients who recently exacerbated had significantly worse function than those in stable state independently of hypoxemia, disease severity, cerebrovascular risk or smoking history, and that this impairment persisted even after 3 months [150]. Among exacerbating subjects, up to 57% had cognitive dysfunction in the "impairment" range, and 20% were deemed to have suffered a pathologic loss in processing speed. Cognitive impairment was also associated with lower health-related quality of life as measured using the SGRQ and longer hospital stay. Consequently it is thought that exacerbations may be associated with permanent cognitive deficiency, whereby more frequent exacerbations accelerate deterioration.

2.7.2 Metabolic syndrome and diabetes Mellitus

Diabetes is found in approximately 12% of people with COPD, with increasing prevalence as disease worsens [151]. It is not yet clear why COPD patients may be more susceptible to diabetes and insulin resistance, however it is thought that it may be due to systemic inflammation, particularly involving IL-6 and TNF-α [152]. High blood sugar during COPD exacerbations has been associated with worse patient outcomes in terms of acute non-invasive ventilation, length of inpatient hospital stay and in-hospital mortality [153, 154]. Additionally, patients with hyperglycaemia tend to have greater bacterial colonization in their sputum during hospital admission [154], suggesting that they may have impaired immune function. Further research will help elucidate the mechanisms and implications of this.

2.7.3 Skeletal muscle dysfunction

Skeletal muscle dysfunction is common in COPD patients and has a direct impact on exercise capacity, fatigue and general activity [155]. It is estimated that 32% of COPD patients have muscle dysfunction (as tested using quadriceps strength) and that this increases with increasing disease severity [156]. Dysfunction is thought to arise due to systemic corticosteroid use, inflammation that may contribute to cachexia, decreased physical activity that results in muscle disuse and atrophy, hormonal imbalance, prolonged hypoxia and oxidative stress [18, 155]. Cachexia is an independent risk factor for morbidity, including exacerbation susceptibility, and mortality [18]. Increased serum TNF-α levels have also been associated with cachexia in COPD patients [157]. Most of the factors associated with muscle dysfunction worsen at exacerbation, and consequently it may be that with frequent exacerbations, patients may have more peripheral muscle dysfunction, which leads to further impairment.

2.7.4 Osteoporosis

It is estimated that 35-70% of COPD patients have osteoporosis [158-160]. Osteoporosis has been linked to COPD disease severity, the degree of computerized tomography (CT)-scan-assessed emphysema, systemic inflammation, body mass index (BMI) and physical activity [155, 159-161]. This suggests that several pathophysiological processes are involved including increased proteolytic activity, inflammation, muscle function, and smoking.

Patients who frequently exacerbate tend to have a greater annual loss in bone mineral density when compared to infrequent exacerbators, and as a result, are more prone to fractures [162]. Consequently, it is likely that these pathophysiological processes are aggravated with repeated exacerbations and thereby render frequent exacerbators more susceptible to osteoporosis.

2.7.5 Gastroesophageal reflux disease (GERD)

Up to 30-60% of COPD patients have GERD [155], and there is evidence that patients with GERD have higher exacerbation frequency [85, 163, 164]. It is thought that micro-aspirations of stomach contents can enter the airways and cause irritation that induces inflammation [117]. Reflux including both non-acid and gaseous components is thought to be very common in COPD. A single-blind trial of 100 COPD patients showed that exacerbation rates can be decreased over twelve months with reflux treatment [165]. Yet there remains large uncertainty with regards to the causes and mechanisms of GERD in COPD, and consequently, further research is needed.

2.7.6 Lung Cancer

COPD is an independent risk factor for lung cancer in smokers, where presence of chronic bronchitis and/or emphysema increases risk two-five fold when compared to smokers without COPD [144, 166, 167]. The National Health And Nutrition Examination Survey (NHANES) I study showed that, in 5402 subjects who were followed for 22 years, there was an inverse correlation between airflow obstruction and lung cancer risk [167]. Inflammation plays an important role in tumor promotion by inhibiting apoptosis, interfering with cell repair, and promoting angiogenesis, and CS is the most important risk factor for increased cytokine production [168]. However, the link between COPD and lung cancer is also independent of active smoking, whereby even after smoking cessation, lung cancer risk remains elevated [169].

2.7.7 Summary

It is clear that many of the most common comorbidities seen in COPD patients are linked to exacerbation frequency, and have a significant impact on the overall prognosis of patients. As such, management of COPD must include the identification and treatment of comorbid conditions in addition to the treatment of COPD itself [4].

2.8 Cardiovascular comorbidity, COPD and exacerbation

CVD represent the largest group of comorbidities among COPD patients [117]. The overall prevalence of these is thought to be 20% in COPD patients [151]. Airflow obstruction and COPD have been associated independently with CV mortality and increased incidence of coronary artery disease (CAD), chronic heart failure and arrhythmias [170]. Yet, shared risk factors and similar underlying mechanisms do not fully explain this relationship [171]. It has become apparent that many different pathobiologic processes may be involved and may contribute to the development of COPD and CVD, whereby the predominant common link is thought to be systemic and pulmonary inflammation [144].

CVD is an important risk factor for poor outcomes in COPD patients, whereby CV events are the second most frequent cause of mortality in COPD [14]. There is a large epidemiological body of evidence to support this. The TOwards a Revolution in COPD health (TORCH) study [15] showed that although 35% of deaths in COPD patients were due to pulmonary causes, 27% were due to CV disease, while 21% were due to cancer and 17% due to other conditions. Whereas, the Tucson Epidemiologic Study of Airways Obstructive Disease showed that CVD is responsible for over 50% of deaths in COPD patients [172]. In a study of 405 COPD patients followed for over four years, a diagnosis of heart failure was associated with significantly increased all-cause

mortality [173]. The Lung Health Study showed that 42% of first time hospitalizations, and 44% of second time hospitalizations in patients with mild COPD were due to CV-related causes [174]. In patients with chronic airway obstruction admitted with acute respiratory failure, cardiac arrhythmias were associated with 70% in-hospital mortality and no survival after 2.4 years [175].

Conversely, COPD also has a significant impact on CV outcomes. In a study of 2481 patients with MI who were followed for one year, the presence of COPD was found to be associated with significantly greater rates of hospital readmission and mortality [176]. Another study of 3438 subjects who suffered from MI found that the five-year survival rate for those with concomitant COPD was 46% vs. 68% for those without COPD [177]. COPD has also been found to be an independent predictor of in-hospital mortality or cardiogenic shock (adjusted odds ratio (OR), 1.83) in patients with MI who underwent percutaneous coronary intervention [178]. In another study of over 8,000 patients with acute coronary syndrome, COPD was associated with greater incidence of heart failure and longer hospital stay, but not with in-hospital mortality [179]. Exacerbations are thought to be closely linked to CVD, and it is likely that they contribute to an increased risk of CV events, which in turn may increase susceptibility to exacerbations. In the four-year UPLIFT study, it was found that the period immediately following an exacerbation was associated with increased risk of acute CV events [22]. Among 2,289 COPD patients, the relative risk of cardiac failure, MI and stroke were 10.71, 3.20 and 2.31 for the 180 days following their first exacerbation when compared to the 180 days prior [22]. This observation was confirmed in another study of over 25,800 COPD patients, which showed that there was a 2.27 fold increased risk of MI in the first five days following an exacerbation, and a 1.26 fold increased risk of stroke in the first 49 days [23]. Moreover, patients who experienced MI or stroke had a greater annual exacerbation frequency than those who did not.

Pulmonary embolisms are another complication of exacerbations that tend to lead to poor outcomes, although there is much variability in the prevalence of these [180, 181].

In the East London COPD Cohort, patients with comorbid ischemic heart disease had longer exacerbations, worse health status, increased dyspnea and a lower exercise capacity than patients without [182]. A small study of COPD patients undergoing echocardiography showed that those with diastolic dysfunction had a higher exacerbation frequency [183], and severe exacerbations have been associated with diastolic dysfunction in 30% of COPD patients [184]. Taken together, it is clear that there is a relationship between COPD exacerbations and CV comorbidity, however there is still a lack of understanding with regards to the mechanisms that may mediate this.

2.8.1 Overview of shared risk factors of CVD and COPD

Part of the natural history of CVD and COPD is attributed to the cumulative exposure to several risk factors. Among these, several major risk factors are shared include aging and senescence, lung function impairment, smoking, physical inactivity, hypertension, and exposure to certain environmental factors.

2.8.1.1 Aging and senescence

A common feature of most age-related diseases is chronic inflammation, which plays an important role in causing structural damage to organs and tissues [171]. While cumulative exposure to oxidative stress causes permanent DNA damage, which with telomere shortening, further impair repair processes [185].

There is a strong positive relationship between the prevalence of COPD and age [4]. Loss of elastic recoil and airspace enlargement occur as a natural part of aging in the lungs [186]. It is thought that COPD represents a condition of accelerated aging due to exposure to elevated levels of chronic inflammation and oxidative stress [187]. Circulating leukocytes, and alveolar epithelial and endothelial cells of COPD patients have been found to have shorter telomere lengths (a marker of aging) than those of healthy control subjects [188, 189]. When telomeres reach a critical length, cells enter cell cycle arrest known as senescence, which may be considered as "cellular aging" and leads to apoptosis. Cellular senescence can contribute to the progression of COPD by causing pulmonary epithelial and endothelial cell apoptosis, resulting in loss of alveolar cells and emphysema [190].

Like COPD, the prevalence of CVD increases with age. The prevalence of CAD is 17-fold higher in subjects who are older than 65 years of age when compared to those who are 18-44 years old [191]. The Framingham study showed that the prevalence of chronic heart failure increases from 0.8% to 4.9% in subjects 70-79 years old when compared to subjects 50-59 years, and this increases to 9.1% for those over 80 [192]. Like COPD, there is evidence that accelerated aging plays a role in the development of atherosclerosis and CVD. Endothelial cell senescence is postulated to play a role in endothelial dysfunction and atherogenesis [193], and shortened leukocyte telomeres have been reported in association with large artery stiffness, a marker of CV risk, in patients with CAD [194].

Therefore it seems that aging, both natural and accelerated, plays an important role in the pathogenesis of both COPD and CVD.

2.8.1.2 Lung function

There is very strong epidemiological evidence supporting that decreased lung function, as what characterizes the progression of COPD, is a marker for CV mortality. The NHANES I study showed that patients with low lung function had the highest risk of CV mortality independent of smoking status [167]. Moreover, risk of mortality from ischemic heart disease was over 5-fold greater for those in the lowest quintile of lung function than those in the highest. The Lung Health Study showed that every 10% decrease in FEV_1 lead to a 28% increase in fatal coronary events and 20% increase in non-fatal coronary events in subjects with mild to moderate COPD [195]. The relationship between increased CV mortality with reduced lung function has been reported in many other studies, including the Framingham Heart Study and the Copenhagen Heart Study [196, 197].

2.8.1.3 Smoking

Cigarette smoking is the predominant risk factor for the development of COPD, and is a risk factor for CAD [198]. The population-attributable risk of smoking for the development of COPD is 51-70% [199]. There is a three to six-fold increase in the incidence of MI in chronic smokers than in never smokers [200]. The INTERHEART study showed that the population-attributable risk of a first MI due to smoking was 36% [201]. Post-mortem studies have shown that there is a causal dose-response relationship between cigarette smoking and the burden of atherosclerosis [202]. It seems that chronic lung exposure to small particles found in CS accelerates the progression of atherosclerotic plaque, independently of dyslipidemia, and this is most likely to be mediated by chronic lung and systemic inflammation [203].

2.8.1.4 Physical inactivity

Physical inactivity is a risk factor for decline in lung function and development of COPD, as well as for the development of CVD. A population-based study of 6,790 subjects showed that moderate to high levels of regular physical activity were associated with less decline in lung function and reduced risk of COPD among smokers over time [204]. It is thought that the anti-inflammatory effects of physical activity may be responsible for this [205]. COPD patients tend to avoid exercise and adopt a sedentary lifestyle [206]. Exacerbations significantly impact physical activity, and contribute to the adoption of an inactive lifestyle [206]. Sedentarism leads to muscle disuses and the development of skeletal muscle dysfunction, and further overall impairment.

Physical activity leads to several important protective mechanisms that help prevent CVD, including increased fibrinolysis, which decreases the risk of thrombus formation [207, 208], increased beneficial vascular remodelling [209], decreased blood pressure, and decreased low-density lipoprotein (LDL) and cholesterol with increased high-density lipoprotein (HDL) [210-212]. Moreover, physical activity decreases the risk of obesity. Consequently, when subjects are sedentary, they do not benefit from the protective effects of exercise and physical activity, and become at increased risk of developing COPD and CVD.

2.8.1.5 Hypertension

It is thought that 40-60% of patients with COPD are hypertensive [151]. There is a strong association between hypertension and COPD even in milder disease (GOLD stage 2) [151]. Chronically elevated blood pressure decreases vascular distensibility via increased collagen production and intima-media thickening, which can change the “mechanical resilience” of blood

vessels leading to increased pulsatile shear stress and pressure, which results in endothelial dysfunction and vascular disease [213, 214]. Indeed, the Framingham Heart Study provided strong evidence that elevated blood pressure is associated with significantly increased risk of CVD [215]. Sympathetic over-activation and decreased baroreflex sensitivity are common in COPD, and may contribute to increasing blood pressure [216, 217].

2.8.1.6 Environmental factors

As discussed, many different environmental factors are thought to contribute to COPD, whereby dust and air pollution (especially small particulate matter) exposure may be major contributors [4]. Air pollution [218], second-hand CS exposure [219], diesel exhaust fumes [220] have all been associated with increased risk of CV-events and mortality [221]. These environmental factors all lead to increased lung injury and inflammation, as well as systemic inflammation, which are known mechanisms that contribute to both COPD and CVD [203].

2.8.2. Beyond shared risk factors- increased CV susceptibility in COPD

Studies suggest that development of CV comorbidity in COPD goes beyond exposure to shared risk factors [186]. It is thought that inflammation, oxidative stress, and certain physiologic and metabolic factors may also contribute to the concomitant development of these two diseases. These processes have all been shown to be aggravated, or their progression accelerated, with repeated exacerbations.

2.8.2.1 Overview of inflammatory mechanisms

Chronic inflammation, a key characteristic of COPD, plays an important role in the development of CVD [17]. As discussed, COPD is characterized by chronic inflammation, which increases with disease severity. Inflammation in the lung parenchyma leads to the activation of downstream inflammatory mediators, including acute phase proteins, cytokines and chemokines, while also stimulating the bone marrow to release leukocytes and platelets, which become activated in conjunction with the vascular endothelium [18]. These can in turn contribute to amplifying and aggravating pulmonary inflammation, which will further aggravate systemic inflammation and thereby initiate a detrimental inflammatory cycle [222]. As discussed, there is evidence to show that inflammatory mediators produced in the lungs in response to injury can escape into the blood stream and contribute to downstream inflammation [223]. This can then promote atherosclerotic progression and contribute to increased susceptibility to acute CV events.

The rupture of vulnerable plaque leads to the partial or complete occlusion of an artery via thrombus formation, which typically results in an acute ischemic event [203]. Vulnerable plaques are characterized by having a large lipid core, thin fibrous cap and increased inflammatory content [224]. Neutrophils play an important role in mediating vulnerability, and it appears that neutrophil activation results from upstream inflammatory mediators [203]. As discussed, neutrophilia is an important part of the inflammatory response at exacerbation, and so, the contribution of exacerbations to increased CV susceptibility become clear.

When considering specific inflammatory mediators, several key mediators have been well described in both diseases and more specifically, in COPD patients that have CV comorbidity. Circulating CRP levels are elevated in COPD patients in stable state and CRP is well known to be predictive of CV risk [225]. CRP is released under conditions of vascular injury and will bind to

injured tissue and activate the complement cascade resulting in endothelial damage and tissue inflammation [226]. CRP has adverse effects on vasomotor endothelial function as it inhibits endothelial NO synthase, which impairs vasorelaxation [226]. When in circulation, CRP can up-regulate other inflammatory cytokines and interact with endothelial cells to stimulate IL-6 and endothelin-1 production [227]. CRP also inhibits fibrinolysis and induces the production of inflammatory factors that induce a pro-coagulated state, which is associated with increased risk of ischemic CV events and atherothrombotic events [228]. IL-6, typically elevated in COPD patients, becomes even more elevated in COPD patients with CVD [229], and frequent exacerbators have been found to have greater IL-6 levels than infrequent exacerbators [119]. At exacerbation, increases in serum IL-6 are associated with increases in plasma fibrinogen (an independent risk factor for CAD), which are in turn associated with more purulent and symptomatic exacerbations [75]. Fibrinogen is directly involved in atherosclerosis, by inducing plaque growth, stimulating platelet and leukocyte adhesion to the vascular endothelium and promoting smooth muscle proliferation and migration [230].

In COPD patients, circulating monocytes are known to release greater quantities of matrix metalloproteinases (MMP), such as MMP-9, MMP-2 and elastases such as NE than in healthy controls, leading to a protease/anti-protease imbalance [231]. MMP-9, MMP-2 and NE also play an important role in the pathogenesis of atherosclerosis including plaque formation, destabilization and rupture, thrombus formation and vessel wall remodelling [186, 232]. Overall, it is very likely that the inflammatory responses that occur during COPD exacerbations render the vasculature more vulnerable to injury and atherosclerotic plaque more susceptible to activation, rupture and thrombus formation [18]. Moreover, there is evidence that lung inflammation [233], and vascular activation and atherosclerosis induced through particulate

matter exposure can be attenuated by treatment with statins [234], which have also been shown to improve COPD symptoms and reduced exacerbation frequency [222].

2.8.2.2 Overview of oxidative stress mechanisms

Oxidative stress plays an important role in the pathophysiology of COPD and CVD. Airway inflammation in COPD is associated with increased oxidative stress [115]. Reactive oxygen species induce oxidative injury to tissues that can result in pro-inflammatory mediator activation that act locally in the lung or systemically. Moreover, oxidative stress can induce lipid peroxidation [115], and oxidized LDL are important mediators of atherosclerotic progression via the up-regulation of adhesion molecules on the vascular endothelium [235]. Reactive oxygen species can also induce proliferation of vascular smooth muscle, endothelial cell apoptosis, activate MMPs as well as modulate vasomotor tone, which all contribute to atherosclerosis [236-238]. As mentioned, oxidative stress increases significantly during exacerbations, and it is thus likely that these excess free radicals contribute to CV pathology in COPD patients susceptible to frequent exacerbations [116].

2.8.2.3 Other physiologic and metabolic factors

Hypoxia can occur in COPD either chronically or intermittently with exercise or exacerbation. Hypoxia may contribute to atherogenesis by inducing systemic inflammation and oxidative stress, causing increased macrophage lipid loading- resulting in foam cell production, upregulating the production of cell adhesion molecules and inducing hemodynamic stress [239, 240]. Hypoxia, as well as hyperglycaemia and/or hyperinsulinemia- also common in COPD, can activate the renin angiotensin aldosterone system (RAAS) and expression of angiotensin type 1

receptor on vessel surfaces [213]. Over-stimulation of the RAAS can result in endothelial dysfunction by increasing the collagen content of the extracellular matrix and promoting vascular smooth muscle cell proliferation, which lead to persistent vasoconstriction [241, 242]. Abnormalities in the vessel walls such as increased arterial stiffness or endothelial dysfunction also contribute to the development of CV susceptibility in COPD. A detailed description of arterial stiffness is provided in section 2.8.3.3. The vascular endothelium is important for the regulation of blood flow, coagulation, fibrinolysis, and inflammation [30]. Endothelial dysfunction arises when there is an imbalance between factors with vasodilating, anti-mitogenic and antithrombotic properties and factors with vasoconstricting, prothrombic and proliferative properties [243]. Endothelial dysfunction is associated with atherosclerosis and other CV risk factors as well as being a predictor of CV events (which is discussed in section 2.8.3.2). The severity of airflow obstruction is an important determinant of endothelial function in COPD patients, and increased physical activity can favourably modulate this [244]. With inflammation and oxidative stress, advanced glycation end products can also impair endothelial function and induce inflammation [245]. These can bind to collagen via irreversible cross-link formation, which renders blood vessels more rigid and less elastic, and thereby contributing to CV remodelling.

2.8.3 Overview of markers of CV risk in COPD

In subjects at mid-to elevated CV risk, identifying early changes to the CV system prior to any major clinical events such as MI or stroke remains critical [213]. There are some subclinical markers of CV risk that have been validated such as measuring carotid intima-media thickness, endothelial function and arterial stiffness. Among these, arterial stiffness has been shown to have

a very strong predictive value for CV events beyond that of classical CV risk factors and appears to be most practical for the clinical setting [246, 247].

2.8.3.1 Carotid intima-media thickness

Measuring the thickness of the carotid intima-media has been a validated and reliable marker of atherogenesis [248]. This involves scanning the carotid arteries using a high-resolution ultrasound and determining the mean maximal intima-media thickness. Data from the Rotterdam Study, a population-based analysis, showed that there is an association between COPD and carotid intima-media thickening [249], providing support for its use in monitoring atherogenesis in COPD. However, its routine use is limited by two major factors including its highly technical basis and the high cost of using an ultrasound [250].

2.8.3.2 Endothelial function

In COPD, the most commonly used technique to assess endothelial function is measuring flow-mediated vasodilation after forearm or upper-arm occlusion [244, 251, 252]. Assessing endothelial function in peripheral vessels reflects endothelial function in the coronary vessels [253], and is predictive of CV events even in subjects without atherosclerosis [254, 255].
In COPD, endothelial dysfunction appears to be related to the severity of airflow obstruction, degree of emphysema, and increased inflammation [251]. Yet, Maclay *et al.* [30] showed that COPD is associated with increased arterial stiffness, without evidence of vascular dysfunction. They proposed that although abnormal endothelial function may occur in COPD patients, this likely results from the effects of age and CS-exposure, and so increased arterial stiffness in COPD could represent a mechanistic link between COPD and increased CV risk. Moreover, there

is evidence suggesting that flow mediated dilation may be unreliable when measured in stiffened arteries [256], and COPD patients have been shown to have significantly increased arterial stiffness [26-28]. As a result, measuring endothelial function in COPD may provide important information about vascular function, but may not be the best predictor of CV risk in this population. Furthermore, the lack of standardized measurement methods and the high cost of the systems used for measurement further limit the routine clinical measurement of endothelial function in COPD patients [213].

2.8.3.3 Arterial stiffness

Arterial stiffness, particularly that of the larger central vessels, has rapidly become recognized as an important predictor of CV events and mortality [257-260]. Stiffening of the large arteries occurs naturally with aging through the deterioration of the media and increased vessel-wall collagen content. This may be accelerated with cumulative exposure to factors such as hypertension, oxidative stress and chronic inflammation [213]. This induces structural and functional changes to the heart by increasing the load imposed on the ventricles leading to cardiac hypertrophy [259, 261]. Moreover, stiffer vessels cause the pressure wave reflected at peripheral arteries to return to the heart prematurely during systole rather than during end-systole or early-diastole, and as a result, it no longer contributes to helping with cardiac perfusion [261]. Elevated central pulse pressures can also damage small arterial vessels by exposing them to large pressure fluctuations, leading to target organ damage such as in the kidneys or brain [259].
Therefore, increased arterial stiffness is a precursor to atherosclerosis and represents a useful subclinical marker of CV risk, particularly in COPD [213]. There are now many studies that have addressed and support the use of arterial stiffness in COPD in the clinical setting and as an

objective and reproducible outcome in clinical trials [24, 25, 27, 28, 32, 213, 262-266]. In COPD, increased arterial stiffness has been associated with disease severity [26], the degree of airflow obstruction [28, 29, 31, 267-270], inflammation and oxidative stress [27, 28], the degree of emphysema [28], systemic elastin degradation [29], age, blood pressure and aortic calcification [32]. As mentioned, increased arterial stiffness in COPD can occur independently of endothelial dysfunction [30], and so it may be abnormalities in the extracellular matrix of large vessels that contribute to stiffening.

Recent studies have shown that it may be possible to modify arterial stiffness in COPD patients via endurance training [262], pulmonary rehabilitation [271], inhaled therapies [272] or oxygen supplementation [273]. Consequently it represents an attractive outcome to assess in the context of CV risk and COPD.

To date only one study has been published on arterial stiffness and COPD exacerbations. Patel *et al.* [31] showed that there is an acute increase in aortic PWV, a marker of arterial stiffness, during community-treated exacerbations. They showed that it may take up to 35 days or more for aortic PWV to return to stable state levels, and that in stable state, aortic PWV was higher in subjects who frequently exacerbated ($\geq$ 2 exacerbations/previous year) than in infrequent exacerbators. Furthermore, increases in aortic PWV at exacerbation were related to inflammation and associated with myocardial injury (as measuring using cardiac inflammatory markers).

2.8.3.3.1 Measurement of arterial stiffness

The most simple and reproducible non-invasive method of assessing arterial stiffness is to measure the arterial waveform (obtained by applanation tonometry), from which can be derived PWV [259, 274]. Applanation tonometry is a well established technique used to measure multiple parameters of arterial stiffness and is based on the principle that when the curved surface of an artery is partially flattened by a tonometer (pressure sensor), pressures are normalized and can be accurately recorded by the tonometer [275].

PWV is inversely related to arterial distensibility and represents the speed of the pressure wave travelling through the arteries. Cf-PWV is considered the 'gold-standard' measurement of aortic stiffness [259, 274]. Measured along the aortic and aorto-iliac pathway, it is the most clinically relevant estimate of arterial stiffness, as the aorta and its first branches are responsible for most severe pathophysiological implications of arterial stiffness. Cf-PWV is an important determinant of both left ventricular function and coronary blood flow, and is associated with aortic elasticity and thickness [276]. It has been used extensively and has the greatest epidemiological evidence to support its predictive value for cardiovascular events in the general and diseased populations [258-260, 277-289]. In addition to PWV, arterial stiffness can be assessed by pulse wave analysis (PWA), which measures several parameters of arterial stiffness, as well as central blood pressure; these include augmentation pressure (AP), augmentation index (AIx), central pulse pressure (PP), and central systolic pressure. The pressure waveform recorded along the arterial tree is the sum of the forward-traveling waveform (generated by ejection), and the backward-traveling wave (the 'echo' of the forward-traveling wave that is reflected as it encounters narrower vessels at peripheral sites).

2.8.4 Summary

The development of CV comorbidity in COPD, and vice versa is partly due to exposure to common risk factors. However, it is also clear that there is a susceptibility for these conditions that go beyond exposure to shared risk factors, and that although there is still much research needed to elucidate what this may be, it is thought that inflammation, oxidative stress and other physiologic and metabolic factors may play a role. There is much evidence that measuring CV risk in COPD provides important information on the CV status of patients. Measuring arterial stiffness by assessing cf-PWV provides a useful means to predict CV risk in COPD, as well as representing a modifiable outcome. There are many plausible links between COPD and increased arterial stiffness. Exacerbations likely have an impact on arterial stiffening, however it is not yet clear whether the observed relationship between exacerbation frequency and arterial stiffening persists over time, and what the long-term determinants of increases in arterial stiffness following exacerbations may be.

CHAPTER 3: RATIONALE, OBJECTIVES AND HYPOTHESES

3.1 Rationale

As discussed in chapter 2, COPD exacerbations have significant impacts on patient health outcomes and wellbeing, as well as on the healthcare system. The consequences of these manifest themselves through changes in inflammatory mediator expression, to the altered function of other organ systems, to increasing the healthcare burden of COPD at the populational level.

Given the complexity and heterogeneity of COPD and its exacerbations, it is important to consider the influence that these different impacts have on one another and on the course of COPD itself. One way to do this is by employing a translational approach to research. This may involve integrating and translating the impacts of exacerbations at different levels (e.g. cellular, physiologic, etc.) but it may also involve taking into account the range of persons who are affected by exacerbations; from individuals with mild and asymptomatic disease to those heavily burdened. The latter consideration is a challenge, as most subjects who are included in studies are those being treated for their COPD who already have advanced disease. However, given the right research platform, such as a population-based cohort, one may gain access to those subjects with undiagnosed COPD. This group of individuals offers a unique opportunity to assess the factors that render subjects susceptible to exacerbations and to better understand the impacts of these events on various health outcomes, disease progression and healthcare utilization. The link, or translation, of this information must then be made to those subjects who have advanced disease. These are the subjects with the worst health outcomes and quality of life, who most need treatment to control their COPD and associated comorbidities. By looking at the molecular, cellular and biological impacts of exacerbation on more severe subjects, the identification of novel therapeutic targets becomes possible, in addition to providing more information on the pathogenesis of this

disease. By considering all of this together, it becomes clear that translational research can act as a bridge between different systems and populations and thereby allowing for a more complete understanding of the impacts of COPD exacerbations to be gained.

This research thesis addresses several key research questions using a translational approach in 2 research projects. The first project was an analysis of the existing population-based CanCOLD cohort study, and the second project was a local study of severe COPD patients who were hospitalized for acute exacerbations and followed for six months. This allowed for the assessment of the impacts of exacerbations from inflammation, to CV outcomes, to populational healthcare impacts in patients from opposite spectrums of disease; thus fulfilling the "bench to bedside" requirements of translational research.

3.2 Objectives and hypotheses

The general objectives of this thesis were to adopt a translational research approach in order to better understand the consequences of COPD exacerbations on inflammation, cardiovascular comorbidity and populational burdens among patients with varying disease severity. More specifically, this thesis had four main research objectives, which are addressed in four different manuscripts within this thesis.

Research objective 1 (manuscript 1): populational impacts

The **overall objective** was to assess exacerbation-like respiratory events in a subset of subjects with diagnosed and undiagnosed COPD belonging to a population-based cohort. The **specific objectives** were to assess in subjects with undiagnosed COPD and those having received a

previous physician diagnosis who are part of a population-based sample of subjects 40 years and older: 1) whether there are differences between the characteristics of subjects in these two subgroups at study entry, and between subjects from these subgroups who report exacerbation-like respiratory events; 2) the proportion of subjects reporting exacerbation-like respiratory events in these two subgroups, and whether there are differences in the odds of using medications or health services, e.g., using medication, physician and hospital emergency department visits and admissions. It was **hypothesized** that there would be a high number of subjects with undiagnosed COPD who have characteristics at study entry that are milder and less symptomatic, and that the proportion of subjects reporting exacerbation-like events would be lower among these subjects than those with diagnosed disease, but that the factors associated with reporting exacerbation like events would be similar between these two groups, despite a lower use of health services by undiagnosed subjects.

Research objective 2 (manuscript 2): cardiovascular impacts

The **overall objective** was to assess during exacerbations requiring hospitalization and subsequent recovery, the course of change in a measurement of arterial stiffness; and to identify several physiological and clinical variables that can predict increases in arterial stiffness over time in subjects with severe COPD and at elevated CV risk. The **specific objectives** were to: 1) to assess the extent of change of cf-PWV and pressure measurements over the time course and recovery of severe exacerbations requiring hospital admission and its relationship with exacerbation frequency; and 2) to assess whether previously studied clinical variables such as lung function, as well as laboratory variables that have not yet been studied such as blood cell counts, lipids and electrolytes over time can predict increases in cf-PWV over time. It was

hypothesized that patients who experience COPD exacerbations requiring hospital admission have increases in cf-PWV and associated pressures during exacerbation; and that some subjects will have continued increases in arterial stiffness following exacerbation that may be predicted by certain clinical factors.

Research objective 3 (manuscript 3): inflammatory impacts

The **overall objective** was to assess RelB expression, an NF-κB family member and suppressor of pulmonary inflammation, relative to other markers of inflammation as well as its association with CV and pulmonary features of COPD patients at stable-state and exacerbation. The **specific objectives** were to: 1) assess systemic RelB mRNA expression relative to other inflammatory markers relevant to both COPD pathogenesis and whose expression is regulated by RelB (e.g. Cox-2, IL-8, IL-1β) in stable-state and exacerbating COPD patients; and (2) assess associations between these two subject groups in relation to acid-base, cardiovascular and pulmonary patient variables. It was **hypothesized** that systemic RelB expression is reduced compared to that of other pro-inflammatory mediators at exacerbation when inflammation is typically increased, and is associated with CV outcomes in COPD, particularly at exacerbation.

Research objective 4 (manuscript 4): inflammatory impacts

The **overall objective** was to evaluate the course of change in the lung-specific inflammatory markers CC-16 and SPD as well as the novel marker RelB during severe COPD exacerbations and subsequent recovery in association with changes in cf-PWV. The **specific objectives** were to: 1) assess the course of change in CC-16, SPD, and RelB protein concentrations over the time course of exacerbations that require hospitalization, and their subsequent recovery; 2) determine

whether increases in CC-16, SPD and RelB protein concentrations are associated with changes in cf-PWV over time; and 3) determine whether increases in CC-16, SPD and RelB protein concentrations are associated with increased risk of subsequent exacerbations during follow-up. It was **hypothesized** that systemic alterations in CC-16, SPD and RelB are associated with changes in cf-PWV over time, and that CC-16 and SPD are associated with increased exacerbation frequency during follow-up.

CHAPTER 4: POPULATIONS & STUDY DESIGNS

4.1 Study 1: the CanCOLD study

4.1.1 Study settings and ethical considerations

The first manuscript of this thesis is an analysis of 545 subjects that is embedded within the existing prospective multi-center population-based CanCOLD study. CanCOLD was built on the previously ongoing cross-sectional COLD study that employs the methodology of the international BOLD initiative. CanCOLD is the first population-based longitudinal study on COPD in Canada with the aim of better understanding the heterogeneity of COPD and its progression. It is carried out through nine sites across including: Calgary, Halifax, Kingston, Montréal, Ottawa, Québec, Saskatoon, Toronto and Vancouver, and is funded by the Canadian Institute of Health Research (CIHR/Rx&D Collaborative Research Program Operating Grants-93326). Research Ethics Boards approval was provided for all sites and all subjects provided written informed consent via the approved consent form (**Appendix I**).

4.1.2 Eligibility criteria and subject selection

Subjects, including both men and women, who are 40 years and older were identified by random telephone digit dialling by NRG Research Group (Vancouver, BC, Canada) and invited to participate in the study. The CanCOLD cohort comprises of two COPD balanced subsets (GOLD 1 or GOLD 2 and higher) and two subsets of subjects without COPD (normal (healthy, never-smokers, no signs of airflow obstruction), and at risk (ever-smokers, no signs of airflow obstruction)), all matched for age and sex. Post-bronchodilator spirometry was used to classify participants into one of four disease status groups and COPD was defined based on the GOLD definition $FEV_1/FVC < 0.70$. For the analysis included in this thesis a subset of 545 subjects were

selected because they had at least twelve months of exacerbation questionnaire (**Appendix II**) follow-up and had either been previously diagnosed by a Physician as having COPD or had never received such a diagnosis, but upon study entry, had evidence of spirometrically defined COPD.

4.1.3 Study design and follow-up

Study participants undergo several measurements during study visits including: questionnaire completion, pulmonary function test and field and laboratory exercise assessment, CT scanning of the chest, and blood sample collection. Study visits are conducted initially at study entry and then every eighteen months thereafter. In addition to this, follow-up of exacerbation-like respiratory events is conducted using a questionnaire that is administered by online and/or telephone interview every three months.

4.2 Study 2: COPD exacerbations: Understanding the course of change and recovery in extra-pulmonary outcomes

4.2.1 Study settings and ethical considerations

The other three manuscripts included in this thesis were based on a local observational study that monitored changes in arterial stiffness, circulating inflammatory mediator concentrations and other clinical and physiological measurements during the time course of an exacerbation and subsequent recovery in COPD patients. This study was conducted from August 2012 to August 2014 at the MCI. It was funded as an investigator initiated grant by GSK Canada. The study protocol was approved by the McGill University Faculty of Medicine Institutional Review Board. Written informed consent was obtained from all study participants (**Appendix I**).

4.2.2 Eligibility criteria and subject selection

Patients were recruited between August 2012 and August 2013 with the following eligibility criteria: 1) admitted to the MCI with COPD exacerbation as a primary diagnosis requiring treatment with antibiotics and systemic corticosteroids; 2) a known history of CVD or CV risk factors; 3) regular medication use to treat their COPD. A subset of patients with a known history of frequent exacerbations (≥2/year) and CVD/CV risk factors, who were admitted to the day-hospital at the MCI with a COPD exacerbation, were also recruited. Patients were excluded from the study if they presented the following criteria: 1) acute medical condition other than COPD exacerbation (cancer, ischemic heart event, etc.); 2) unwillingness/inability to sign consent form.

A total of 70 patients were recruited including: 40 hospitalized patients, and 30 patients recruited prior to hospitalization at the day-hospital at the MCI. However, 12 of the day-hospital patients refused to participate in the study after enrollment, and so a total of 58 patients were included in final analyses.

4.2.3 Study design and follow-up

This study was a prospective observational study. **Table 4.1** shows the study follow-up schedule. Initial patient assessments were conducted within 48 ± 24 hours of hospital admission, followed by every 72 ± 24 hours during hospitalization, and once discharged, they were follow every week up to one month (since initial assessment). Follow-up assessments were conducted at three (± 2 weeks) and six (± 4 weeks) months. In the subset of patients with a known history of frequent exacerbations, an additional stable-state assessment was carried out prior to the exacerbation assessments. If patients were re-hospitalized within 30 days of discharge, the measurement

protocol outlined was repeated for the assessments undertaken during hospitalization (every 72 ± 24 hours, and every week for 1 month). For the three- and six-month post-exacerbation assessments, the timeline was based on the initial hospitalization event of the patient (i.e. first exacerbation), so that each patient had a total follow-up period of six months.

Table 4.1: Study follow-up schedule

Measurement points	Hospitalized patients	Patients with a history of frequent exacerbations (day-hospitalized)
1	----	Baseline (prior to an exacerbation)
2	Within 48 ± 24 hours of hospitalization due to an exacerbation	Within 48 ± 24 hours of hospitalization due to an exacerbation
3	Every 72 ± 24 hours during hospitalization	Every 72 ± 24 hours during hospitalization
4	Every week for 1 month, after the beginning of an exacerbation	Every week for 1 month, after the beginning of an exacerbation
5	1 month after the beginning of the exacerbation	1 month after the beginning of the exacerbation
6	3 (± 2 weeks) months after the beginning of the exacerbation	3 (± 2 weeks) months after the beginning of the exacerbation
7	6 (± 4 weeks) months after the beginning of the exacerbation	6 (± 4 weeks) months after the beginning of the exacerbation

CHAPTER 5: MANUSCRIPT 1 "EXACERBATIONS IN UNDIAGNOSED CHRONIC OBSTRUCTIVE PULMONARY DISEASE GREATLY IMPACT USE OF HEALTH SERVICES: THE CANCOLD STUDY"

PREFACE TO MANUSCRIPT 1

This manuscript is based on an analysis of exacerbation-like respiratory events in a subset of subjects participating in the existing Canadian Cohort Obstructive Lung Disease study. These subjects were selected as they had twelve months of exacerbation follow-up and had either previously received a physician diagnosis of COPD, or had not, but had evidence of spirometrically defined COPD at study entry.

Exacerbations are the principal contributor to the global burden of COPD. Despite their impact on patients and the healthcare system, COPD remains a significantly under-diagnosed disease [9, 290]. Little is known about subjects with undiagnosed COPD in the population and their potential contribution to the burden on the healthcare system. This study is the first to compare the characteristics of subjects with diagnosed and undiagnosed disease within a population-based cohort, and to identify those characteristics associated with reporting exacerbation-like events. It is also the first estimate of the proportion of subjects reporting exacerbation-like events among these subjects and report on health service utilization.

The references in the following manuscript have been renumbered and are included in the combined bibliography at the end of this thesis in numerical order following the sequence in which they appear throughout the entire thesis. Previously defined acronyms within this thesis are redefined within this manuscript as it has been formatted for submission to the European Respiratory Journal. The questionnaire used in this analysis is found in **Appendix II**.

TITLE PAGE

Exacerbations in undiagnosed chronic obstructive pulmonary disease greatly impact use of health services: The CanCOLD Study

L E Labonté[1], W Tan[2], P Z Li[1], P Mancino[1], S Aaron[3], A Benedetti[1], KR Chapman[4], R Cowie[5], M Fitzgerald[2], P Hernandez[6], F Maltais[7], DD Marciniuk[8], D O'Donnell[9], D Sin[2], J Bourbeau[1] for the Canadian Respiratory Research Network and the CanCOLD study group

[1]McGill University Health Centre, McGill University, Montreal, Quebec

[2]University of British Columbia, Vancouver, British Columbia

[3]Ottawa University, Ottawa, Ontario

[4]University of Toronto, Toronto, Ontario

[5]University of Calgary, Calgary, Alberta

[6]Dalhousie University, Halifax, Nova Scotia

[7]Université Laval, Quebec, Quebec

[8]University of Saskatchewan, Saskatoon, Saskatchewan

[9]Queens University, Kingston, Ontario, Canada.

Corresponding author:

Jean Bourbeau, M.D.,
Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute,
3650 St. Urbain, Office K1.32, Montréal (QC), Canada H2X 2P4,
phone: (514) 934-1934 ext. 32185; fax (514) 843-2083,
email: jean.bourbeau@mcgill.ca

ABSTRACT

Background: Many individuals with airflow obstruction never get diagnosed with chronic obstructive pulmonary disease (COPD), and are susceptible to exacerbation-like respiratory events that might impact health and burden the healthcare system.

Objectives: Differences between characteristics of subjects with undiagnosed and diagnosed COPD were assessed. Characteristics associated with reporting exacerbation-like respiratory events, proportions of subjects reporting events, and differences in the odds of using medication and/or health services were determined.

Methods: Subjects sampled from the general population participating in the Canadian Cohort Obstructive Lung Disease (CanCOLD) study, with at least 12 months of exacerbation-like event follow-up who were classified as having physician-diagnosed or undiagnosed COPD were assessed. Exacerbation-like events were captured using a questionnaire administered every three months.

Results: 355 subjects were undiagnosed, while 190 were diagnosed. Undiagnosed subjects were less symptomatic and functionally impaired, prescribed fewer respiratory medications, and had better health-related quality of life. Frequency of reported exacerbation-like events was 50% higher in diagnosed subjects, and increased in both groups with disease severity. Lower health status was associated with reporting events in both groups, while impacts on medication use was greater in diagnosed subjects. Despite this, health service utilization was similar between groups.

Conclusion: Undiagnosed subjects represent a significant proportion of individuals, who are less symptomatic and impaired, which may partly explain lack of diagnosis. Remarkably, they contribute similarly to the healthcare burden of COPD in Canada as diagnosed subjects; ensuring accurate diagnosis is important while emphasizing exacerbation-therapy prevention.

INTRODUCTION

The natural course of chronic obstructive pulmonary disease (COPD) is perturbed by episodes of acute symptom worsening, known as exacerbations [74]. These events are associated with accelerated lung function decline [12], impaired health status [11, 291], increased hospitalization [10], and increased mortality [13]; as a result, they contribute greatly to the increasing cost and burden of COPD [292]. Exacerbations are most commonly caused by viral and/or bacterial infections [293], and become more frequent as disease progresses [12, 293, 294].

The longitudinal ECLIPSE cohort study showed that, among COPD patients, there is a stable, independent "exacerbation-susceptibility" phenotype [85]. Accordingly, the best way of identifying subjects susceptible to exacerbations is through their exacerbation history, where frequent exacerbations predict risk of future events. To date however, most studies on exacerbations have focused on diagnosed and treated patients. As a result, there is a lack of knowledge and understanding of the impacts of these events in people with undiagnosed COPD, who may have milder and less symptomatic disease, but may still be affected by the consequences of these acute respiratory events, and importantly, contributing to their burden.

Hill et al., [290] found that in the Canadian primary care setting, approximately 20% of patients aged 40 years and older with at least 20 years of smoking history had COPD, but that among these, only 30% were previously diagnosed. Untreated and uninformed individuals about their disease and exacerbation management may experience recurrent exacerbations leading to increasing emergency room visits and hospitalizations [295]. Little is known about subjects with undiagnosed COPD in the population and their potential burden on the healthcare system, especially how their exacerbations impact health service utilization compared to individuals having received a physician diagnosis of

COPD. Being able to recognize characteristics associated with an inherent susceptibility to exacerbations [85] in undiagnosed subjects could help design and implement new approaches to identify these individuals for early disease management to reduce exacerbations and related complications, as well as providing new insight into why these individuals remain undiagnosed.

In this study, we evaluated subjects aged 40 years and older participating in the population-based Canadian Cohort Obstructive Lung Disease (CanCOLD) study who had spirometrically-defined airflow obstruction in order to compare the demographic and clinical characteristics of the two subgroups, with and without a prior self-reported physician diagnosis COPD. In particular, we compared the proportion of subjects who report symptom-based and event-based exacerbation-like respiratory events in these subgroups. We also determined the clinical factors, patient–reported outcomes, medication and health service use associated with exacerbation-like events in these subgroups.

METHODS

Study design and subjects

Data from 1532 people from the general population aged 40 years and older participating in the prospective longitudinal CanCOLD study were initially evaluated. To address the aims of our analysis, data from a subset of subjects with at least 12 months of exacerbation follow-up who had either received a previous physician diagnosis of COPD or who had not, but had evidence of spirometrically defined COPD at study entry, were analyzed. The details of the study design and protocol have been previously published [296]. The CanCOLD study was built on the cross-

sectional population-based COLD study which utilized the study protocol from the global Burden of Obstructive Lung Disease (BOLD) initiative [35]. All patients provided written informed consent, and the study was approved by relevant ethics and review boards.

Briefly, subjects 40 years and older were identified by random telephone digit dialling by NRG Research Group (Vancouver, BC, Canada) and invited to attend a clinic visit at one of nine sites across Canada to complete interviewer-administered questionnaires and perform pre and post-bronchodilator spirometry. Subjects were classified as having COPD based on the Global Initiative for Obstructive Lung Disease (GOLD) definitions for spirometrically defined COPD with post-bronchodilator forced expired volume in one second (FEV_1) to forced vital capacity FVC ratio <0.70 [74]. Subjects were grouped into one of four disease status groups: normal (healthy, never-smokers, no signs of airflow obstruction), at risk (ever-smokers, no signs of airflow obstruction), GOLD 1 and GOLD 2 or higher.

Full subject assessments were performed every 18 months (see Bourbeau *et al.*, [296], for full details). Additionally, a questionnaire administered by telephone interview or online every three months for a period of 12 months was used to capture exacerbation-like respiratory events.

"Exacerbation" definition

Exacerbation-like respiratory events were the main outcome for this analysis. In the CanCOLD study two different operational definitions were used (table 5.1). The definition could be 'symptom-based', requiring a change in at least one major symptom (dyspnea, sputum purulence, sputum volume), or 'event-based', requiring symptom change and use of medication/health

services. The purpose of using both definitions was to capture all types of respiratory episodes, with varying levels of severity (requiring treatment or not), in the cohort.

Method of measurement of exacerbation-like respiratory events

Exacerbation-like respiratory events were captured using a questionnaire administered every three months by telephone interview or online. This questionnaire included questions on respiratory symptoms and their duration, medication use, impact on work absenteeism/presenteeism, and healthcare resource utilization. It was developed to minimize recall bias, based on previously used questionnaires that measured exacerbations in large cohorts [83, 84, 297, 298]. If subjects reported having experienced at least one major symptom that persisted for at least two days, this was identified as an exacerbation-like event, which was then classified as being either symptom-based or event-based using criteria outlined in table 5.1.

"Undiagnosed" and "diagnosed" COPD definitions

COPD was defined spirometrically (post bronchodilator FEV1/FVC<0.7). Subjects with spirometrically defined COPD who reported having received a previous physician-diagnosis of COPD upon entering the CanCOLD study were identified as "diagnosed" subjects, and subjects with spirometrically-defined COPD, who never received a diagnosis of COPD prior to entry in the CanCOLD study, were identified as having "undiagnosed" COPD.

Statistical analysis

Descriptive statistics are shown as counts and percentages for categorical data and means and

standard deviations (SD) for continuous variables. T-tests (normal continuous variables) or Wilcoxon signed rank test (not normal continuous variables) or Chi-square test (categorical variables) were two-tailed in nature; we considered a p-value of 0.05 or less to be significant. Only spirometric data that fulfilled the American Thoracic Society acceptability and repeatability criteria were used for analyses. To determine factors associated with reporting exacerbation-like events, exacerbations were analyzed as a binary variable (a patient did have or did not have an exacerbation during year 1), and a univariate and multiple logistic regression analyses were used. Adjusted odd ratios (OR) and 95% confidence intervals (CI) were estimated adjusting for sex, age, body mass index (BMI), and smoking history. All statistically significant factors in the univariate model were then included in a stepwise logistic model to explore the predictors of an exacerbation in the first year. To determine determinants and impact/outcomes of susceptibility to exacerbation-like respiratory events, multiple logistic regression analyses were used. Adjusted ORs and 95% CIs were estimated adjusting for sex, age, BMI and smoking history. All analyses were performed using statistical software (Statistical Analysis Software, V.9.1; SAS Institute; Cary, North Carolina, USA).

RESULTS

This analysis included 545 CanCOLD participants who had at least 12 months of exacerbation follow-up using questionnaire evaluation (table 5.2). These subjects included 190 individuals with diagnosed COPD and 355 individuals with undiagnosed COPD; that is about two-thirds of subjects with spirometrically-defined COPD were not diagnosed by their physician. "Undiagnosed COPD" included more men, while "diagnosed COPD" included more women.

Characteristics of subjects with undiagnosed and diagnosed COPD at study entry

Table 5.2 shows differences in demographic, lung function, disease status, and clinical characteristics at study entry between subjects with undiagnosed and diagnosed COPD, and between subjects who reported exacerbation-like respiratory events during prospective follow-up in these subgroups. Compared with diagnosed subjects, undiagnosed subjects included fewer ever-smokers, with lower lifetime exposures to tobacco smoking, better lung function and less severe COPD, had fewer comorbidities and used fewer respiratory medications.

Factors associated with reporting exacerbation-like respiratory events in subjects with undiagnosed and diagnosed COPD

Among individuals who reported exacerbation-like events during follow-up, undiagnosed subjects tended to be less dyspneic, have better health-related quality of life, better physical health status, and less anxiety than diagnosed subjects (table 5.3). Table 5.4 shows factors significantly associated with increased odds of reporting exacerbation-like respiratory events during prospective follow-up, whether using a symptom-based or event-based definition (see supplementary table 5.1 for all factors considered). For both subgroups, reporting exacerbation-like events was associated with lower health status. Factors associated with reporting exacerbation-like respiratory events only in undiagnosed subjects included decreased lung function, and reporting allergy and anxiety comorbidities. Factors associated with reporting exacerbation-like respiratory events only in diagnosed subjects included increased dyspnea, a history of exacerbation-like events at study entry, and reflux-heartburn.

Proportion of subjects reporting exacerbation-like respiratory events and differences in medication and health service utilization between subjects with diagnosed COPD and those with undiagnosed COPD who report exacerbation-like respiratory events

Figure 5.1 shows the proportion of subjects (undiagnosed and diagnosed) who reported at least one exacerbation-like respiratory event during 12 months of follow-up, defined by symptom-based or event-based criteria. Symptom-based exacerbation-like events were reported by a higher proportion of subjects overall. Undiagnosed subjects reported approximately 50% fewer exacerbation-like events than diagnosed subjects regardless of which definition is used. The proportion of reported events increased with disease severity regardless of definition or diagnosis status.

Figure 5.2 shows a comparison between the odds of medication or health service utilization in diagnosed subjects compared to undiagnosed subjects (reference group), who reported exacerbation-like respiratory events during prospective follow-up. Subjects with diagnosed COPD had increased odds of reporting increased medication use at study entry, and increased antibiotic and/or prednisone use to treat exacerbation-like events. However, there are no statistical differences between subjects with diagnosed and undiagnosed COPD in terms of the odds of using health services to treat exacerbation-like events, including hospitalizations, emergency room visits and doctor visits.

DISCUSSION

In this population-based study of subjects with undiagnosed and diagnosed COPD, we show that subjects who never received a physician-diagnosis of COPD, despite having spirometric evidence of airflow obstruction, have milder obstruction, are less symptomatic, have

better patient reported outcomes, and are less likely to be treated with respiratory medication than subjects with diagnosed COPD. However, like diagnosed subjects, undiagnosed subjects who report exacerbation-like respiratory events have lower health-related quality of life, in addition to also having more allergy, anxiety and depression, and lower lung function than subjects not reporting events. Undiagnosed subjects also report fewer exacerbation-like respiratory events and are less likely to be treated with antibiotics and prednisone for these. Despite this, remarkably, they use health services in an equivalent manner as subjects who receive a previous COPD diagnosis. As such, subjects with undiagnosed COPD contribute to a similar extent as subjects with diagnosed COPD to the healthcare burden of COPD in Canada.

CanCOLD is the first prospective, longitudinal, population-based cohort designed specifically to study COPD. It offers a unique opportunity to assess the consequences of this disease on members of the population at large, the gap in the clinical practice of undiagnosed COPD and its potential consequences. Exacerbations are one of the most important factors determining functional impairment and health status [11, 12]. Furthermore, it is the primary cause of hospital admission among major chronic diseases as recently demonstrated from the Canadian Institute for Health Information [47]. Unsurprisingly, the most likely cause for hospital admission is delayed treatment and management of exacerbations [117].

We found that the proportion of reported exacerbation-like respiratory events was lower among subjects with undiagnosed COPD than among those with a physician diagnosis. COPD exacerbations may be a trigger for patients to come to the attention of the healthcare system and for a physician to consider the diagnosis of COPD. Exacerbation prevalence is well known to increase with disease severity [4, 12, 293, 294]. In our analysis, at study entry, undiagnosed

subjects were less symptomatic and less impaired in terms of lung function, comorbidities, health-related quality of life and other psychosocial attributes. This could explain a lower proportion of patient-reported events. Other studies have found that subjects with undiagnosed COPD have fewer symptoms, better health status, milder airflow obstruction, and fewer comorbidities than diagnosed patients [299-302]. Most individuals seeking healthcare that could lead to an initial COPD diagnosis tend to do so because they are experiencing debilitating dyspnea [4], a major risk factor for exacerbations [303]. This may explain why milder less symptomatic subjects remain undiagnosed.

Having lower health-related quality of life was associated with increased odds of reporting exacerbation-like events for both subject subgroups. Frequent exacerbators (experiencing both treated and untreated events) have been shown to have poorer health-related quality of life in general [11, 136, 137]. Even individuals with unreported exacerbations tend to have lower health status [56]. Thus our finding is not surprising, and further supports the detrimental impacts of exacerbations on health status. Our findings also showed that reporting exacerbation-like events was associated with lower lung function, allergy, anxiety and depression in undiagnosed subjects; and increased dyspnea, a history of exacerbations, and reflux/heart burn in diagnosed subjects. All of these factors except allergy, anxiety and depression were among those identified in the ECLIPSE study [85] as being associated with increased risk of exacerbations. This difference with regards to undiagnosed subjects may relate to their milder and less symptomatic disease state. It is likely that anxiety and depression result from experiencing exacerbation-like events and unmanaged symptoms. In diagnosed subjects, having reflux/heartburn may be related to a more advanced, and comorbid-associated disease status. Overall, our data lends further support that there are intrinsic risk factors associated with exacerbation susceptibility among subjects

with more severe airflow obstruction. Yet it remains to be determined how these change over time in milder subjects.

Although it is not surprising that subjects with diagnosed COPD report more medication use at study entry and to treat exacerbation-like events, it is quite surprising that the odds of using health services during exacerbation-like events were similar to those of subjects with undiagnosed disease. Intriguingly this suggests that these subjects with COPD, could be admitted to hospital, visit the emergency room or their doctors for treatment of exacerbation-like events regardless of diagnosis status. Moreover, even individuals with "milder" COPD, who are less symptomatic and less functionally impaired, contribute significantly to the burden of exacerbations. This highlights an important consequence of COPD. It also suggests that there is a challenge for the management of exacerbation-like events and COPD by healthcare providers, resulting in an increased healthcare burden with potentially serious economic consequences. Hospital admissions for COPD exacerbations in Canada typically result in a 10-day hospital stay, costing $10,000 per stay, totalling approximately $1.5 billion a year [49]. Yet, the most effective way of reducing hospital admissions for COPD remains exacerbation prevention through appropriate use of preventive inhaled medication and disease management by the healthcare team and patient. Globally, approximately 50% of subjects with COPD remain undiagnosed [35], and COPD prevalence is expected to continue to rise [4]. In Canada, the COPD prevalence study COLD showed that COPD prevalence was approximately 4-times greater than previously thought (16.4%) [9]. Correct COPD diagnosis would ensure prompt delivery of care with appropriate medication, patient-education, and disease management plans to anticipate and handle exacerbations, significantly improving health-related quality of life and decreasing hospitalizations and doctor visits [304].

Our study includes several strengths and limitations. The former include that this was a population-based study reflecting events occurring in the population at large that impact the health and wellbeing of people, as well as the healthcare system. A unique and novel aspect of the CanCOLD study is that it provides information on subjects from all GOLD stages, particularly those with mild disease, and those potentially at-risk of disease development. These groups are often under-represented in cohorts, but represent important populations, as they are most likely to benefit from early intervention. Our analysis also included men and women, represented in approximately equal proportion, a challenge in many cohorts. Another strength is the evaluation of symptom- and event-based exacerbation-like events. This allowed for the inclusion of milder events that may not warrant medication or health service use, but may still impact quality of life [56]; as well as more severe events requiring treatment. Finally a follow-up of at least 12 months on subjects provides stronger and robust data.

Limitations include the potential presence of asthmatics in our COPD-based cohort. To address this, a substudy is being undertaken to identify subjects with asthma or asthma-COPD overlap syndrome in CanCOLD. Once data has been analyzed for the entire cohort, we will be in a better position to evaluate the contribution of these subjects to this current exacerbation analysis. There is still no definitive way of distinguishing between COPD and asthma with post-bronchodilator fixed airflow obstruction. Even in the absence of smoking, it remains difficult to differentiate between these conditions, since there is evidence that COPD in never-smokers is more prevalent than previously thought [305]. It is also possible that subjects did not report having received a previous physician diagnosis of COPD at study entry despite having received one, and as a result, were misclassified in this analysis. Other acute events that may present with exacerbation-like symptoms or manifestation e.g. coronary events could have been included in our analysis.

Finally, although the administration of the exacerbation questionnaire every three months was undertaken to ensure improved subject-recall, there remains inaccuracies in all symptom-based criteria.

Our study highlights the serious impacts on health service utilization resulting from exacerbation-like respiratory events reported by subjects with undiagnosed COPD. This is especially important when considering that these are treated as acute events without consideration for future management of the underlying COPD. If these patients were recognized as having COPD, then providing proper preventive therapies has the potential to reduce or prevent complications such as emergency room visits and hospital admissions. Continued follow-up and analysis of this cohort will hopefully provide the information required to identify susceptibility-phenotypes in at-risk subjects or those in the early stages of disease. Moreover, it will be interesting to see how health impacts and outcomes of exacerbation-like events change as subjects with undiagnosed COPD progress in their disease.

In summary, the results of our study show the urgent need of identifying subjects susceptible to exacerbation-like respiratory events, regardless of whether they are occurring in previously diagnosed or undiagnosed individuals, as these have serious impacts on health service utilization. Successfully targeting these individuals could result in decreased morbidity, mortality, better quality of life for patients, as well as significantly fewer COPD-related healthcare expenditures. Further research will provide more information that may help in the recognition of a phenotype for identifying early and/or milder disease susceptibility to exacerbations.

ACKNOWELDGEMENTS

We would like to thank all the CanCOLD staff and study participants for their help with this study.

TABLES

Table 5.1: Exacerbation definitions and methods of capture

Recall period	**3 months**	
Type of definition	**Symptom-based**	**Event-based**
COPD exacerbation definition	*The presence of at least 1 major symptom (increased dyspnea. increased sputum volume or increased sputum purulence) for at least 48 hours*	*The presence of at least 1 major symptom (increased dyspnea. increased sputum volume or increased sputum purulence) for at least 48 hours and utilization of antibiotics or corticosteroids or healthcare use (ER or doctor visit, hospitalization)*
Cohort	***CanCOLD***	
Method to capture exacerbations	Telephone interview/online questionnaire administered every 3 months (recall of last 3 months)	
Question asked to capture exacerbations	In the last 3 months (or since this questionnaire was last administered) have you experienced an episode with new or changes in any respiratory symptoms (i.e. cough, phlegm, wheeze, breathlessness…) that became worse for at least 2 days? *If yes*, subject completes the following sections: 1. Respiratory symptoms 2. Medication (due to respiratory symptoms) 3. Work (affected by respiratory symptoms) 4. Healthcare visits (due to respiratory symptoms)	

Table 5.2: Characteristics at study entry of subjects with undiagnosed and diagnosed COPD (n=545), and by exacerbation-status (reporting at least one exacerbation-like respiratory event during 12 months of follow-up)

Characteristics	All subjects			Subjects reporting symptom-based exacerbation-like respiratory events			Subjects reporting event-based exacerbation-like respiratory events		
	Undiagnosed COPD N=355	Diagnosed COPD N=190	P-value	Undiagnosed COPD N=78	Diagnosed COPD N=74	P-value	Undiagnosed COPD N=50	Diagnosed COPD N=60	P-value
Demographic characteristics									
Age in years [mean (sd)]	67.7 (10.3)	67.3 (9.5)	0.519	67.1 (11.0)	67.0 (9.2)	0.908	69.1 (11.2)	66.5 (9.5)	0.155
Sex, male gender [n (%)]	224 (63.1)	88 (46.3)	<0.001*	43 (55.1)	29 (39.2)	0.049*	25 (50.0)	24 (40.0)	0.293
Ever-smokers [n (%)]	232 (65.4)	153 (80.5)	<0.001*	47 (60.3)	62 (83.8)	0.001*	29 (58.0)	49 (81.7)	0.007*
Cigarette smoking pack-years [mean (sd)]	17.3 (21.6)	30.3 (27.1)	<0.001*	17.3 (21.5)	34.1 (31.0)	<0.001*	16.9 (21.6)	35.8 (32.4)	<0.001*
Lung function and disease status									
Post-bronchodilator spirometry [mean (sd)]									
FEV_1 (Liter)	2.5 (0.7)	2.1 (0.8)	<0.001*	2.3 (0.8)	1.9 (0.8)	0.006*	2.1 (0.8)	1.9 (0.9)	0.121
FEV_1 % predicted	86.6 (17.9)	76.6 (21.8)	<0.001*	81.6 (18.8)	72.4 (22.0)	0.01*	80.5 (18.7)	70.7 (22.9)	0.015*
FEV_1/FVC %	62.6 (6.6)	60.9 (12.0)	0.12	60.6 (7.7)	58.9 (13.6)	0.611	59.7 (8.3)	58.4 (14.4)	0.855
GOLD Classification (fixed FEV_1/FVC)** [n (%)]									
Normal	0 (0.0)	13 (6.8)	<0.001*	0 (0.0)	3 (4.1)	0.113	0 (0.0)	3 (5.0)	249
At Risk	0 (0.0)	27 (14.2)	<0.001*	0 (0.0)	11 (14.9)	<0.001*	0 (0.0)	9 (15.0)	0.004*
GOLD 1	226 (63.7)	53 (27.9)	<0.001*	42 (53.8)	18 (24.3)	<0.001*	26 (52.0)	12 (20.0)	<0.001*
GOLD 2	120 (33.8)	77 (40.5)	0.119	32 (41.0)	29 (39.2)	0.817	21 (42.0)	24 (40.0)	0.832
GOLD 3+	9 (2.5)	20 (10.5)	<0.001*	4 (5.1)	13 (17.6)	0.015*	3 (6.0)	12 (20.0)	0.033*
Clinical characteristics									
Self-reported comorbidities [n (%)]									
CVD	92 (25.9)	52 (27.4)	0.714	21 (26.9)	20 (27.0)	0.988	14 (28.0)	15 (25.0)	0.722
Angina	11 (3.1)	17 (8.9)	0.003*	1 (1.3)	11 (14.9)	0.002*	0 (0.0)	8 (13.3)	0.008*

Myocardial infarction	13 (3.7)	10 (5.3)	0.376	3 (3.8)	6 (8.1)	0.318	2 (4.0)	3 (5.0)	1.000
Diabetes	34 (9.6)	23 (12.1)	0.358	6 (7.7)	10 (13.5)	0.242	4 (8.0)	9 (15.0)	0.257
Musculoskeletal	108 (30.4)	85 (44.7)	<0.001*	28 (35.9)	38 (51.4)	0.055	20 (40.0)	30 (50.0)	0.294
Reflux/heart burn	79 (22.3)	53 (27.9)	0.143	13 (16.7)	27 (36.5)	0.006*	7 (14.0)	24 (40.0)	0.003*
Allergy	131 (36.9)	98 (51.6)	<0.001*	40 (51.3)	45 (60.8)	0.237	21 (42.0)	37 (61.7)	0.040*
Generalized anxiety disorder	9 (2.5)	13 (6.8)	0.015*	5 (6.4)	8 (10.8)	0.332	3 (6.0)	6 (10.0)	0.507
Major depression	22 (6.2)	17 (8.9)	0.235	8 (10.3)	10 (13.5)	0.534	5 (10.0)	9 (15.0)	0.433
Respiratory medications reported in the past 12 months [n (%)]									
SABD	20 (5.6)	17 (8.9)	0.143	2 (2.6)	6 (8.1)	0.159	0 (0.0)	5 (8.3)	0.062
LABA or LAMA	0 (0.0)	13 (6.8)	<0.001*	0 (0.0)	8 (10.8)	0.003*	0 (0.0)	7 (11.7)	0.015*
ICS alone	22 (6.2)	22 (11.6)	0.028*	9 (11.5)	9 (12.2)	0.905	8 (16.0)	7 (11.7)	0.51
ICS combined with LABA/LAMA	38 (10.7)	61 (32.1)	<0.001*	19 (24.4)	32 (43.2)	0.014*	13 (26.0)	29 (48.3)	0.016*
Any above medications	80 (22.5)	113 (59.5)	<0.001*	30 (38.5)	55 (74.3)	<0.001*	21 (42.0)	48 (80.0)	<0.001*

** based on post-bronchodilator spirometry. Normal: never smokers and Post-FEV_1/FVC ≥ 0.70; At Risk: ever smokers and Post-FEV_1/FVC ≥ 0.70; GOLD1: Post-FEV_1/FVC < 0.70 and FEV_1%pred ≥ 80; GOLD2: Post-FEV_1/FVC < 0.70 and 80 > FEV_1%pred ≥ 50; GOLD3+: Post-FEV_1/FVC < 0.70 and 50 > FEV_1%pred > 0. Undiagnosed COPD based on no self-reported of previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Diagnosed COPD based on a self-reported previous Physician diagnosis of COPD at study entry. FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, CVD: cardiovascular disease, SABD: short-acting bronchodilator, LABA: long-acting β2 agonist, LAMA: long-acting muscarinic antagonists, ICS: inhaled corticosteroid. *Denotes statistical significance. P-values were obtained by performing T-test (normal continuous variables) or Wilcoxon signed rank test (not normal continuous variables) or Chi-square test (category variables)

Table 5.3: Patient-reported outcomes of subjects who report at least one exacerbation-like respiratory event over 12 months with undiagnosed and diagnosed COPD

Patient-reported outcomes	Symptom-based			Event based		
	Undiagnosed COPD N=78	Diagnosed COPD N=74	P-value	Undiagnosed COPD N=50	Diagnosed COPD N=60	P-value
BOD score	0.3 (0.6)	0.8 (1.1)	0.064	0.3 (0.6)	0.8 (1.2)	0.164
MMRC dyspnea scale (1-5)	1.6 (0.7)	2.0 (1.0)	0.009*	1.7 (0.8)	2.1 (0.9)	0.042*
CAT score	7.6 (5.3)	13.3 (8.2)	<0.001*	7.5 (5.9)	13.3 (8.4)	<0.001*
SGRQ total score	16.1 (13.2)	29.4 (19.6)	<0.001*	17.9 (14.1)	30.3 (19.7)	<0.001*
COPD specific module total score	9.8 (7.6)	18.0 (11.5)	<0.001*	10.3 (8.2)	18.2 (11.5)	<0.001*
SF36V2						
Physical component scale	50.7 (7.9)	45.4 (10.7)	0.002*	51.3 (7.9)	44.9 (11.2)	0.002*
Mental component scale	49.1 (8.8)	48.0 (9.9)	0.511	49.8 (8.6)	48.5 (10.0)	0.595
HADS						
Anxiety score	4.0 (3.1)	5.2 (3.7)	0.029*	3.5 (2.7)	5.1 (3.6)	0.02*
Depression score	2.8 (2.0)	3.6 (3.0)	0.22	2.7 (1.9)	3.5 (3.1)	0.375
CHAMPS caloric expenditure per week						
Moderate and greater intensity. ≥ 3 METs (KC)	2.0 (2.1)	1.5 (1.8)	0.17	1.9 (2.3)	1.6 (1.9)	0.902
All activities	3.6 (2.4)	3.4 (2.4)	0.55	3.6 (2.6)	3.5 (2.5)	0.957

Values are shown as mean (SD). Undiagnosed COPD based on no self-reported of previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Diagnosed COPD based on a self-reported previous Physician diagnosis of COPD at study entry. BMI: body mass index, BOD: BMI-obstruction- dyspnea, MMRC: modified medical research council, SGRQ: St-George's respiratory questionnaire, CAT: COPD assessment test, SF: short-form, HADS: hospital anxiety and depression scale, CHAMPS: community healthy activities model program for seniors. *Denotes statistical significance. P-values were obtained by performing T-test (normal continuous variables) or Wilcoxon signed rank test (not normal continuous variables).

Table 5.4: Factors associated with reporting exacerbation-like respiratory events during 12 months of follow-up

Factor	Undiagnosed COPD (n=355)				Diagnosed COPD (n=190)			
	Symptom-based		Event-based		Symptom-based		Event-based	
	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
FEV_1 Liter − per 100-ml decrease			1.05 (1.01-1.10)	0.049				
SGRQ total score − per increase of 4 points	1.21 (1.10-1.32)	<0.001*	1.21 (1.09-1.34)	<0.001*	1.12 (1.04-1.22)	0.005*	1.11 (1.02-1.21)	0.016*
MMRC dyspnea scale − 3, 4 or 5 vs. 1 or 2					3.02 (1.05-8.73)	0.041*		
History of at least 1 exacerbation-like respiratory event in the past 12 months at study entry − yes vs. No**					3.02 (1.05-8.73)	0.041*	3.85 (1.45-10.21)	0.007*
Allergy − yes vs. No	1.89 (1.10-3.23)	0.021*						
Generalized anxiety disorder − yes vs. No	4.44 (1.07-18.42)	0.040*						
Reflux/heartburn − yes vs. No							1.95 (1.02-4.04)	0.048*

Undiagnosed COPD based on no self-reported of previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Diagnosed COPD based on a self-reported previous Physician diagnosis of COPD at study entry. ** Exacerbation-like respiratory events reported in 12 months prior to study entry based on subject recall. FEV_1: forced expiratory volume in 1 second, SGRQ: St-George's Respiratory Questionnaire.*Denotes statistical significance. P-values were obtained using a stepwise multivariable model adjusted for age, sex, BMI and smoking history.

Supplemental Table 5.1: Factors associated with reporting exacerbation-like respiratory events (n=545)

Baseline characteristics	Undiagnosed COPD (n=355)				Diagnosed COPD (n=190)			
	Symptom-based		Event-based		Symptom-based		Event-based	
	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Post-bronchodilator spirometry								
FEV1. Liter − per 100-ml decrease	1.07 (1.02-1.13)	0.006*	1.10 (1.03-1.17)	0.004*	1.04 (0.99-1.09)	0,142	1.05 (0.99-1.10)	0,081
FEV1. % predicted − per 5% decrease	1.11 (1.02-1.20)	0.011*	1.13 (1.03-1.24)	0.010*	1.07 (0.99-1.15)	0,073	1.09 (1.01-1.18)	0.031*
FEV1/FVC. % − per 1% decrease	1.06 (1.02-1.10)	0.003*	1.06 (1.02-1.11)	0.003*	1.03 (1.00-1.06)	0.032*	1.03 (1.00-1.06)	0.040*
GOLD Classification (fixed FEV1/FVC) **								
Non-COPD and GOLD 1								
GOLD 2	1.59 (0.93 - 2.72)	0,093	1.70 (0.89 - 3.23)	0,107	1.07 (0.55 - 2.05)	0,846	1.17 (0.58 - 2.34)	0,657
GOLD 3+	3.16 (0.76 - 13.09)	0,113	3.69 (0.79 - 17.21)	0,097	3.72 (1.31 - 10.55)	0.014*	4.34 (1.54 - 12.21)	0.005*
Self-reported comorbidities − yes vs. no								
CVD	1.15 (0.64-2.07)	0,647	1.08 (0.54-2.17)	0,83	0.87 (0.42-1.76)	0,691	0.73 (0.34-1.56)	0,414
Angina	0.36 (0.05-2.94)	0,343	0.00 (0.00-I)	0,98	3.00 (1.00-8.99)	0.049*	1.75 (0.60-5.13)	0,307
Myocardial infarction	1.28 (0.34-4.85)	0,720	1.28 (0.27-6.16)	0,757	2.61 (0.68-10.00)	0,162	0.97 (0.23-4.05)	0,964
Diabetes	0.82 (0.32-2.11)	0,686	0.84 (0.28-2.55)	0,755	1.04 (0.40-2.69)	0,936	1.34 (0.51-3.54)	0,55
Musculoskeletal	1.31 (0.76-2.27)	0,333	1.41 (0.74-2.67)	0,295	1.36 (0.74-2.53)	0,324	1.23 (0.64-2.34)	0,532

Reflux/heartburn	0.63 (0.32-1.22)	0,172	0.51 (0.22-1.19)	0,118	1.85 (0.95-3.59)	0,069	2.28 (1.15-4.51)	0.019*
Allergy	2.09 (1.25-3.52)	0.005*	1.19 (0.64-2.22)	0,583	1.75 (0.96-3.21)	0,07	1.72 (0.91-3.25)	0,097
Generalized anxiety disorder	4.50 (1.16-17.50)	0.030*	3.64 (0.84-15.75)	0,084	2.66 (0.80-8.85)	0,111	1.71 (0.53-5.53)	0,37
Major depression	2.05 (0.81-5.19)	0,129	1.95 (0.66-5.75)	0,225	1.99 (0.69-5.72)	0,2	2.10 (0.74-5.98)	0,165
Have reported exacerbation-like respiratory events in the past 12 months − yes vs. No	2.16 (0.67-6.99)	0,198	3.37 (1.02-11.13)	0.046*	3.97 (1.59-9.90)	0.003*	5.95 (2.37-14.96)	<0.001*
BOD score − per increase of 1 point	1.55 (0.94-2.56)	0,084	1.66 (0.95-2.89)	0,073	1.28 (0.91-1.79)	0,155	1.32 (0.93-1.87)	0,123
MMRC dyspnea scale − 3, 4 or 5 vs. 1 or 2	3.94 (1.40-11.11)	0.009*	3.75 (1.26-11.15)	0.018*	4.29 (1.61-11.40)	0.004*	3.80 (1.48-9.72)	0.005*
SGRQ total score − per increase of 4 point	1.26 (1.14-1.39)	<0.001*	1.27 (1.14-1.42)	<0.001*	1.15 (1.06-1.25)	<0.001*	1.15 (1.06-1.25)	<0.001*
CAT score − per increase of 2 point	1.21 (1.09-1.35)	<0.001*	1.16 (1.03-1.31)	0.012*	1.14 (1.04-1.25)	0.003*	1.12 (1.02-1.22)	0.013*

** based on post-bronchodilator spirometry, Non-COPD and GOLD1: Post-FEV_1/FVC < 0.70 and FEV_1%pred ≥ 80; GOLD2: Post-FEV_1/FVC < 0.70 and 80 > FEV_1%pred ≥ 50; GOLD3+: Post-FEV_1/FVC < 0.70 and 50 > FEV_1%pred > 0. Undiagnosed COPD based on no self-reported of previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Diagnosed COPD based on a self-reported previous Physician diagnosis of COPD at study entry. FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, CVD: cardiovascular disease, BOD: body mass index-obstruction-dyspnea, MMRC: modified medical research council, SGRQ: St-George's Respiratory Questionnaire, CAT: COPD assessment test.* Denotes statistically significance. P-values obtained using multivariable logistic regression adjusted for age, sex, BMI and smoking history.

FIGURE LEGENDS AND FIGURES

FIGURE LEGENDS

Figure 5.1. The proportion of subjects reporting exacerbation-like respiratory events by definition in CanCOLD subjects with undiagnosed (dark grey bars, n=355) and diagnosed (light grey bars, n=190) COPD across disease statuses during 12 months of follow-up. Exacerbation-like events were classified as being symptom-based or event-based, based on established criteria (table 5.1). Undiagnosed COPD based on no self-reported of previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Diagnosed COPD based on a self-reported previous Physician diagnosis of COPD at study entry. Normal: never smoker and $FEV_1/FVC \geq 0.70$ (n=13); At Risk: ever smokers and $FEV_1/FVC \geq 0.70$ (n=27); GOLD1: $FEV_1/FVC < 0.70$ and $FEV_1\%pred \geq 80$ (n=279); GOLD2: $FEV_1/FVC < 0.70$ and $80 > FEV_1\%pred \geq 50$ (n=197); GOLD3+: $FEV_1/FVC < 0.70$ and $50 > FEV_1\%pred > 0$ (n=29).

Figure 5.2. Comparison of odds of medication or health service utilization in subjects with diagnosed COPD vs. subjects with undiagnosed COPD (the reference group) who report exacerbation-like respiratory events over at least 12 months in the CanCOLD cohort. Undiagnosed COPD based on no self-reported previous Physician diagnosis of COPD prior to study entry, but with spirometric evidence of obstruction. Reported COPD based on a self-reported previous Physician diagnosis of COPD at study entry**.** ORs adjusted for age, sex, BMI and smoking history. *Denotes statistical significance, $p<0.05$. ER: emergency room.

FIGURES

Figure 5.1

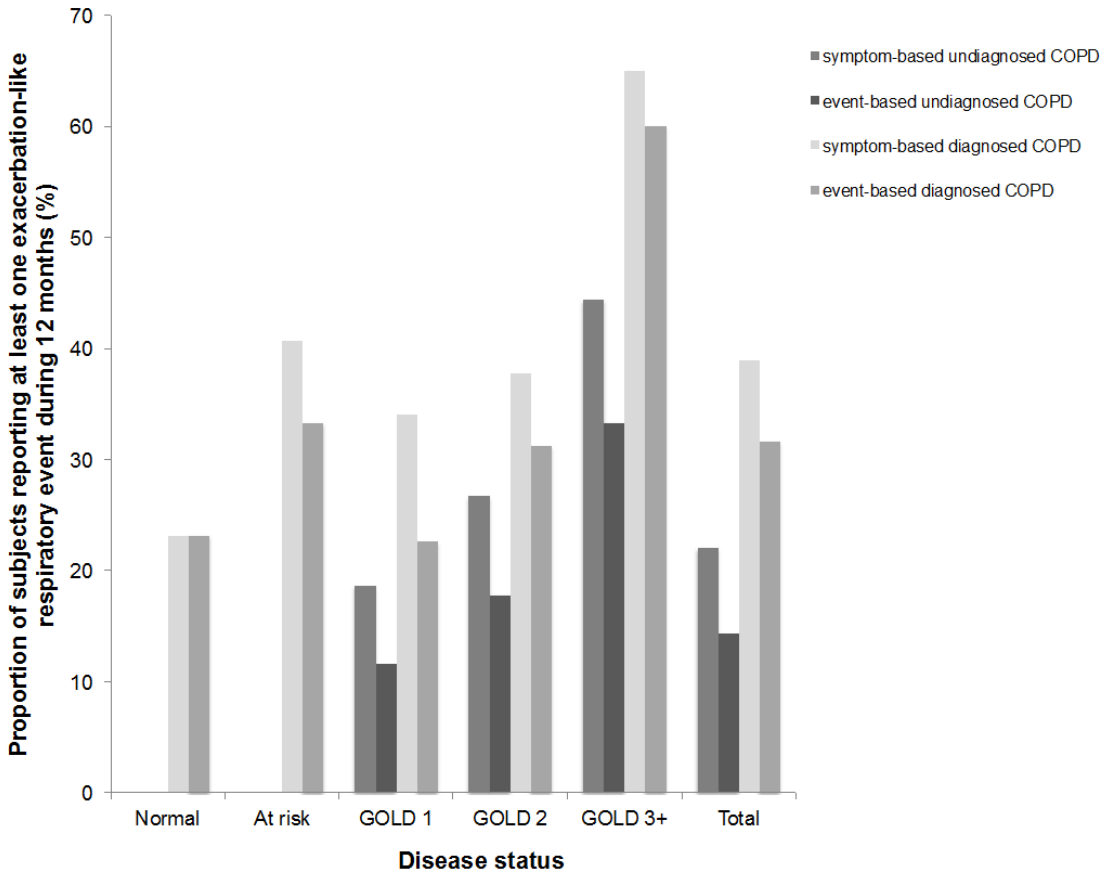
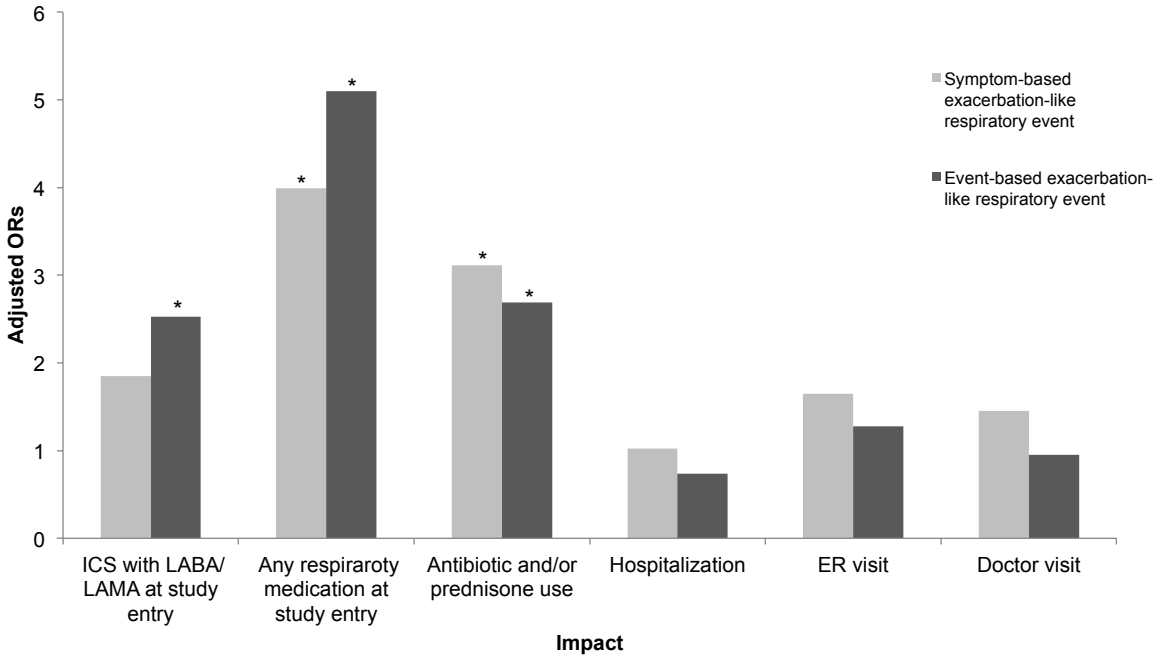


Figure 5.2


6
5
4
3
2
1
0
Adjusted ORs
*
*
*
*
*
*
Symptom-based exacerbation-like respiratory event
Event-based exacerbation-like respiratory event
ICS with LABA/ LAMA at study entry
Any respiraroty medication at study entry
Antibiotic and/or prednisone use
Hospitalization
ER visit
Doctor visit
Impact

CHAPTER 6: MANUSCRIPT 2 "LONGITUDINAL PREDICTORS OF INCREASED ARTERIAL STIFFNESS DURING COPD EXACERBATIONS AND RECOVERY"

PREFACE TO MANUSCRIPT 2

This manuscript examines the longitudinal predictors of increased arterial stiffness during COPD exacerbations and subsequent recovery in patients with severe airflow obstruction, who frequently exacerbate, have elevated CV risk and were admitted to hospital for exacerbation.

CVD and acute CV events are significant causes of morbidity and mortality in COPD patients [14]. Assessing arterial stiffness has shown to be a useful predictor of CV risk in COPD patients [24, 25, 27, 28, 32, 213, 262-266]. Yet little is known on its longitudinal predictors. This study is the first to report on the longitudinal predictors of changes in arterial stiffness during COPD exacerbations that warrant hospital admission, and their subsequent recovery.

The references in the following manuscript have been renumbered and are included in the combined bibliography at the end of this thesis in numerical order following the sequence in which they appear throughout the entire thesis. Previously defined acronyms within this thesis are redefined within this manuscript as it has been formatted for journal submission.

TITLE PAGE

Longitudinal predictors of increased arterial stiffness during COPD exacerbations and recovery

Labonté L[1,2], Daskalopoulou S[1], Baglole CJ[1,3], Metz K[2], Coulombe P[1], Garland K[1], Patel M[2], Bourbeau J[1,2]

[1] McGill University, Montreal, Québec, [2] Respiratory and Epidemiology Clinical Research Unit, Research Institute of the McGill University Health Center, Montreal, Québec, [3]Meakins Christie Laboratories, McGill University, Montreal, Québec.

Corresponding author:

Jean Bourbeau, M.D.,
Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute,
3650 St. Urbain, Office K1.32, Montréal (QC), Canada H2X 2P4,
phone: (514) 934-1934 ext. 32185; fax (514) 843-2083,
email: jean.bourbeau@mcgill.ca

Author contributions: LL, CJB, SD & JB were responsible for study design and implementation, review and approval of the manuscript. LL, KM, PC, KG & MP were responsible for data acquisition, analysis, review and approval of the manuscript.

This study was funded by an investigator-initiated grant from GSK Canada.

Running title: Predictors of arterial stiffness with exacerbation

Total word count: 3228

ABSTRACT

Rationale: Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are associated with increased risk of cardiovascular events. Arterial stiffness, a key predictor and potentially modifiable risk factor of cardiovascular events and mortality, increases with AECOPD. Little is known on longitudinal predictors of increased stiffness after AECOPD.

Objectives: To assess during AECOPD requiring hospitalization and subsequent recovery, the course of change in arterial stiffness and pressure measurements, and identify several physiological and clinical variables that can predict increases in arterial stiffness over time.

Methods: 40 subjects with COPD and a history of cardiovascular disease or risk factors were recruited during hospital admission for AECOPD. Subjects were assessed every 3 days during hospitalization, every week after discharge up to 30 days, and at 90 and 180 days after onset. Lung function and other physiological measurements were taken at each assessment. Arterial stiffness and pressures were measured using carotid-femoral pulse wave velocity (cf-PWV) and pulse wave analysis. Total cell blood count, lipids and electrolytes were measured from venous blood.

Measurements and main results: Cf-PWV and pressure measurements increased during AECOPD. Longitudinal increases in cf-PWV were predicted by subsequent AECOPD, decreased lung function, low-density lipoproteins, cellular hemoglobin and increased circulating potassium. Increased odds of having the most elevated cf-PWV over time were associated with subsequent AECOPD, increased creatinine and potassium; decreased odds were associated with increased lung function and low-density lipoproteins.

Conclusions: Increases in arterial stiffness are predicted by prospective AECOPD frequency as well as several other routinely measured clinical variables. These may represent modifiable targets that could be used to regulate arterial stiffness, and in doing so, cardiovascular risk in COPD patients.

Abstract word count: 269

INTRODUCTION

The natural course of chronic obstructive pulmonary disease (COPD) can be aggravated by exacerbations and extra-pulmonary comorbidities such as cardiovascular disease (CVD) [15, 16, 86, 306]. It is well established that exacerbations significantly contribute to the increasing global burden of COPD [10, 307-310]. These acute events of worsened respiratory symptoms associated with increased inflammation lead to increased hospitalization [10], declined overall patient health status [11, 12] and increased mortality [13].

Cardiovascular (CV) events are the 2nd most frequent cause of mortality in COPD patients [14, 15]. There is growing interest in understanding the association between CV risk and COPD exacerbations, where it has been proposed that exacerbations directly influence susceptibility to CV events. In the 4-year UPLIFT trial [22], relative risks of heart failure, myocardial infarction (MI) and stroke were 10.71, 3.20 and 2.31, respectively, during the 180 days following their first exacerbation versus the 180 days before it. Arterial stiffness independently predicts risk of CV events and all-cause mortality [258, 260, 279, 280]. Arteries stiffen with age due to the cumulative detrimental effects of CV risk factors (e.g. smoking, obesity, etc.) on the arterial tree [260]. Carotid-femoral pulse wave velocity (cf-PWV), considered the gold standard measure of arterial stiffness, has been shown to be a useful, safe and non-invasive way to measure central artery stiffness [277, 311]. Cf-PWV can independently predict CV risk and mortality [258, 260, 279] and increases independently of smoke exposure in COPD [26]. Recently, Patel *et al.* [31] showed that aortic PWV, becomes significantly elevated from stable-state levels during community-treated exacerbations, and may take over 35 days to return to stable-state levels. Moreover in stable-state, patients who frequently exacerbate (≥2 exacerbations/year) appear to have higher aortic PWV than in subjects who infrequently exacerbate. However whether the course of change in PWV is similar in a more

severely exacerbating COPD population remains to be determined. There is currently no data on factors that can predict increases in arterial stiffness over time following COPD exacerbations.

We hypothesize that patients who experience COPD exacerbations requiring hospital admission and who are at risk of serious CV events (as they have a history of CV disease and/or CV risk factors), have increases in arterial stiffness and associated pressure during exacerbation. Some subjects will have continued increases in arterial stiffness following exacerbation that may be predicted by certain clinical factors. The objectives of our study were thus: 1) to assess the extent of change of cf-PWV and pressure measurements over the time course and recovery of severe exacerbations requiring hospital admission and its relationship with exacerbation frequency; and 2) to assess whether previously studied clinical variables such as lung function, as well as laboratory variables that have not yet been studied such as blood cell counts, lipids and electrolytes over time can predict increases in cf-PWV over time.

Our study may provide novel information on the clinical factors that link COPD exacerbations and increased arterial stiffness over time. This may be useful in the context of identifying novel potentially modifiable targets aimed at improving CV outcomes in COPD patients who are susceptible to frequent exacerbations and who are at high CV risk.

METHODS

Subject selection

COPD patients with a known history of CVD or CV risk factors were recruited when admitted to the Montreal Chest Institute (MCI) with COPD exacerbation (severe exacerbation) as a primary diagnosis requiring treatment with antibiotics and/or systemic corticosteroids and hospitalization. Subjects were all currently treated for their COPD by physicians at the MCI. Exclusion criteria included: 1) acute medical condition other than COPD exacerbation (cancer, ischemic heart event, etc.) or 2) unwillingness/inability to sign consent form. Ethics approval was obtained from the McGill University Faculty of Medicine Institutional Review Board. All subjects provided written informed consent prior to assessment.

Study adjudication and definitions of exacerbation and cardiovascular risk and disease

One pulmonologist from the MCI who was not involved in our study adjudicated each reported exacerbation and every patient for the presence of cardiovascular risk and disease. Review was done using subjects charts to ensure participants did indeed have COPD and were currently treated for this (based on COPD medications included in GOLD guidelines [4]), had a previous history of CV risk factors and CVD and that admission to the MCI was based on COPD exacerbation as a primary diagnosis. The number of exacerbations during the duration of the study, e.g. 6 months were verified and adjudicated by this same pulmonologist. Exacerbations were defined as a period during which a subject experienced two or more of three major symptoms (increase in dyspnea, sputum purulence, and increased sputum volume), or any major symptom with a minor symptom (including increased nasal discharge, wheeze, sore throat, cough

or fever) that worsened beyond day-to-day and persisted for a minimum of 2 consecutive days, in addition to requiring treatment with corticosteroid and/or antibiotics and hospitalization [53, 60, 77]. Exacerbation onset was determined to be the first of the 2 consecutive days of symptom change. Exacerbations were considered new events if there was at least 14 days between exacerbation onset and the end date of the previous event during which subjects were no longer treated with antibiotics/corticosteroids or hospitalized, and the subject felt that they were back to their baseline.

CV risk factors and CVD were defined as established evidence from clinical signs or imaging studies of: being treated for hypercholesterolemia , hypertension, diabetes mellitus or peripheral vascular disease and/or having subject-reported physician diagnosis of coronary artery disease , peripheral vascular disease , previous stroke , MI or diabetes mellitus with target organ disease.

Study assessment

Subjects were assessed within 48 ± 24 hours of hospitalization, and every 72 hours (± 24 hours) thereafter during hospitalization. Once subjects were discharged, they were assessed once a week up to 30 days since exacerbation onset, and at days 90 and 180 following onset (figure 6.1). All measurements were taken in the morning after subjects had been ask to refrain from smoking for at least 12 hours prior. Subjects underwent post-bronchodilator spirometry, venous blood sample collection, pulse and oxygen saturation measurements, capillary gas assessment, and arterial stiffness/pressure measurements

If subjects required re-hospitalization within 30 days of discharge, the measurement protocol outlined was repeated for the assessments undertaken during hospitalization (every 72 ± 24 hours, and every week for 30 days). For the days 90 and 180 post-exacerbation assessments, the timeline was based on the initial hospitalization event (*i.e.* first exacerbation), so that each study participant had a total follow-up of 180 days.

Arterial stiffness and pressure measurements

Peripheral blood pressure, PWV, and pulse wave analysis were measured in duplicate at rest using the SphygmoCor system (AtCor Medical, Sydney, Australia) after a subject had been resting for 10 minutes in a supine position. Subjects were asked to refrain from speaking or falling asleep during measurements. Peripheral blood pressure was measured by cuff sphygmomanometry (HEM-705CP, Omron Corp.), along with heart rate, PWV, and pulse wave analysis. A high-fidelity micromanometer on the tip of a hand-held tonometer (SPC-301; Millar Instruments, Houston, TX, USA) was applied to the surface of the skin overlying the radial artery to flatten but not occlude the artery to accurately record radial artery pressure waveforms, and derived a corresponding central aortic pressure waveform (as well as the central pressures (pulse pressure (PP), augmentation pressure (AP)) and the augmentation index (AIx)). Cf-PWV was measured on the subject using the tonometer and a 3 lead electrocardiogram. By measuring the distance between the two recording sites, PWV was calculated [PWV = distance (m)/transit time (s)].

Analysis

Analyses were performed using SAS version 9.3 software (SAS Institute. Inc., Cary, North Carolina). For mean comparisons, two-tailed T-tests (normal distribution) or Wilcoxon signed-rank tests (non-normal distribution) were used; Chi-squared tests were used for dichotomous variables. A p-value of 0.05 or less was deemed statistically significant. Mixed-effects linear models were used to estimate the association of exacerbation frequency and change in cf-PWV over time. The model included random intercepts to capture individual-specific change in levels and a spatial power correlation structure [spl (pow)] to account for varied time intervals between repeated measurements, adjusted for age, sex, and body mass index (BMI). Significant interaction terms (variable*exacerbation) were also put into the models to look at the modifier effects of other variables on this association. To assess the associations between other clinical laboratory variables and cf-PWV over time, the most clinically or statistically significant clinical laboratory variables from univariate analyses were chosen for multivariate analysis. Significant associations were identified using backward elimination in multivariable models. Odds ratios were also calculated using non-linear mixed models comparing subjects with the highest quartile of cf-PWV to those with the lower to examine associations between clinical laboratory variables and cf-PWV over time.

RESULTS

Characteristics of study participants

Table 6.1 shows the characteristics of the study participants. Subjects were approximately 72 years of age with an extensive smoking history, including slightly more men than women. The most prevalent CV risk factor was hypertension and CVD was coronary artery disease.

Changes in arterial stiffness and pressure during exacerbation and recovery

Clinical characteristics, arterial stiffness and pressure measurements collected during the first assessment during acute exacerbation are shown in table 6.2. Figure 6.2 shows the course of change in mean absolute cf-PWV, PP, and AP during exacerbation and subsequent recovery for subjects. Overall, these measurements acutely rose at exacerbation, declined on days 6-15, and rose again at days 21-30. At day 90, measurements remained elevated, while at day 180 values were lower than the initial values measured at acute exacerbation.

Relationship between exacerbation frequency and cf-PWV over time

Table 6.3 shows the relationship between exacerbation frequency and cf-PWV over time. For every additional exacerbation subjects experienced over 6 months of follow-up, cf-PWV increased by 0.33 metres per second. We assessed modifiers of this interaction and determined that systolic blood pressure, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and platelet count had significant interaction effects on this relationship.

Associations between changes in lung function and clinical laboratory variables with changes in cf-PWV over time

Table 6.4 shows the most significant univariate (see supplementary table 6.1 for all variables) and multivariate associations between lung function and clinical laboratory variables with the change in cf-PWV over time. The number of exacerbations experienced during 6 months of follow-up, lung function (FEV_1/FVC), LDL, hemoglobin and circulating potassium levels were most associated with cf-PWV over time after multivariable analysis, with lung function having the greatest association. We also divided subjects into quartiles of cf-PWV at first study assessment. Increased odds of having greater cf-PWV over time were associated with several clinical laboratory variables (supplementary table 6.2). Multivariate analysis revealed that increased odds of having the most elevated cf-PWV over time were associated with the number of exacerbations experienced during 6 months of follow-up (OR 1.32, 95% confidence interval (CI) 1.01 to 1.73, p=0.044), increases in creatinine (OR 1.02, 95% CI 1.00 to 1.05, p=0.046), and increases in potassium (OR 2.84, 95% CI 1.12 to 8.42, p=0.042), while decreased odds were associated with increases in FEV_1/FVC (OR 0.01, 95% CI 0.00 to 0.48, p=0.020) and increases in LDL (OR 0.57, 95% CI 0.31 to 0.99, p=0.049).

DISCUSSION

In this study, we validate and extend the findings that there are significant changes in cf-PWV and associated pressures during and following exacerbations of COPD that require hospitalization in subjects who frequently exacerbate and have elevated CV risk. We also identify several important predictors of increased cf-PWV over time including: subsequent AECOPD, decreased lung function, low-density lipoproteins, cellular hemoglobin and increased circulating potassium; as well as identifying clinical factors associated with increased odds of having the most elevated cf-PWV over time. These factors may include potential determinants of increased arterial stiffness, which could be modifiable through appropriate intervention.

In subjects with COPD who are at mid-to elevated CV risk, identifying early changes to the CV system prior to any major clinical events such as MI or stroke would be greatly beneficial [213]. Arterial stiffness is a precursor to atherosclerosis, and has been shown to have a very strong predictive value for CV events beyond that of classical CV risk factors in addition to being practical for use in the clinical setting [246, 247]. Recent studies have shown that it may be possible to modify arterial stiffness in COPD patients via endurance training [262], pulmonary rehabilitation [271], inhaled therapies [272] or oxygen supplementation [273]. As a result, it represents a useful subclinical, modifiable marker of CV risk in COPD [213]. However, there remains a lack of understanding with regards to the longitudinal changes in arterial stiffness in COPD patients who exacerbate and the factors that may be driving continued increases in stiffness following exacerbations and their recovery.

Our results showed that there are significant changes in the course of cf-PWV (and associated pressures) during, and subsequent to COPD exacerbations. This is in line with the

findings of Patel *et al.* [31] who showed that aortic PWV rises acutely during COPD exacerbations, and decreases thereafter with recovery. However, we extend this observation from the community-treated exacerbation setting to include subjects in the hospitalized setting who are at elevated CV risk. The period immediately following acute exacerbation is known to be associated with significantly increased risk of CV events [22, 23]. Donaldson *et al.* [23] reported that in the first 5 days following exacerbation onset, risk of MI was 2.27 greater, and in the 49 days following onset, risk of stroke was 1.27 greater than in stable state. Those subjects who experienced MI or stroke had a greater annual exacerbation frequency than those who did not [23]. Patel *et al.* [31] also showed that subjects with COPD reporting frequent exacerbations (≥2) in the previous year had increased aortic PWV in the stable state. Similarly, our results suggest that cf-PWV is more elevated over time in subjects experiencing the highest number of exacerbations following an exacerbation. Interestingly, we found that clinical variables that could be modifying this relationship included increased systolic blood pressure, cholesterol, HDL, LDL and platelet count (with a very modest effect). To date there have been no studies on the factors that could be influencing the association between exacerbation frequency and arterial stiffness, and so our findings provide novel evidence on these. Although we provide novel evidence of possible modifiers, further research is needed to validate these, and determine whether they can be modulated through certain interventions, and thereby affect the relationship between exacerbation frequency and arterial stiffness.

We also examined whether lung function and other clinical laboratory variables may be predictors of increases in cf-PWV over time. As previously reported in smokers and subjects with COPD [266, 267, 270, 312], we found a strong association between changes in lung function and cf-PWV over time, whereby increases in lung function were associated with decreases in arterial

stiffness. Moreover, we found that together with subsequent exacerbations and declines in lung function; decreases in LDL, decreases in cellular hemoglobin and increases in circulating potassium were predictors of increases in cf-PWV over time- associations not yet described in the context of arterial stiffness in COPD. The inverse association between LDL and cf-PWV is surprising, as it has previously been shown that these are positively associated among a population-based sample of 2375 subjects [313]. However, we found that this association was positive in our univariate analysis, which suggests that co-variation with other factors included in the multivariable model may have caused this inversed association. To date, results on the relationship between anemia and arterial stiffness have been conflicting [314-316]. It is possible that decreased cellular hemoglobin in our subjects reflects chronic anemia, which has been shown to be an independent risk factor for CV complications [317] and CV morbidity and mortality [318]. Further research is needed to better understand this relationship. The positive relationship between circulating potassium and increased cf-PWV has we observed, has not been described previously. A potential mechanism for this may be related to the chronic acid-base disturbances that occur in COPD patients. These may cause a shift in the movement of potassium from the intracellular to the extracellular compartment [319], which can activate the renin-angiotensin-aldosterone-system. This system is well known to modulate arterial stiffness via various mechanisms [241, 242, 320].

Our study had several strengths and limitations. The former include our analysis of a particularly high-risk COPD population including those patients with a history of CV comorbidity and who are susceptible to frequent exacerbations. Patients with severe COPD exacerbations are particularly difficult to recruit, as they are very ill and usually refuse to participate in research studies. Another strength of our study is that we assessed patients within the first 72 hours of hospitalization. This allowed us to capture important physiological and clinical data on changes

occurring systemically and locally within the lung due to exacerbation. Moreover, the 90- and 180-day measurement time points provide additional longitudinal data on the changes we detected at exacerbation. Despite these strengths, there are several limitations associated with our study. First, the sample size was relatively small and as a result we may have limited power. Second, we could not assess patients before their exacerbations when in stable-state. This would have allowed for a better understanding of the severity of changes in arterial stiffness observed at and subsequent to exacerbation. As patients had received treatment by the time we could assess them, these treatments may have mediated some of the systemic and CV effects we measured, and as we did not have prior stable-state measurements, we were unable to assess the extent of this. However, since the overall trend of change in cf-PWV that we saw during exacerbation mirrored that described by Patel *et al*. [31], who were able to assess patients before they were given antibiotic and corticosteroids, it is likely that their potential effects are negligible. A final limitation is that despite our best efforts and with study adjudication of exacerbations during study follow-up, we may not know the exact dates of all subsequent exacerbations during follow-up and whether subjects experienced other acute events, which were unreported and may have influenced day 90- and 180- measurements.

Future studies should aim to have a larger sample size and longer more frequent patient follow-up. This would allow for the validation of our findings as well as the potential identification of other important predictors of increased arterial stiffness in COPD patients. Moreover, research is needed on the ability of certain interventions to modify the predictors of increased arterial stiffness we identified, in order to determine whether these could be used to modulate arterial stiffness.

Overall, we validated and extended the finding that arterial stiffness is elevated over the course of an exacerbation. We also provide novel evidence that increased cf-PWV over time is

predicted by having more frequent subsequent AECOPD, decreased lung function, low-density lipoproteins, cellular hemoglobin and increased circulating potassium. This is particularly clinically relevant as these measurements are routinely taken in COPD subjects whether in stable state or at exacerbation. Consequently, they may help identify patients who are at increased risk of elevated arterial stiffness overtime, and as a result, are susceptible to increased CV risk. Continued research will help us better pinpoint the determinants of elevated arterial stiffness in COPD, and in doing so, allow for the investigation of targeted treatments that could decrease CV risk in these patients.

ACKNOWLEDGMENTS

We would like to thank all research staff, in particular Stefania Dzieciolowska for help with study assessments and Pei Z. Li for her help with statistical analyses, and the COPD patients who participated in this study.

TABLES

Table 6.1: Characteristics and cardiovascular disease/risk in subjects with COPD (n=40)

Mean (± SD) age, years	71.7 (13.3)
Male (%)	60
Smoking status (%)	
Ex	82.5
Current	17.5
Mean (± SD) smoking pack-year history	54.3 (36.1)
Use of long-term oxygen therapy (%)	22.5
Mean number of exacerbations reported in previous year	2.67
Mean (± SD) body mass index, kg/m2	25.3 (3.79)
CV disease type/ risk factor (%)	
Hypertension	50
High cholesterol	15
Angina	12.5
Coronary artery disease	10
Other	17.5

SD: standard deviation

Table 6.2: Clinical characteristics, arterial stiffness and pressure measurements on admission for acute exacerbation in subjects with COPD (n=40)

Mean (± SD) FEV_1, % predicted	33.0 (14.8)
Mean (± SD) FEV_1, (L)	0.758 (0.346)
Mean (± SD) FEV_1/FVC	0.472 (0.084)
Mean (± SD) systolic BP, mmHg	125.1 (7.1)
Mean (± SD) diastolic BP, mmHg	65.9 (21.2)
Mean (± SD) MAP, mmHg	75.8 (8.1)
Mean (± SD) pulse	91.9 (9.9)
Mean (± SD) pulse oximetry, %	94.1 (0.71)
Arterial stiffness and pressure measurements	
Mean (± SD) cf-PWV (m/s)	11.56 (2.68)
Mean (± SD) PP (mmHg)	43.61 (9.85)
Mean (± SD) AP (mmHg)	8.97 (5.25)
Mean (± SD) Aix%	26.08 (7.19)

SD: standard deviation, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, BP: blood pressure, MAP: mean arterial pressure, CC-16: club cell-16, SPD: surfactant protein D, cf-PWV: carotid-femoral pulse wave velocity, PP: pulse pressure, AP: augmentation pressure, AIx: augmentation index.

Table 6.3: Relationship between exacerbation frequency and change in cf-PWV over time & interaction effects of significant modifiers on this association

	Change in cf-PWV (m/s) over time	
	ß (95% CI)	**P-value**
Number of exacerbations during 6 months of f/u	0.330 (0.055 - 0.604)	0.020*
Modifiers	**Change in the association between cf-PWV (m/s) and number of exacerbations over time**	
	ß (95% CI)	**P-value**
Systolic blood pressure (mmHg)	0.019 (0.003 - 0.035)	0.022*
Cholesterol (mmol/L)	0.325 (0.044 - 0.607)	0.024*
HDL (mmol/L)	1.064 (0.535 - 1.594)	<0.001*
LDL (mmol/L)	0.671 (0.264 - 1.078)	0.001*
Platelet count (10^9/L)	0.005 (0.001 - 0.009)	0.011*

β represents the corresponding change in cf-PWV in m/s resulting from a 1 unit change in number of exacerbations or modifiers.* denotes statistical significance $p<0.05$. CI: confidence interval, f/u: follow-up, HDL: high-density lipoprotein, LDL: low-density lipoprotein. n=38 for this analysis.

Table 6.4: Associations between changes in clinical laboratory variables and changes in cf-PWV over time

Clinical laboratory variables	Change in cf-PWV (m/s) over time			
	Univariate model		Multivariable model	
	ß (95% CI)	P-value	ß (95% CI)	P-value
Number of exacerbations during 6 months of f/u	0.330 (0.055 - 0.604)	0.020*	0.450 (0.036 - 0.776)	0.028*
Systolic blood pressure (mmHg)	0.026 (-0.002 - 0.054)	0.064		
FEV1/FVC	-3.914 (-7.189 - -0.640)	0.02*	-6.570 (-10.704 - -2.436)	0.002*
Triglycerides (mmol/L)	0.420 (0.064 - 0.776)	0.021*		
LDL (mmol/L)	0.024 (0.002 - 0.047)	0.034*	-0.907 (-1.608 - -0.206)	0.012*
RBC (10^12/L)	0.141 (0.018 - 0.265)	0.026*		
Hct (L/L)	0.280 (0.040 - 0.521)	0.022*		
Mean cell Hg (pg/cell)	-0.124 (-0.219 - -0.028)	0.011*	-0.452 (-0.879 - -0.024)	0.039*
Abs. Eosinophil (10^9/L)	0.407 (0.128 - 0.686)	0.005*		
Bicarbonate Level (mmol/L)	0.060 (0.022 - 0.099)	0.003*		
Creatinine (umol/L)	0.022 (0.000 - 0.044)	0.049*		
Potassium (mmol/L)	1.622 (0.393 - 2.852)	0.01*	1.364 (0.067 - 2.660)	0.039*

* denotes statistical significance $p<0.05$. f/u: follow-up, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, LDL: low-density lipoproteins, RBD: red blood cells, Hct: hematocrit, Hg: hemoglobin, Abs. absolute. n=38 for this analysis

Supplementary table 6.1: Associations between changes in clinical laboratory variables and changes in cf-PWV over time

Clinical laboratory variables	Change in cf-PWV (m/s) over time	
	Total	
	ß (95% CI)	P-value
Number of exacerbations during 6 months of f/u	0.330 (0.055 - 0.604)	0.02*
Systolic blood pressure (mmHg)	0.026 (-0.002 - 0.054)	0.064
Diastolic blood pressure (mmHg)	-0.011 (-0.050 - 0.027)	0.556
O2 saturation (%)	0.096 (-0.102 - 0.293)	0.341
FEV1 % predicted	-0.032 (-0.064 - 0.000)	0.053
FEV1/FVC	-3.914 (-7.189 - -0.640)	0.02*
Triglycerides (mmol/L)	0.420 (0.064 - 0.776)	0.021*
HDL (mmol/L)	0.476 (-0.258 - 1.209)	0.201
LDL (mmol/L)	0.024 (0.002 - 0.047)	0.034*
Cholesterol/HDL (RR)	-0.243 (-0.868 - 0.382)	0.443
WBC (10^9/L)	0.035 (-0.002 - 0.073)	0.065
RBC (10^12/L)	0.141 (0.018 - 0.265)	0.026*
Hemoglobin (g/L)	0.013 (-0.002 - 0.027)	0.086
Hct (L/L)	0.280 (0.040 - 0.521)	0.022*
Mean cell Hg (pg/cell)	-0.124 (-0.219 - -0.028)	0.011*
Platelet (10^9/L)	0.006 (0.001 - 0.012)	0.03*
Platelet Hct	0.119 (-0.684 - 0.922)	0.77
Abs. Lymphocyte (10^9/L)	-0.530 (-1.207 - 0.147)	0.124
Abs. MNC (10^9/L)	-0.474 (-1.986 - 1.038)	0.536
Abs. Neutrophil (10^9/L)	0.090 (-0.008 - 0.189)	0.071
Abs. Eosinophil (10^9/L)	0.407 (0.128 - 0.686)	0.005*
Bicarbonate Level (mmol/L)	0.060 (0.022 - 0.099)	0.003*
Anion Gap (mmol/L)	-0.072 (-0.293 - 0.149)	0.521
Creatinine (umol/L)	0.022 (0.000 - 0.044)	0.049*
Glucose Random (mmol/L)	0.018 (-0.029 - 0.066)	0.447
Chloride (mmol/L)	-0.097 (-0.250 - 0.056)	0.211
Potassium (mmol/L)	1.622 (0.393 - 2.852)	0.01*
Sodium (mmol/L)	-0.057 (-0.262 - 0.148)	0.582

Associations determined using univariate mixed models. β represents the corresponding change in cf-PWV in m/s resulting from a 1-unit change in clinical laboratory variables. * denotes statistical significance $p<0.05$. CI: confidence intervals, f/u: follow-up, O2: oxygen, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, HDL: high-density lipoproteins, LDL: low-density lipoproteins, WBC: white blood cells, RBD: red blood cells, Hct: hematocrit, Hg: hemoglobin, Abs: absolute. n=38 for this analysis

Supplementary table 6.2: Impact of changes in clinical laboratory variables on changes in cf-PWV in subjects with highest cf-PWV over time

Clinical laboratory variables	Univariate model		Multivariable model	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Number of exacerbations during 6 months of f/u	1.30 (1.09 - 1.55)	0.004*	1.32 (1.01 - 1.73)	0.044*
Systolic blood pressure (mmHg)	1.01 (0.99 - 1.03)	0.315		
FEV1/FVC	0.17 (0.02 - 1.80)	0.139	0.01 (0.00 - 0.48)	0.020*
Triglycerides (mmol/L)	1.38 (1.09 - 1.74)	0.007*		
LDL (mmol/L)	1.02 (1.00 - 1.04)	0.014*	0.57 (0.31 - 0.99)	0.049*
RBC (10^12/L)	1.12 (1.02 - 1.21)	0.013*		
Hct (L/L)	1.26 (1.05 - 1.51)	0.012*		
Mean cell Hg (pg/cell)	0.92 (0.86 - 0.98)	0.010*		
Abs. Eosinophil (10^9/L)	1.40 (1.08 - 1.81)	0.011*		
Bicarbonate Level (mmol/L)	1.04 (1.01 - 1.07)	0.007*		
Creatinine (umol/L)	1.02 (1.00 - 1.03)	0.012*	1.02 (1.00 - 1.05)	0.046*
Potassium (mmol/L)	2.81 (1.20 - 6.55)	0.017*	2.84 (1.12 - 8.42)	0.042*

Associations determined using non-linear mixed models. cf-PWV was divided into two groups using 25% baseline distribution (≥13.35 m/s as higher group). * denotes statistical significance $p<0.05$. f/u: follow-up, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, LDL: low-density lipoproteins, RBD: red blood cells, Hct: hematocrit, Hg: hemoglobin, Abs. absolute. n=38 for this analysis

FIGURE LEGENDS AND FIGURES

FIGURE LEGENDS

Figure 6.1. Timeline of study

Figure 6.2. Mean absolute change in arterial stiffness measurements at acute exacerbation of COPD and during subsequent recovery for subjects. Panel A shows the absolute change in cf-pwv (m/s) over 6 months of follow-up, panel B shows the absolute change in PP (mmHg) over 6 months of follow-up, and panel C shows the absolute change in AP (mmHg) over 6 months of follow-up.

FIGURES

Figure 6.1

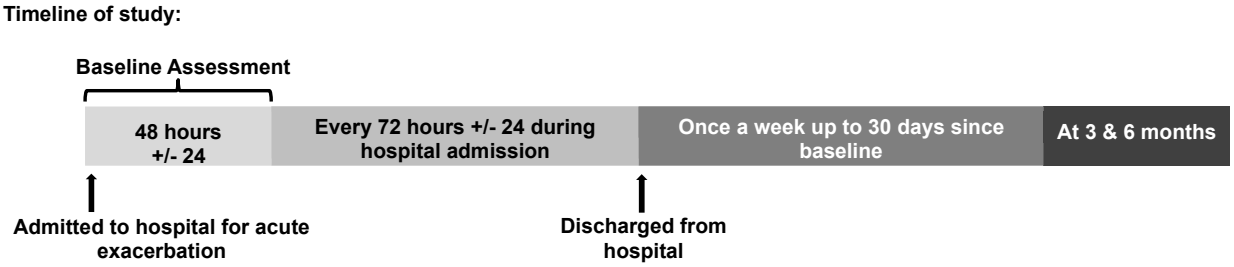
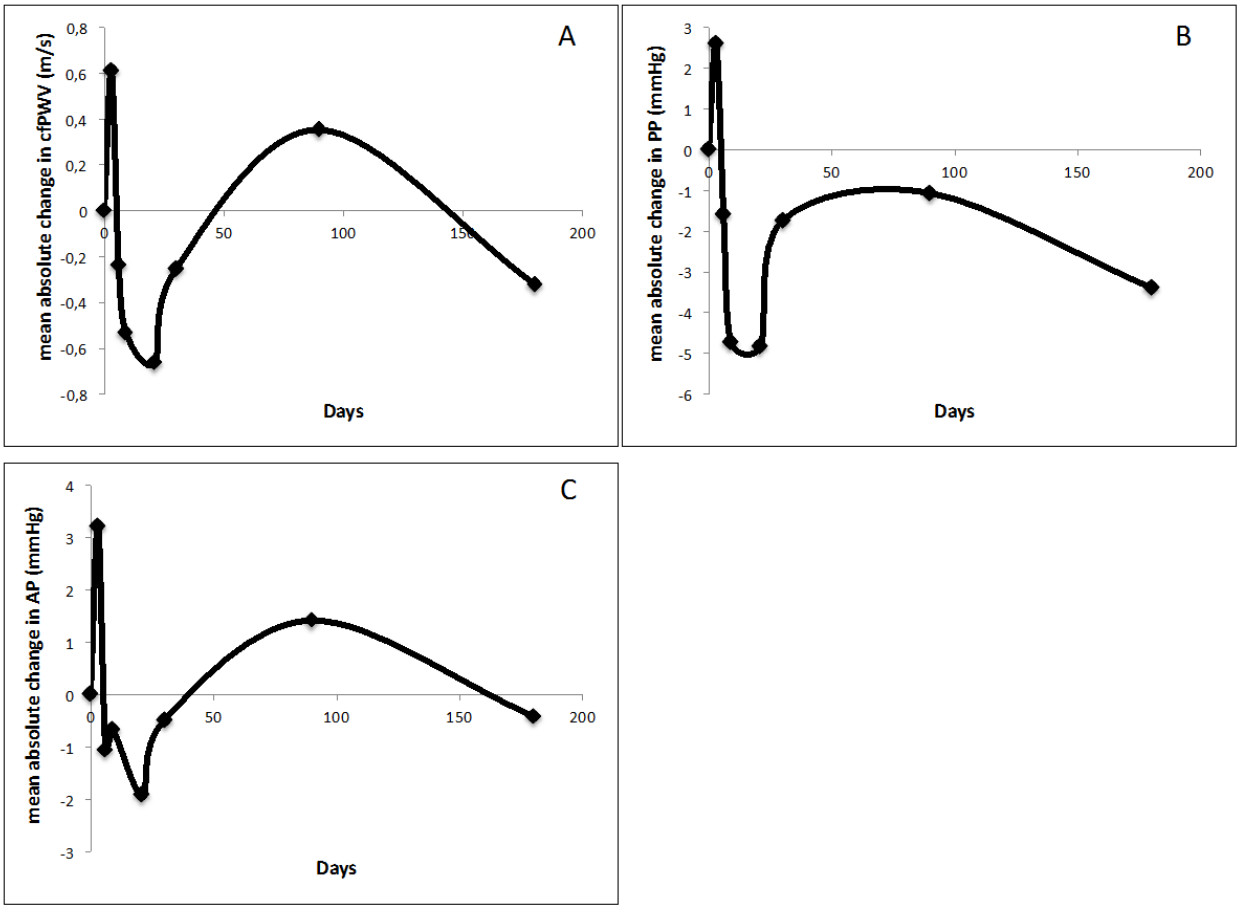


Figure 6.2


A
mean absolute change in cfPWV (m/s)
0,8
0,6
0,4
0,2
0
-0,2
-0,4
-0,6
-0,8
0
50
100
150
200
Days
B
mean absolute change in PP (mmHg)
3
2
1
0
-1
-2
-3
-4
-5
-6
0
50
100
150
200
Days
C
mean absolute change in AP (mmHg)
4
3
2
1
0
-1
-2
-3
0
50
100
150
200
Days

CHAPTER 7: MANUSCRIPT 3 "ALTERATIONS IN THE EXPRESSION OF THE NF-Κ B FAMILY MEMBER RELB AS A NOVEL MARKER OF CARDIOVASCULAR OUTCOMES DURING ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE"

PREFACE TO MANUSCRIPT 3

This manuscript describes a comparison of RelB, an NF-κB family member, mRNA expression at stable-state and during exacerbation in COPD patients who have severe airflow obstruction, frequently exacerbate, and have elevated CV risk.

Finding biomarkers of health outcomes in COPD is a principal goal of research. RelB has been attracting growing interest as a potent suppressor of CS-induced inflammation [321-324]. However its expression in humans remains unknown, and as cigarette smoking is a primary cause of COPD, RelB may represent a clinically-relevant biomarker of this disease. This study is the first to report on RelB in COPD patients, and to identify its association with important CV outcomes at exacerbation.

The references in the following manuscript have been renumbered and are included in the combined bibliography at the end of this thesis in numerical order following the sequence in which they appear throughout the entire thesis. Previously defined acronyms within this thesis are redefined within this manuscript as it has been formatted for journal publication. The published version of this manuscript is found in **Appendix III**.

TITLE PAGE

Alterations in the Expression of the NF-κB Family Member RelB as a Novel Marker of Cardiovascular Outcomes During Acute Exacerbations of Chronic Obstructive Pulmonary Disease

Laura Labonté, MSc[1,2], Patrick Coulombe BSc[2], Michela Zago PhD[1,3], Jean Bourbeau MD[1,2] and Carolyn J. Baglole PhD[1,3*]

[1]Department of Medicine, McGill University. Montreal, Quebec, Canada; [2]Respiratory Epidemiology and Clinical Research Unit (RECRU), Montreal Chest Institute, McGill University Health Centre. Montreal, Quebec, Canada; [3]Meakins-Christie Laboratories, McGill University. Montreal, Quebec, Canada

[*]Correspondence to: Carolyn Baglole, PhD, Meakins-Christie Laboratories, McGill University, 3626 St. Urbain Street, Montreal, Quebec Canada H2X 2P2; Email: carolyn.baglole@McGill.ca

ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) exacerbations are acute events of worsened respiratory symptoms and enhanced inflammation partly mediated by NF-κB activation. RelB, an NF-κB family member, suppresses cigarette smoke-induced inflammation but its expression in COPD is unknown. Moreover, there is no information on its association with clinical features of COPD. The objectives of this study were to assess RelB expression relative to markers of inflammation as well as its association with cardiovascular and pulmonary features of COPD patients at stable-state and exacerbation.

Methods: Data from 48 COPD patients were analyzed. Blood samples were collected from stable-state and exacerbating patients. After RNA isolation, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to assess RelB, Cox-2, IL-8 and IL-1β mRNA expression and their associations with measured clinical variables.

Results: Of the 48 COPD subjects, 18 were in stable-state and 30 were in exacerbation. RelB mRNA expression was lower than that of Cox-2, IL-8, and IL-1β in all cases (all $p<0.001$, except for IL-8 at exacerbation ($p=0.22$)). Cox-2, IL-8 and IL-1β were significantly associated with clinical features of patients in both stable-state and at exacerbation. There was no association with RelB expression and any clinical features in COPD subjects at stable-state. RelB mRNA levels were significantly associated with cardiovascular events such as systolic blood pressure during exacerbation.

Conclusions: RelB mRNA expression is lower than that of the other inflammatory mediators. Expression of Cox-2, IL-8 and IL-1β were related to clinical features in both stable-state and at exacerbation. However, RelB expression was associated with clinical features of patients only during exacerbation, suggesting that RelB may represent a novel marker of health outcomes, in particular cardiovascular, during exacerbation in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, not fully reversible airflow limitation [325] and chronic inflammation [326]. The course of COPD is perturbed by acute events of worsened respiratory symptoms known as exacerbations [75, 79, 80, 327, 328]. Exacerbations are associated with heightened pulmonary and systemic inflammatory responses [73], and lead to significant morbidity and mortality, especially due to cardiovascular events, as well as decreased health-related quality of life and an accelerated decline in lung function [11, 12, 64, 75, 80].

COPD inflammation in stable-state and at exacerbation is partly mediated by nuclear factor-κB (NF-κB) activation [329-331]. NF-κB is a ubiquitous transcription factor family composed of five proteins [332] activated by cigarette smoke (CS) that collectively are involved in the regulation of gene expression for pro-inflammatory cytokines, chemokines and adhesion molecules [329, 333]. These include interleukin (IL)-1β, a key orchestrator of the immune response in COPD[331] that can activate NF-κB and is produced by airway epithelial cells in response to CS or acute injury [331, 334]. Other mediators include IL-8, which is involved in inflammatory cell recruitment in COPD [18, 81, 96, 335], especially with bacterial infection at exacerbation [81, 96]; and cyclooxygenase-2 (COX-2), an inducible enzyme that catalyzes arachidonic acid transformation into thromboxane and prostaglandins [329]. COX-2 induction can also occur via IL-1β activation of the NF-κB pathway and is enhanced in response to noxious stimuli such as CS [329, 336].

Although activation of NF-κB is typically regarded as pro-inflammatory, we recently identified RelB, another member of the NF-κB family, as a potent suppressor of CS-induced inflammation

[321-324, 337]. Weih *et al.* [338, 339] showed that mice deficient in RelB have severely increased multi-organ inflammation, suggesting that RelB may have a role in suppressing inflammation. Our *in-vitro* and *in-vivo* data provide further evidence for an anti-inflammatory role for RelB. We have shown that loss of RelB expression due to smoke exposure promotes pro-inflammatory mediator production (including IL-8 and COX-2 expression), whereas RelB reconstitution reduces inflammation associated with CS [322, 324]. Reciprocally, overexpression of pulmonary RelB in mice exposed to CS is associated with decreased lung neutrophil infiltration, pro-inflammatory cytokine and chemokine production and COX-2 /prostaglandin production [323].

Despite increasing experimental evidence regarding the anti-inflammatory abilities of RelB against CS, neither expression of RelB nor its expression relative to other inflammatory mediators has been studied in the context of COPD at stable state or during exacerbation. It is also unknown whether RelB is associated with any clinically-relevant outcomes in COPD, including acid-base balance and cardiovascular events. Recent experimental evidence in human lung epithelial cells has shown that increased nuclear RelB correlates with hypercapnia-induced protection against lung injury. This suggests that RelB is carbon dioxide-sensitive and may contribute to the benefits of hypercapnia in pulmonary inflammatory diseases [340]. Given that in COPD acid-base disturbances are common, particularly during exacerbations [341] and are associated with poor health outcomes, we hypothesize that RelB may be associated with clinical variables involved in acid-base maintenance during COPD and its exacerbations. It is also well-described that the period following COPD exacerbations has been associated with enhanced risk of acute cardiovascular events [23, 31, 342] possibly due to increased inflammation. Experimental evidence supports a protective role for RelB in the cardiovascular system, including

our data where RelB controls pulmonary endothelial intercellular adhesion molecule-1 (ICAM-1) levels in response to CS [343]. RelB-deficient mice also have inflammatory cell infiltrates in the heart [344]. Thus our data and that of others support that RelB expression is an essential suppressor of pulmonary and cardiovascular inflammation. We therefore hypothesize that systemic RelB expression will be associated with cardiovascular outcomes in COPD, particularly at exacerbation when inflammation is typically increased.

The objectives of this study were: (1) to assess systemic RelB mRNA expression relative to other inflammatory markers relevant to both COPD pathogenesis and whose expression is regulated by RelB (*e.g.* Cox-2, IL-8, IL-1β) in stable-state and exacerbating COPD patients and (2) to assess associations between these two subject groups in relation to acid-base, cardiovascular and pulmonary patient variables. Our results reveal for the first time that RelB is expressed in stable-state and at exacerbation in COPD. Although circulating RelB levels did not correlate with any clinical parameters at stable-state, we show for the first time that RelB expression is associated with both acid-base and cardiovascular features of COPD patients at exacerbation, suggesting that RelB may represent a novel biomarker of cardiovascular outcomes during COPD exacerbations.

METHODS

Study subjects

48 COPD patients were recruited at the Montreal Chest Institute between February-September 2013, including 18 in stable-state (no-exacerbation in the 4 weeks before assessment) and 30 who were hospitalized with a primary diagnosis of exacerbation. Exacerbations were defined based on

symptom-change lasting at least two consecutive days requiring treatment with corticosteroid and/or antibiotics and hospitalization [53, 60]. All subjects had to be ≥40 years old, previously diagnosed with COPD[325] (post-bronchodilator forced expired volume in one second (FEV_1) to forced vital capacity (FVC) ratio <0.70) and have clinical evidence of cardiovascular disease and/or established risk factors (clinical signs or imaging studies; coronary artery disease; peripheral vascular disease; previous stroke or myocardial infarction (MI); diabetes mellitus with target organ disease; or treatment for hypercholesterolemia, hypertension, diabetes mellitus or peripheral vascular disease). This study was conducted in accordance with the amended Declaration of Helsinki. The McGill University Faculty of Medicine Institutional Review Board approved the protocol and written informed consent was obtained from all patients.

Clinical assessment and blood sample collection

Subjects underwent post-bronchodilator spirometry, blood collection (biomarker, lipid profile, complete cell blood count, and metabolic panel analyses), pulse, oxygen saturation, capillary gas, and hemodynamic measurements (carotid-femoral pulse wave velocity (cfPWV) and pulse wave analysis). Peripheral blood was collected using PAXgene blood RNA tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). For those in stable-state, assessments occurred during respiratory clinic visits and for exacerbating subjects, assessments were made during the first 72 hours of hospitalization. Samples were frozen at -80 until analysis.

Analysis of gene expression

RNA was isolated using PAXgene blood RNA kits (PreAnalytiX GmbH), and quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware). Reverse

transcription of total RNA was carried out using iScriptII Reverse Transcription Supermix (Bio-Rad Laboratories, Mississauga, Canada). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed by adding 1uL cDNA and 0.5mM primers (Table 7.1) to SsoFast EvaGreen (Bio-Rad). PCR amplification was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad). Melt curves were analyzed to confirm that non-specific products were absent. The fluorescence detection threshold was set above the non-template control background within the linear phases of PCR amplifications and the cycle threshold (Ct) of each reaction was detected. Gene expression was analyzed using the ΔΔCt method and results are presented as fold-differences normalized to housekeeping gene (β-actin).

Statistical analysis

Analyses were performed using SAS version 9.3 software (SAS Institute. Inc., Cary, North Carolina). For mean comparisons, two-tailed T-tests (normal distribution) or Wilcoxon signed-rank tests (non-normal distribution) were used; Chi-squared tests were used for dichotomous variables. A p-value of 0.05 or less was deemed statistically significant. Associations between clinical variables and mediators were assessed using Pearson correlation coefficients. Multiple regression models were used to determine whether changes in mediator expression could predict changes in clinical variables (adjusted for age, sex, body mass index and smoking history).

RESULTS

Patient characteristics

Both the stable-state and exacerbation subjects groups were statistically-similar in most of the clinical features examined except that the hospitalized group had a lower mean FEV_1 (%

predicted and liters), included more current smokers, and had significantly greater blood pressure and elevated heart rate (Table 7.2).

Stable-state and exacerbation inflammatory mediator expression relative to RelB levels

Figure 7.1 shows the relative fold-difference in mRNA expression of RelB compared to Cox-2, IL-8 and IL-1β. When considering the Stable-state group only, RelB mRNA expression was 69.01 fold less than that of Cox-2, 4.42 fold less than that of IL-8 and 26.97 fold less than that of IL-1β ($p<0.001$ for all cases). When considering the subjects who were in exacerbation, RelB mRNA expression was 65.46 fold less than that of Cox-2 ($p<0.001$), 1.48 fold less than that of IL-8 ($p=0.22$) and 32.16 fold less than that of IL-1β ($p<0.001$). Moreover in exacerbating patients, RelB mRNA expression was 1.43 fold lower than in stable-state patients ($p<0.001$) (Figure S7.1).

Associations between inflammatory mediators and patient clinical features during Stable-state or Exacerbation

In the stable-state patients (Table 7.3), Cox-2 mRNA expression correlated negatively with FEV_1 (liters), FEV_1/FVC and the ratio of cholesterol to high-density lipoprotein (HDL). IL-8 mRNA correlated negatively with systolic and diastolic blood pressure, and absolute basophil count, and positively with red blood cell (RBC) diameter width. IL-1β mRNA expression correlated positively with pack-year smoking history, white blood cell (WBC) count, absolute monocyte count and absolute neutrophil count. RelB mRNA expression was not correlated to any clinical variables in stable-state.

When considering mRNA expression in exacerbating subjects (Table 7.3), Cox-2 mRNA expression correlated negatively with systolic blood pressure, hematocrit (%) and anion gap, and positively with pack-year smoking history, calcium levels, RBC diameter width and absolute eosinophil count. IL-8 mRNA expression correlated negatively with systolic blood pressure, pH, anion gap and glucose level, and positively with the partial pressure of carbon dioxide (PCO_2), augmentation index (AIx) and absolute eosinophil count. IL-1β mRNA expression correlated negatively with cholesterol level, RBC count, hemoglobin level and hematocrit level. RelB mRNA expression correlated negatively with systolic blood pressure, anion gap, and glucose level.

Predictors of change in patient clinical features

When only considering the predictive relationships between inflammatory mediator mRNA expression and clinical features of stable-state patients (Figure 7.2a; Table 7.4), Cox-2 could predict negative changes in diastolic blood pressure, PCO_2, cfPWV, mean arterial pressure, AIx, cholesterol level, and low density lipoprotein (LDL). IL-8 expression could predict negative changes in systolic blood pressure and absolute basophil count, and positive changes in RBC diameter width. IL-1β expression could predict positive changes in pH, WBC count, and absolute neutrophil count. The expression of RelB could not significantly predict changes for any variables in stable-state.

For exacerbating patients (Figure 7.2b; Table 7.5), Cox-2 mRNA expression could predict negative changes in anion gap and positive changes in PCO_2, blood calcium level, RBC width, absolute eosinophil count, absolute basophil count and bicarbonate level. IL-8 mRNA expression

could predict negative changes in systolic blood pressure, pH, and anion gap, and positive changes in PCO_2, augmentation pressure, AIx and absolute eosinophil count. IL-1β expression could predict negative changes in FEV_1/FVC, RBC count, hemoglobin level, and hematocrit level, and positive changes in calcium level. RelB mRNA expression could predict negative changes in systolic blood pressure and anion gap.

DISCUSSION

Finding biomarkers of patient-relevant outcomes is a primary goal of COPD research [345]. Acute inflammatory changes that occur during exacerbations make it important to differentiate between biomarkers that might be useful for assessing disease activity and/or outcomes in stable-state from those found during exacerbation [345]. One such biomarker that offers potential in COPD is RelB, an NF-κB family member that is constitutively expressed in human lymphocytes and dendritic cells [346], suppresses cytokine production in lung epithelial cells[347] and is vital for thymus development and T cell function [348, 349]. Importantly, RelB suppresses CS-induced inflammation by interacting with the aryl hydrocarbon receptor (AhR) [321-324, 337] to collectively control Cox-2 expression in lung fibroblasts [324]. Previous studies of the importance of RelB on CS and lung diseases have been restricted to experimental *in-vitro* and *in-vivo* models. Although these studies conclusively support an anti-inflammatory role for RelB against CS, there is no data on RelB expression in COPD or associations with relevant clinical outcomes. We report RelB expression for the first time in COPD patients and provide novel evidence that RelB may be associated with clinically-relevant features of COPD patients during exacerbations. Consequently, our study is an important step to provide insights into RelB expression and its potential role in COPD. It reports for the first time the associations

between RelB and clinical parameters during COPD exacerbations.

One of our most intriguing findings is that although RelB mRNA expression was not associated with clinical outcomes in stable-state, at exacerbation RelB expression was negatively associated with several clinical parameters including systolic blood pressure. Our finding on RelB expression and systolic blood pressure is novel, as a relationship between RelB and blood pressure has not been reported to-date in humans. Experimentally RelB has been associated with balloon catheter injury in the rat carotid artery [350] and may be downregulated in response to treatment with DETA-NONOate- a nitric oxide donor [351]. Moreover, our recently published data show that RelB may suppress pulmonary ICAM-1 levels in response to CS [343]. When considered together with our current data, this supports a role for RelB in modulating endothelial function and blood pressure. This is potentially of importance, as it is well known that the period immediately following a COPD exacerbation is associated with increased risk of acute cardiovascular events [23, 31, 342]. In a separate analysis, we grouped stable-state and exacerbating subjects and found RelB expression to be negatively correlated to and able to predict negative changes in several outcomes including heart rate and PCO_2, while predicting positive changes in pulse pressure (Table S1). Thus, identifying novel biological targets that could be capable of maintaining cardiovascular stability in COPD is of significant clinical value.

In addition to cardiovascular events, RelB expression was also related to anion gap, a parameter commonly used to identify acid-base disorders and disturbances [352]. In COPD, acid-base disturbances occur frequently [341] and lead to poor patient outcomes. Although it was recently shown that RelB is carbon dioxide-sensitive and contributes to the benefits of hypercapnia in pulmonary inflammatory diseases [340], our study is the first to report on RelB expression in

relation to anion gap. This renders it possible that RelB may play a role in acid-base regulation during COPD exacerbations. Together, these data lend further support for an association between RelB and cardiovascular function as well as the involvement of RelB in acid-base maintenance. Thus, given the relationship between RelB and CS-induced inflammation, it might be reasonable to speculate that alterations in RelB expression and/or activity during exacerbations in COPD contribute to cardiovascular manifestations.

RelB dampens the expression of numerous COPD-relevant inflammatory mediators. Therefore we also examined associations between these (*i.e.* Cox-2, IL-8 and IL-1β) and patient clinical features. As with RelB expression, several of these mediators exhibited strong associations with cardiovascular alterations. Cox-2 is induced by HDL [353] and LDL [354], and although an association between Cox-2 and blood pressure has been described [355, 356], our study is first to suggest a relationship with cfPWV and AIx, two measures of arterial stiffening associated with cardiovascular risk. Moreover, the positive relationship between Cox-2 and RBC diameter width is novel and may be of clinical significance, as RBC diameter width-distribution is a powerful outcome predictor in chronic and/or acute left heart failure, and in COPD, can help identify right ventricle failure [357]. Associations between IL-8 and cardiovascular outcomes have been reported [358, 359], although none specifically on AIx and augmentation pressure. In our study IL-8 was also linked to acid-base parameters (pH, anion gap and PCO_2) and fluctuations in these have been shown to alter pH and neutrophil IL-8 release [360]. Alterations in pH can also control IL-1β production by monocytes, not only supporting the relationship between IL-1β and pH [361] but also perpetuating and augmenting the inflammatory response in COPD.

Our study has strengths as well as limitations, the former including the fact that we recruited patients both in stable-state COPD and patients who were in acute exacerbation requiring hospitalization (*i.e.* severe exacerbation). Patients with severe COPD exacerbations are often difficult to recruit, as they are very sick and often refuse to participate in research studies. An additional strength of our study is that we also assessed patients within the first 72 hours of hospitalization, which allowed us to capture important physiological and clinical data that occur systemically and locally within the lung. A limitation of our study is that 25 of the 30 exacerbating patients took inhaled corticosteroids prior to blood collection. Corticosteroids can dampen Cox-2 and IL-8 expression via the NF-κB pathway by suppressing gene transcription [362, 363]. Thus the relative expression levels we report may be an under-representation. Another perceived limitation is the reliance on mRNA levels to correlate with clinical parameters, as quantification of blood RelB protein expression remains to be determined. However, RelB protein expression mirrors that of mRNA levels during dendritic cell differentiation [364]. Thus, we expect a similar association between blood RelB mRNA and protein levels in COPD. It would also strengthen our observations presented herein to further investigate the relationship between RelB expression and pulmonary patient outcomes in COPD stages GOLD I-IV; these investigations are currently ongoing. Finally, we recognize that the subjects in our study are unpaired, thereby reducing the statistical power and rendering it possible that inter-subject variability may have impacted our measurements. Thus, investigation of RelB and associated clinical outcomes in paired subjects (stable-state and exacerbation from the same subjects) is warranted. To ultimately determine the suitability of RelB as a biomarker in COPD, a longitudinal relationship between expression and associated outcomes must be examined.

There has been growing evidence that RelB is a potent suppressor of CS-induced inflammation [323, 324, 337, 347, 365]. Thus despite the limitations of our study, the expression and function of RelB in COPD represents a burgeoning area of research, and our data on the associations of RelB expression in COPD are highly novel and clinically relevant. Moreover, the results of our study suggest for the first time that blood RelB expression may be a noteworthy marker of cardiovascular events during COPD exacerbations. Future longitudinal and mechanistic studies will undoubtedly shed light on the functional significance of RelB in COPD pathogenesis and its potential for therapeutic modulation.

ACKNOWLEDGMENTS

We would like to thank all researchers, staff, in particular Katrina Metz for blood sample collection and Pei Z. Li for her help with statistical analyses, and to the COPD patients who participated in this study.

TABLES

Table 7.1 : qRT-PCR Primer sequences

Primer	Forward sequence	Reverse sequence
hCox-2	TCACAGGCTTCCATTGACCAG	CCGAGGCTTTTCTACCAGA
hIL-8	GATGTCAGTGCATAAAGACATACTCCAAAC	GCTCTCTTCCATCAGAAAGCTTTACAATAA
hIL-1β	AAAAGCTTGGTGATGTCTGGTCCATATGAA	CTTATCATCTTTCAACACGCAGGACAGGTA
hRelB	TGTGGTGAGGATCTGCTTCCA G	GGCCCGCTTTCCTTGTTAATT C
β-actin	CTACAATGAGCTGCGTGTG	TGGGGTGTTGAAGGTCTC

Table 7.2: Patient characteristics

	Stable-state (n=18)	Exacerbation (n=30)	P-value
Mean age (years)	71.0	71.1	0.86
No. Male (%)	7 (39)	14 (47)	0.11
Mean FEV_1 % predicted	43.5	34.5	0.046*
Mean FEV_1 L	1.1	0.79	0.0082*
Mean FEV_1/FVC	0.44	0.48	0.30
Smoking status, n (%)			
Ex-smoker	17 (94)	23 (77)	0.083
Current smoker	1 (6)	7 (23)	0.014*
LTOT (%)	22	20	0.62
Mean pack-year smoking history	68.77	57.84	0.42
Mean body mass index	27.9	25.1	0.15
Mean no. reported exacerbations in past 12 months	2	3	0.57
Mean systolic BP	111	125	0.013*
Mean diastolic BP	54	66	0.0049*
Mean heart rate	78	92	0.0030*
Pulse oximetry (%)	94	94	0.57

*Denotes statistical significance. No: number, LTOT: long-term oxygen therapy, BP: blood pressure.

Table 7.3: Associations between inflammatory mediators and clinical features of patients in stable-state and at exacerbation

	Inflammatory mediators							
	Cox-2 expression levels		IL-1β expression levels		RelB expression levels		IL-8 expression levels	
	Correlation coefficients		Correlation coefficients		Correlation coefficients		Correlation coefficients	
Clinical features	Stable-state n=18	Exacerbation n=30	Stable-state n=18	Exacerbation n=30	Stable-state n=18	Exacerbation n=30	Stable-state n=18	Exacerbation n=30
Pack-year smoking history	0.36	0.39*	0.49*	0.13	0.39	-0.020	0.050	0.18
Systolic BP	-0.18	-0.42*	0.13	-0.32	-0.090	-0.41*	-0.52*	-0.52*
Diastolic BP	-0.37	-0.29	-0.060	-0.36	-0.26	-0.17	-0.51*	-0.10
FEV_1 (L)	-0.51*	-0.060	0.11	0.070	-0.19	-0.080	-0.25	-0.20
FEV_1/FVC	-0.56*	-0.11	-0.33	-0.10	-0.37	-0.12	-0.11	-0.17
pH	0.33	0.11	0.42	0.22	0.16	-0.21	0.58	-0.46*
PCO_2 (mmHg)	-0.21	0.26	-0.34	-0.090	-0.10	0.41	-0.13	0.49*
Ca++ (mmol/L)	-0.070	0.47*	0.15	0.40	-0.57	0.35	0.080	0.19
Cholesterol/HDL	-0.51*	-0.060	-0.30	-0.15	0.080	0	-0.090	0.070
Cholesterol (mmol/L)	-0.25	-0.11	-0.34	-0.41*	-0.050	-0.36	-0.25	0.050
Aix (%)	-0.10	-0.15	-0.29	-0.13	-0.11	0.22	-0.17	0.46*
Hct (%)	0.26	-0.47*	0.48	-0.38	-0.030	-0.13	-0.10	-0.30
Hct (L/L)	-0.21	-0.36	0.30	-0.52*	-0.24	-0.11	-0.44	-0.18
WBC [a]	0.24	0.010	0.76**	0.33	0.32	-0.040	0.40	-0.12
RBC (10^12/L)	-0.15	-0.33	0.31	-0.47*	-0.11	-0.14	-0.37	-0.18
RBC diameter width (cV)	0.030	0.52*	0.19	0.29	0.26	0.22	0.67*	0.050
Hemoglobin (g/L)	-0.14	-0.34	0.30	-0.46*	-0.21	-0.16	-0.45	-0.17
Abs. MNC[a]	0.070	-0.05	0.63*	0.080	0.31	0.090	0.28	-0.060
Abs. neutrophil[a]	0.32	0	0.74**	0.35	0.29	-0.10	0.46	-0.15
Abs. basophil[a]	-0.020	0.25	0.21	0.20	-0.060	0.32	-0.61*	0.080
Abs. eosinophil[a]	0.010	0.49*	0.010	0.050	0.20	0.30	0.20	0.55*
Anion gap (mmol/L)	-0.050	-0.47*	0.13	0.020	0.21	-0.55*	0.15	-0.55*
Glucose (mmol/L)	-0.33	-0.31	0.14	0.15	0.050	-0.41*	0.12	-0.39*

Pearson correlation coefficients were used to determine associations.
*Denotes statistical significance $p<0.05$, ** denotes statistical significance $p<0.001$.[a] units are (10^9/L). BP: blood pressure, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, PCO_2: partial pressure of carbon dioxide, Ca++: calcium, HDL: high-density lipoprotein, Aix: augmentation index, Hct: hematocrit, WBC: white blood cell, RBC: red blood cell, Abs: absolute, MNC: monocyte.

Table 7.4: Predictors of clinical feature change in stable-state

	Stable-state COPD (n=18)							
	Cox-2 expression levels		IL-1beta expression levels		RelB expression levels		IL-8 expression levels	
Clinical features	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Systolic BP	-637.13 (-1839.06 - 564.79)	0.27	48.89 (-2856.79 - 2954.56)	0.97	-66325.7 (-193597 - 60945.40)	0.28	-8410.48 (-16147.2 - -673.76)	0.036*
Diastolic BP	-1173.04 (-2117.53 - -228.56)	0.019*	-391.22 (-3170.54 - 2388.10)	0.76	-70756.5 (-191298 - 49784.85)	0.22	-7471.46 (-15178.5 - 235.53)	0.056
pH	2.85 (-0.64 - 6.33)	0.086	8.24 (1.35 - 15.13)	0.029*	182.37 (-357.62 - 722.36)	0.40	28.54 (-6.07 - 63.15)	0.084
PCO_2 (mmHg)	-474.44 (-861.41 - -87.47)	0.027*	-593.12 (-2343.89 - 1157.64)	0.40	-15073.0 (-98917.2 - 68771.15)	0.64	-1125.91 (-8586.88 - 6335.06)	0.70
cfPWV (m/s)	-634.31 (-1006.47 - -262.14)	0.0030*	-807.59 (-1798.15 - 182.97)	0.10	-10424.8 (-62823.5 - 41973.93)	0.67	-557.57 (-4426.40 - 3311.26)	0.76
MAP (mmHg)	-1445.68 (-2228.01 - -663.36)	0.0020*	-616.78 (-3197.05 - 1963.49)	0.60	-61331.8 (-172971 - 50307.08)	0.25	-5757.05 (-13787.5 - 2273.42)	0.14
Aix %	-931.72 (-1859.35 - -4.09)	0.049*	-546.46 (-2782.59 - 1689.67)	0.59	-37079.5 (-138077 - 63918.39)	0.43	-1724.41 (-9533.71 - 6084.89)	0.63
Cholesterol (mmol/L)	-62.21 (-119.63 - -4.80)	0.036*	-65.24 (-282.03 - 151.55)	0.52	86.86 (-8457.91 - 8631.63)	0.98	-302.84 (-1043.82 - 438.15)	0.38
LDL (mmol/L)	-61.38 (-117.57 - -5.19)	0.035*	-85.14 (-294.24 - 123.95)	0.39	1469.95 (-6853.37 - 9793.26)	0.70	-319.33 (-1042.00 - 403.34)	0.35
WBC (10^9/L)	85.65 (-54.29 - 225.59)	0.21	504.49 (186.88 - 822.10)	0.0050*	3843.09 (-12921.6 - 20607.79)	0.62	913.43 (-368.64 - 2195.49)	0.15
RBC diameter width (cV)	44.43 (-136.38 - 225.24)	0.60	270.73 (-259.38 - 800.83)	0.29	8895.13 (-10807.2 - 28597.48)	0.34	1690.40 (386.50 - 2994.31)	0.016*
Abs. neutrophil (10^9/L)	85.84 (-51.73 - 223.41)	0.20	470.02 (138.86 - 801.18)	0.010*	2751.54 (-13865.3 - 19368.40)	0.72	1021.97 (-200.52 - 2244.47)	0.093
Abs. basophil (10^9/L)	-1.23 (-4.08 - 1.62)	0.36	-2.48 (-11.38 - 6.43)	0.55	-111.46 (-435.83 - 212.92)	0.47	-27.49 (-48.50 - -6.48)	0.015*

Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. Inflammatory markers were used as independent variables to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in biomarker expression. *Denotes statistical significance. BP: blood pressure, PCO_2: partial pressure of carbon dioxide, cfPWV: carotid-femoral pulse wave velocity, MAP: mean arterial pressure, Aix: augmentation index, LDL: low-density lipoprotein, WBC: white blood cell, RBC: red blood cell, Abs: absolute.

Table 7.5: Predictors of clinical feature change at exacerbation

	Exacerbation (n=30)							
	Cox-2 expression levels		IL-1β expression levels		RelB expression levels		IL-8 expression levels	
Clinical features	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Systolic BP	-677.58 (-1586.61 - 231.45)	0.14	-1390.54 (-3283.70 - 502.62)	0.14	-50749.6 (-92792.3 - -8706.83)*	0.020*	-12649.1 (-24031.7 - -1266.55)*	0.031*
pH	-0.13 (-2.15 - 1.90)	0.90	1.00 (-2.94 - 4.94)	0.60	-25.42 (-116.18 - 65.33)	0.56	-29.07 (-52.47 - -5.67)*	0.018*
PCO_2 (mmHg)	360.06 (18.51 - 701.60)	0.040*	123.24 (-620.68 - 867.17)	0.73	12518.77 (-3831.36 - 28868.91)	0.13	5085.44 (564.18 - 9606.71)*	0.030*
Ca++ (mmol/L)	2.82 (0.46 - 5.19)	0.023*	5.41 (0.34 - 10.48)*	0.038*	85.87 (-33.15 - 204.90)	0.14	15.66 (-23.12 - 54.43)	0.40
FEV_1/FVC	-2.27 (-9.08 - 4.55)	0.50	-14.75 (-28.27 - -1.23)*	0.034*	-157.61 (-492.65 - 177.42)	0.34	-46.09 (-140.18 - 48.00)	0.32
AP (mmHg)	-115.51 (-500.67 - 269.66)	0.54	-120.55 (-831.09 - 589.99)	0.73	12466.04 (-5030.98 - 29963.05)	0.15	5254.52 (1644.29 - 8864.76)*	0.0070*
Aix (%)	-348.85 (-1173.38 - 475.68)	0.39	-572.74 (-2098.13 - 952.65)	0.44	22125.77 (-16420.3 - 60671.88)	0.24	11956.20 (4295.85 - 19616.55)*	0.0040*
RBC (10^12/L)	-26.85 (-54.28 - 0.57)	0.060	-79.50 (-132.82 - -26.18)*	0.0060*	-833.40 (-2267.21 - 600.42)	0.24	-233.26 (-634.42 - 167.91)	0.24
Hemoglobin (g/L)	-697.08 (-1584.64 - 190.48)	0.12	-2074.77 (-3864.48 - -285.06)*	0.025*	-27604.0 (-72519.4 - 17311.45)	0.22	-5718.07 (-18463.6 - 7027.43)	0.36
Hct (L/L)	-2.18 (-4.87 - 0.51)	0.11	-7.33 (-12.46 - -2.19)*	0.0070*	-63.61 (-202.16 - 74.94)	0.35	-17.94 (-56.53 - 20.65)	0.34
RBC diameter width (cV)	83.94 (12.69 - 155.20)	0.023*	42.68 (-122.55 - 207.91)	0.60	2309.70 (-1537.06 - 6156.46)	0.23	-247.14 (-1305.99 - 811.72)	0.63
Abs. eosinophil (10^9/L)	4.15 (0.79 - 7.51)	0.018*	-1.17 (-9.51 - 7.16)	0.77	134.32 (-45.59 - 314.24)	0.14	63.29 (18.69 - 107.89)	0.0080*
Abs. basophil (10^9/L)	1.89 (0.23 - 3.54)	0.027*	0.97 (-2.45 - 4.40)	0.56	77.14 (-8.13 - 162.41)	0.074	5.87 (-16.07 - 27.82)	0.58
Bicarbonate level (mmol/L)	214.54 (41.93 - 387.15)	0.017*	-28.89 (-431.63 - 373.85)	0.88	8733.02 (-227.46 - 17693.51)	0.056	1700.47 (-753.55 - 4154.48)	0.16
Anion gap (mmol/L)	-183.24 (-289.11 - -77.38)	0.0020*	0.27 (-288.19 - 288.73)	1.00	-8316.41 (-13802.0 - -2830.85)*	0.0050*	-2294.72 (-3798.95 - -790.50)	0.0050*
Sodium (mmol/L)	111.10 (-89.66 - 311.86)	0.26	54.92 (-389.96 - 499.80)	0.80	5829.30 (-4079.80 - 15738.40)	0.24	-346.36 (-3193.78 - 2501.05)	0.80

Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. Inflammatory markers were used as independent variables to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in biomarker expression. *Denotes statistical significance. MAP: mean arterial pressure, PCO_2: partial pressure of carbon dioxide, Ca++: calcium, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, AP: augmentation pressure, Aix: augmentation index, RBC: red blood cell, Hct: hematocrit, Abs: absolute.

Supplemental table 7.1: Associations between RelB expression levels and clinical features of both exacerbating and stable-state patients (n=48) and its ability to predict changes in these features.

	Associations		Ability to predict change in clinical features	
Clinical features	**Correlation coefficient**	**P-value**	**ß (95% CI)**	**P-value**
Systolic BP	-0.42	0.0030*	-51205.3 (-89737.3 - -12673.4)	0.010*
Diastolic BP	-0.33	0.022*	-30750.3 (-62720.6 - 1219.91)	0.059
Pulse (BPM)	-0.38	0.0080*	-43450.1 (-78570.1 - -8330.11)	0.017*
O2 saturation (%)	0.070	0.62	2282.89 (-4423.89 - 8989.67)	0.50
pH	-0.18	0.33	-23.34 (-121.59 - 74.91)	0.63
PO2 (mmHg)	-0.12	0.50	-3703.96 (-31894.9 - 24486.97)	0.79
PCO2 (mmHg)	0.34	0.050*	11295.79 (-3690.84 - 26282.43)	0.13
Na+ (mmol/L)	0.29	0.11	6364.29 (-5672.46 - 18401.04)	0.29
K+ (mmol/L)	-0.29	0.11	-2956.32 (-6340.64 - 428.00)	0.084
Ca++ (mmol/L)	0.15	0.42	22.72 (-115.24 - 160.68)	0.74
Hct (%)	-0.14	0.44	-10363.1 (-26522.9 - 5796.77)	0.20
Approximate number of exacerbations reported during the previous year	-0.1	0.51	-1009.36 (-4995.93 - 2977.21)	0.62
FEV1 L	0.11	0.46	-54.56 (-863.62 - 754.49)	0.89
FEV1 % predicted	-0.07	0.63	-18874.4 (-49897.7 - 12148.90)	0.23
FEV1/FVC	-0.22	0.15	-300.82 (-569.17 - -32.48)	0.029*
Mean cfPWV (m/s)	0.15	0.34	2012.17 (-9002.75 - 13027.09)	0.71
Mean MAP (mmHg)	-0.2	0.21	-16046.9 (-50007.2 - 17913.50)	0.34
Mean PP (mmHg)	0.31	0.051	29042.30 (3063.65 - 55020.94)	0.030*
Mean AP (mmHg)	0.24	0.14	13136.28 (-971.24 - 27243.80)	0.067
Mean Aix (%)	0.15	0.34	18482.26 (-10646.3 - 47610.86)	0.21
Mean crPWV 1 (m/s)	0.12	0.44	841.56 (-2867.24 - 4550.37)	0.65
Cholesterol (mmol/L)	-0.15	0.32	-511.91 (-3010.68 - 1986.86)	0.68
Triglycerides (mmol/L)	0.040	0.82	272.50 (-1368.49 - 1913.49)	0.74
HDL (mmol/L)	-0.14	0.38	-156.49 (-1425.70 - 1112.71)	0.80
LDL (mmol/L)	-0.12	0.46	-478.54 (-2746.65 - 1789.57)	0.67
Cholesterol/HDL	0.030	0.82	-54.97 (-2426.23 - 2316.29)	0.96
WBC (10^9/L)	-0.12	0.42	-2813.16 (-12962.0 - 7335.69)	0.58

RBC (10^12/L)	-0.06	0.70	-737.39 (-2077.71 - 602.93)	0.27
Hemoglobin (g/L)	-0.18	0.23	-31628.3 (-71677.7 - 8421.00)	0.12
Hct (L/L)	-0.14	0.35	-80.22 (-198.90 - 38.46)	0.18
Mean cell vol. (fL)	-0.14	0.36	-3049.93 (-17685.5 - 11585.62)	0.68
Mean cell hemoglobin (pg/cell)	-0.18	0.23	-2086.62 (-7842.80 - 3669.56)	0.47
Mean cell hemoglobin Conc. (g/L)	-0.2	0.17	-11957.5 (-33895.0 - 9980.04)	0.28
RBC diameter Width (cV)	0.29	0.054	2650.85 (-1663.19 - 6964.90)	0.22
Platelet (10^9/L)	-0.18	0.24	-73614.0 (-271159 - 123931.5)	0.46
Platelet Hct	-0.13	0.40	-37.11 (-189.86 - 115.64)	0.63
Mean platelet vol. (fL)	0.16	0.30	563.72 (-1165.91 - 2293.35)	0.51
Platelet Dist. Width (cV)	0.030	0.86	342.31 (-838.73 - 1523.34)	0.56
Abs. Lymphocyte (10^9/L)	0.33	0.025*	1463.58 (-10.50 - 2937.66)	0.052
Abs. MNC (10^9/L)	0.20	0.18	239.08 (-572.63 - 1050.80)	0.56
Abs. Neutrophil (10^9/L)	-0.2	0.17	-5032.65 (-15249.6 - 5184.34)	0.33
Abs. Eosinophil (10^9/L)	0.44	0.0020*	273.19 (39.32 - 507.06)	0.023*
Abs. Basophil (10^9/L)	0.24	0.11	80.54 (-8.99 - 170.08)	0.077
Bicarbonate Level (mmol/L)	0.36	0.016*	7183.79 (344.14 - 14023.45)	0.040*
Anion Gap (mmol/L)	-0.46	0.0010*	-7400.53 (-12612.0 - -2189.07)	0.0070*
Creatinine (umol/L)	-0.08	0.62	-21089.4 (-76711.5 - 34532.62)	0.45
Glucose Random (mmol/L)	-0.45	0.0020*	-11766.3 (-20056.2 - -3476.45)	0.0070*
Chloride (mmol/L)	0.20	0.20	6280.30 (-3149.26 - 15709.86)	0.19
Potassium (mmol/L)	-0.09	0.58	-131.31 (-1059.12 - 796.51)	0.78
Sodium (mmol/L)	0.27	0.069	6063.56 (-1496.57 - 13623.70)	0.11

Pearson correlation coefficients were used to determine associations. Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. RelB was used as an independent variable to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in RelB expression.*Denotes statistical significance $p<0.05$. BPM: beats per minute, O2: oxygen, PO2: partial pressure of oxygen, PCO2: partial pressure of carbon dioxide, Na+: sodium, K+: potassium, Ca++: calcium, Hct: hematocrit, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, BP: blood pressure, cfPWV: carotid-femoral pulse wave velocity, MAP: mean arterial pressure, PP: pulse pressure, AP: augmentation pressure, Aix: augmentation index, crPWV: carotid-radial pulse wave velocity, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WBC: white blood cell, RBC: red blood cell, vol.: volume, Abs.: absolute, MNC: monocyte.

FIGURE LEGENDS AND FIGURES

FIGURE LEGENDS

Figure 7.1. Fold difference in RelB mRNA expression relative to IL-1β, IL-8 and Cox-2 mRNA expression for patients in stable-state (n=18) and those in exacerbation (n=30). * Denotes non-statistically significant difference in expression ($p=0.22$), for all other $p<0.001$.

Figure 7.2. Ability of inflammatory mediators to predict changes in patient clinical features in a) stable-state (n=18) and b) at exacerbation (n=30). A linear regression model was used and adjusted for age, sex, body mass index and smoking pack-years. The inflammatory mediators were used as independent variables to predict changes in all assessed clinical features. ***** Denotes a positive association. BP: blood pressure, MAP: mean arterial pressure, Abs: absolute, AP: augmentation pressure.

Supplemental Figure 7.1. Mean fold difference (± standard error) in RelB mRNA expression at exacerbation (n=30) relative to RelB mRNA expression in stable-state patients (n=18). Fold decrease in RelB expression at exacerbation is 1.43 ($p<0.001$).

FIGURES

Figure 7.1

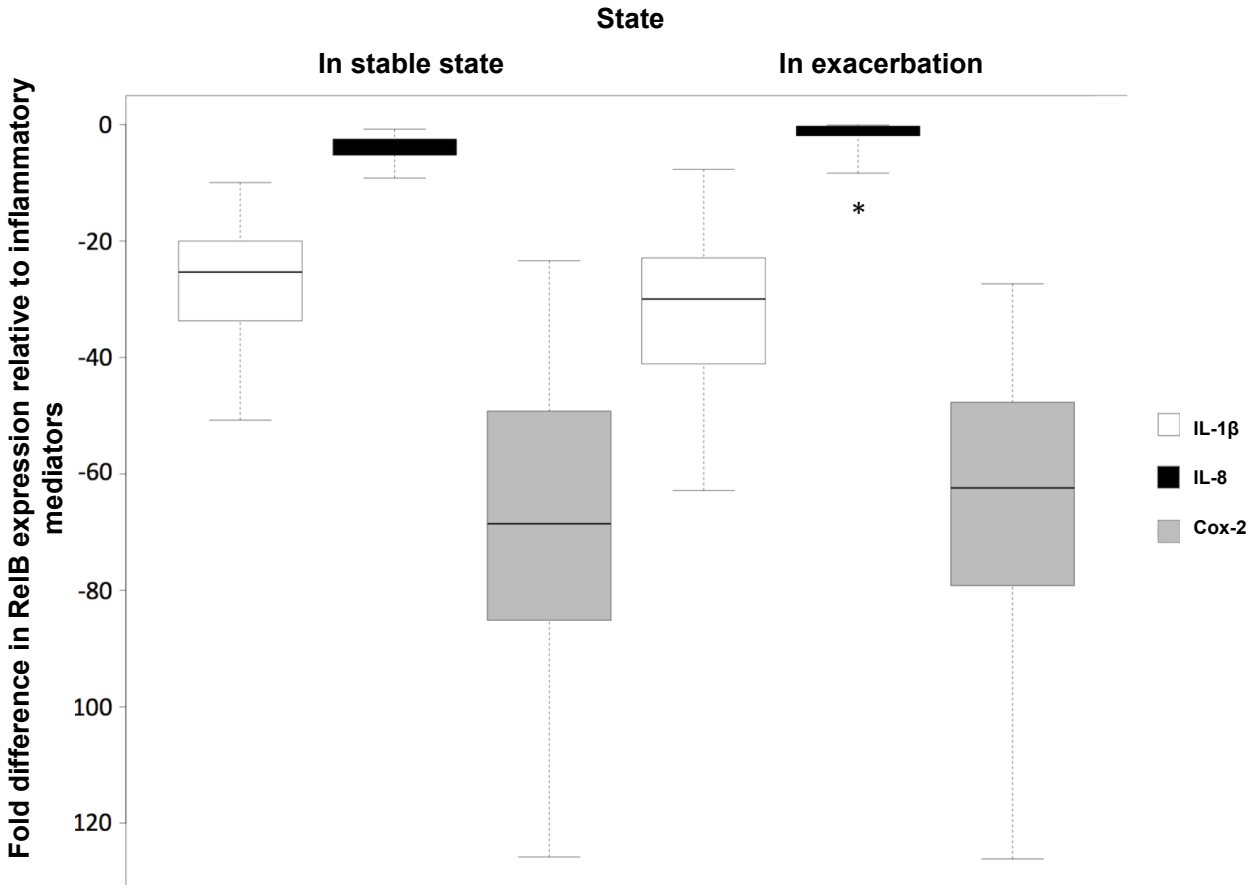
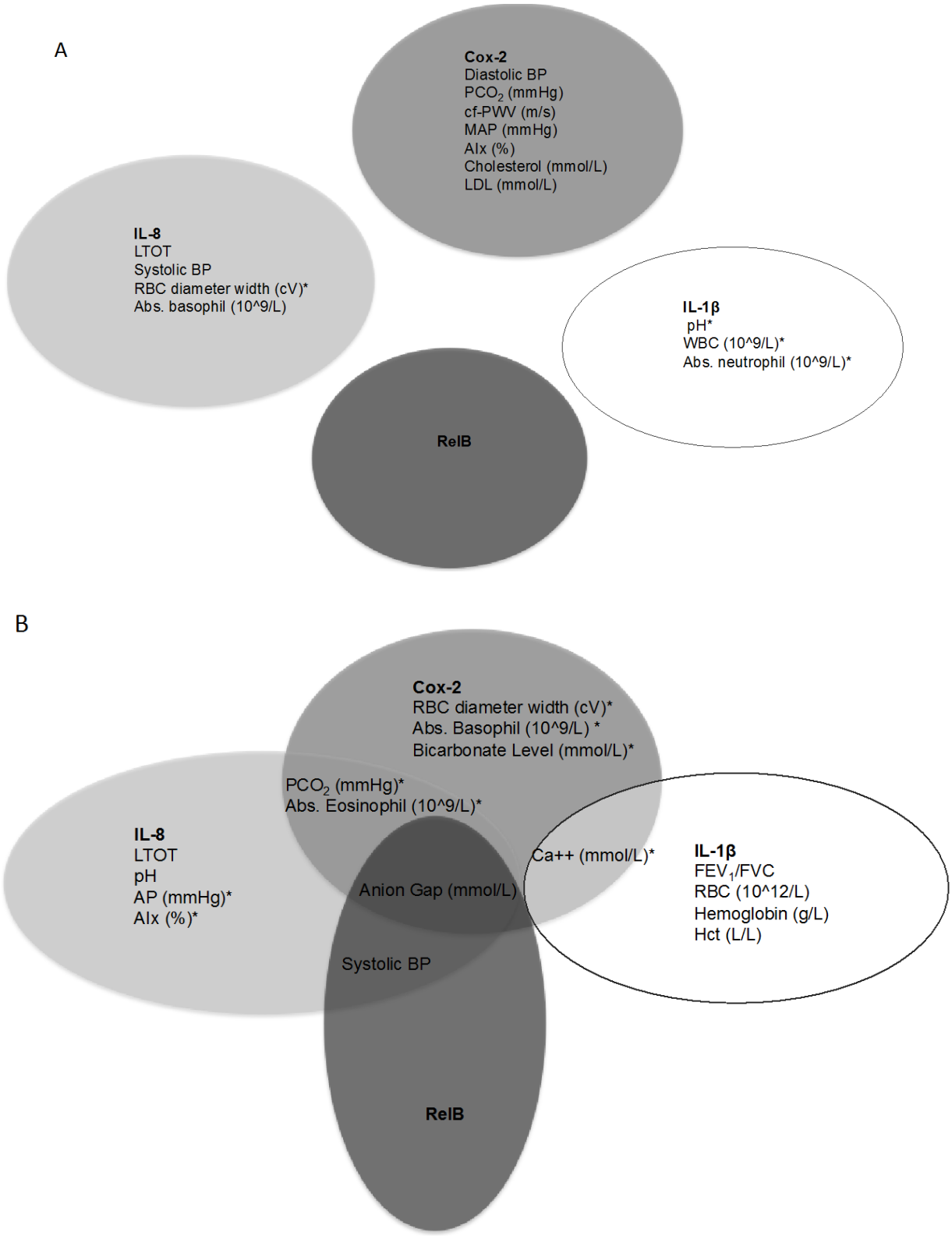
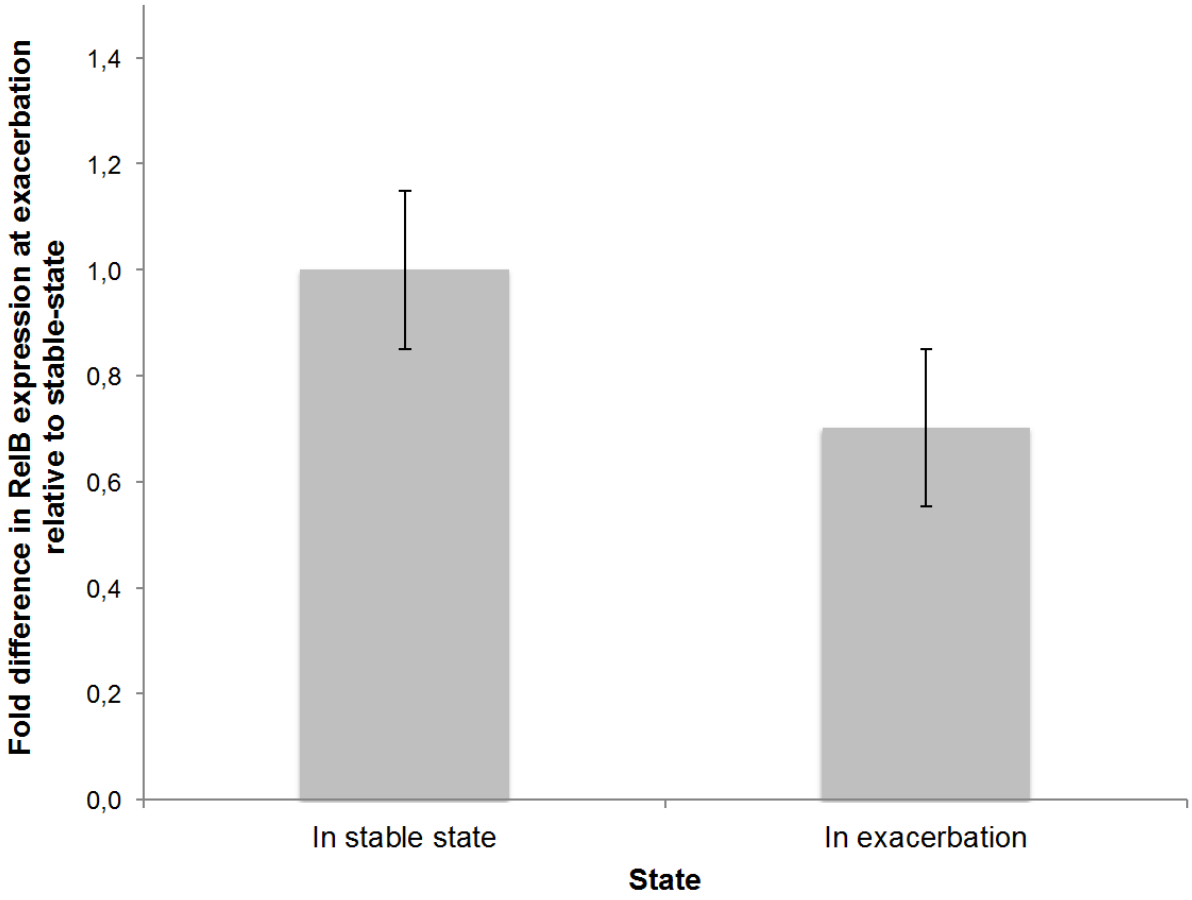


Figure 7.2


A
Cox-2
Diastolic BP
PCO2 (mmHg)
cf-PWV (m/s)
MAP (mmHg)
AIx (%)
Cholesterol (mmol/L)
LDL (mmol/L)
IL-8
LTOT
Systolic BP
RBC diameter width (cV)*
Abs. basophil (10^9/L)
IL-1β
pH*
WBC (10^9/L)*
Abs. neutrophil (10^9/L)*
RelB
B
Cox-2
RBC diameter width (cV)*
Abs. Basophil (10^9/L) *
Bicarbonate Level (mmol/L)*
PCO2 (mmHg)*
Abs. Eosinophil (10^9/L)*
IL-8
LTOT
pH
AP (mmHg)*
AIx (%)*
Ca++ (mmol/L)*
IL-1β
FEV1/FVC
RBC (10^12/L)
Hemoglobin (g/L)
Hct (L/L)
Anion Gap (mmol/L)
Systolic BP
RelB

Supplemental figure 7.1



CHAPTER 8: MANUSCRIPT 4 "CLUB CELL-16 AND RELB: PREDICTORS OF ARTERIAL STIFFNESS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE"

PREFACE TO MANUSCRIPT 4

This manuscript is based on an analysis of two lung-specific inflammatory mediators and a potentially novel biomarker of CV outcomes in COPD patients who have severe airflow obstruction, who frequently exacerbate, who have elevated CV risk and who were admitted to hospital for COPD exacerbation.

CV outcomes significantly impact the course of COPD, yet it remains difficult to identify subjects who will be at elevated CV risk. Lung-specific inflammatory mediators are thought to be important biomarkers of disease in COPD [122, 366, 367]. However, their utility in predicting adverse CV events is not known. RelB, a ubiquitous NF-κB protein that suppresses pulmonary inflammation, was identified as a novel marker of CV outcomes during COPD exacerbations but the association with CV alterations over time remains unknown. This is the first study to evaluate the course of change in lung-specific inflammatory markers and RelB during severe COPD exacerbations and subsequent recovery in association with changes in cf-PWV.

The references in the following manuscript have been renumbered and are included in the combined bibliography at the end of this thesis in numerical order following the sequence in which they appear throughout the entire thesis. Previously defined acronyms within this thesis are redefined within this manuscript as it has been formatted for submission to the American Journal of Respiratory and Critical Care Medicine.

TITLE PAGE

Club cell-16 and RelB: predictors of arterial stiffness in chronic obstructive pulmonary disease

Laura E. Labonté [1,2], Jean Bourbeau [1,2], Stella S. Daskalopoulou [1], Michele Zhang [2], Patrick Coulombe [2], Katie Garland [2], and Carolyn J. Baglole [1,3]*

[1]Departments of Experimental Medicine, McGill University, 1110 Pins Ave. West, Montreal, Québec, Canada.

[2] Respiratory and Epidemiology Clinical Research Unit, Research Institute of the McGill University Health Center, 5252 De Maisonneuve Blvd., Montreal, Québec, Canada.

[3]Meakins Christie Laboratories, Research Institute of the McGill University Health Center, Block E, 1001 Decarie Blvd., Montreal, Québec, Canada.

Corresponding author:
*Carolyn J. Baglole, PhD
Research Institute of the McGill University Health Center
1001 Decarie Blvd., Block E (EM22248), Montreal, Quebec H4A 3J1
Tel: (514) 934-1934 Ext. 76109
E-mail: carolyn.baglole@mcgill.ca

Contributorship
LL was involved in study conception and design, protocol development, study implementation, data collection, data analysis, interpretation of data and drafting of the manuscript.

SD, JB & CB were involved in study conception and design, protocol development, study implementation, and critical review of manuscript.

MZ, PC & KG were involved in study implementation, data collection, data analysis and review of manuscript.

Sources of support
This study was funded as an investigator-initiated grant by GlaxoSmithKline Canada Ltd. LL was funded by a Canadian Institute of Health Research Doctoral Award. Infrastructure for this project was supported by the Canada Foundation for Innovation (CFI) -Leaders Opportunities Fund (CJB). CJB was supported by a salary award from the Fonds de Recherche du Quebec-Sante (FRQ-S).

Running title: CC-16 and RelB determine arterial stiffness in COPD
Descriptor: 9.7

Word count: 2798
At a Glance Commentary:

Scientific Knowledge on the Subject: Patients with chronic obstructive pulmonary disease (COPD) have elevated cardiovascular risk, particularly during and subsequent to exacerbations. Little is known on the utility of lung-specific inflammatory mediators, and a novel potential biomarker of cardiovascular function in COPD (RelB), in mediating adverse cardiovascular outcomes.

What This Study Adds to the Field: Changes in the expression of club cell-16, a lung specific inflammatory mediator, and RelB, a potential biomarker of cardiovascular function, during and subsequent to COPD exacerbations that require hospital admission can determine changes in arterial stiffness over time. Club cell-16 can predict exacerbation frequency over time, and may represent an important biomarker of pulmonary and cardiovascular function in severe COPD patients.

ABSTRACT

Rationale: Cardiovascular disease is a leading cause of mortality amongst chronic obstructive pulmonary disease (COPD) patients. The utility of lung-specific inflammatory mediators such as club cell protein-16 and surfactant protein D in mediating adverse cardiovascular outcomes is not known; nor is that of RelB, a ubiquitous NF-κB protein that suppresses pulmonary inflammation, and novel marker of cardiovascular outcomes during COPD exacerbations.

Objective: To measure systemic club cell protein-16, surfactant protein D and RelB during and subsequent to COPD exacerbations in association with changes in carotid-femoral pulse wave velocity.

Methods: 38 exacerbating COPD subjects admitted to hospital were included. Clinical, physiological and arterial stiffness measurements were taken within 72 hours of admission, every 3 days until discharge, and then once a week until 30 days since first assessment, and at days 90 and 180. Plasma concentrations of inflammatory mediators were measured from venous blood taken at admission and days 15, 30, 90 and 180.

Results: Club cell protein-16 and RelB concentrations were increased at day 15 of exacerbation; surfactant protein D was decreased. The course of change in club cell protein-16 and RelB were inversely associated with that of carotid-femoral pulse wave velocity over time. Increased levels of club cell protein-16 were predictive of fewer exacerbations during follow-up.

Conclusions: Lung-specific (club cell protein-16) and potential novel (RelB) inflammatory biomarkers are associated with systemic cardiovascular changes over time. Club cell protein-16 can predict subsequent exacerbations in severe COPD patients, and may be an important biomarker of pulmonary and systemic stress in COPD.

Abstract word count: 250

Keywords: nuclear-factor-κB, arterial stiffness, chronic obstructive pulmonary disease, exacerbation

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, not fully reversible airflow limitation and chronic inflammation [4]. Its course is perturbed by acute events of worsened respiratory symptoms known as exacerbations that can lead to significant morbidity and mortality. Cardiovascular disease (CVD) is the 2nd most frequent cause of death in COPD [14, 15] and exacerbations are associated with increased risk of acute CV events [23, 31]. There is growing interest in identifying lung-related biomarkers in COPD [122, 366, 367], and given the extra-pulmonary consequences of this disease, there is interest in identifying biomarkers predictive of comorbidity-related health outcomes.

Pulmonary inflammatory mediators of interest in COPD include club cell protein (CC)-16 and surfactant protein D (SPD) [122, 123, 368-371]. CC-16 is thought to play a role in mediating airway inflammation [372, 373]. The ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study showed that serum CC-16 was stable over time and positively associated with lung function over 3 years [132], a finding corroborated by Park *et al.* [368] using the Lung Health Study. SPD is thought to play a role in innate immunity and lung surfactant regulation [374, 375]. Circulating SPD is inversely associated with lung function decline in COPD in addition to predicting exacerbation risk [124, 125]. Despite their clinical promise, little is known about the course of change in circulating CC-16 and SPD with exacerbation in severe COPD subjects. Moreover, little is known about whether they can predict other relevant outcomes in COPD, particularly CVD.

Our group recently identified RelB as a potential biomarker of CV outcomes during COPD exacerbations [376]. RelB, an anti-inflammatory component of the NF-κB family, is increased by

pro-inflammatory stimuli, and suppresses cigarette smoke-induced inflammation [321-324, 337]. We showed that smoker- and COPD-derived cells have reduced RelB protein due to enhanced proteolytic degradation. We have also shown that during COPD exacerbations, increased peripheral RelB mRNA expression is inversely associated with and predictive of systolic blood pressure in COPD patients [376]. While experimentally there is growing evidence that RelB modulates aspects of CV function [343, 350, 351], there is no information on RelB in the context of CVD, making it of considerable interest to examine its role in this context in COPD.

We hypothesized that systemic alterations in CC-16, SPD and RelB are associated with changes in a reliable and non-invasive predictor of CV risk and mortality [258-260, 266], and measurement of arterial stiffness, carotid-femoral pulse wave velocity (cf-PWV), over time; as well as increased exacerbation frequency during follow-up. In recognizing a relationship between CV risk and COPD, studying lung-specific and novel inflammatory mediator expression that may be involved in modulating this relationship is clinically relevant and may represent attractive pharmaceutical targets to reduce CV morbidity and mortality in COPD.

METHODS

Subject selection

Subjects with a confirmed diagnosis of COPD and known history of CVD or CV risk factors were recruited upon admission to the Montreal Chest Institute for COPD exacerbation between August 2012 and August 2013. Exclusion criteria included: 1) acute medical conditions other than COPD exacerbation; or 2) unwillingness/inability to provide informed consent.

Study design, clinical definitions and measurements

The details of our study design, clinical definitions and measurements are as previously described (Labonté *et al.,* see chapter 6). Briefly, subjects were assessed within 48 ± 24 hours of hospital admission and then every 72 ± 24 hour until discharged. Subjects were then assessed once a week up to 30 days since initial assessment, and then at days 90 and 180 since initial assessment. Ethics approval was obtained from the McGill University Faculty of Medicine Institutional Review Board (A04-M20-12B). All subjects provided written informed consent prior to assessment.

Arterial stiffness measurements

Arterial stiffness as measured using cf-PWV was measured in duplicate at rest using the SphygmoCor system (AtCor Medical, Sydney, Australia). Prior to measurements, subjects rested for at least 10 minutes in a supine position and refrained from speaking. Cf-PWV was determined using arterial waveforms measured using a hand-held tonometer (SPC-301; Millar Instruments, Houston, TX, USA) applied to the surface of the skin overlying the carotid and femoral arteries that were gated using a 3-lead electrocardiogram. By measuring the distance between the two recording sites (carotid and femoral arteries), PWV was calculated [PWV = distance (m)/transit time (s)].

Inflammatory mediator analysis

Peripheral venous blood samples were collected from subjects at the first assessment time point, and then at days 15, 30, 90 and 180. Samples were immediately centrifuged for plasma isolation, aliquoted and stored at -80 °C until biomarker analysis. Concentrations of circulating CC-16 and SPD (BioVendor Laboratory Medicine, Modrice, Czech Republic) and RelB (MyBioSource Inc.,

San Diego, CA) were measured in triplicate using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions.

Analysis

Analyses were performed using SAS version 9.3 software (SAS Institute. Inc., Cary, North Carolina). To decrease inter-subject variation, values are presented as absolute changes from initial assessment; if initial values were not obtained, measurements collected at the next available time point were used to anchor absolute change calculations. For mean comparisons, two-tailed T-tests (normal distribution) or Wilcoxon signed-rank tests (non-normal distribution) were used; Chi-squared tests were used for dichotomous variables. A p-value of 0.05 or less was deemed statistically significant. Mixed-effects linear models were used to estimate the association between increases in the absolute change in circulating inflammatory mediator concentrations and cf-PWV over time. The model included random intercepts to capture individual-specific change in levels and a spatial power correlation structure [spl (pow)] to account for varied time intervals between repeated measurements, adjusted for age, sex, forced expiratory volume in 1 second (FEV_1) % predicted and days. The relative risk of exacerbations during follow up based on increases in the absolute change in inflammatory mediator concentrations over time were estimated using Poisson regression models.

Our sponsor GSK has not been involved in the data collection, analysis and editing with regards to the study and the publication.

RESULTS

Clinical characteristics of subjects at exacerbation

Table 8.1 shows the characteristics and clinical measurements of subjects during hospital admission for acute exacerbation. A total of 38 subjects were assessed with a mean age of approximately 72 (range 55-88) years, with slightly more than half of subjects being male. Subjects had severe airflow obstruction and an extensive smoking history.

Absolute changes in inflammatory mediator concentrations over time

Table 8.1 shows the mean concentrations of CC-16, SPD and RelB at initial assessment during hospitalization. The absolute course of change in the concentration of the 3 inflammatory mediators over time compared to those measured during the initial assessment is shown in figure 8.1. At day 15, concentrations of CC-16 (figure 8.1A) and RelB (figure 8.1C) were increased compared to initial levels, whereas that of SPD (figure 8.1B) was decreased. By day 30, both CC-16 and RelB concentrations were decreased compared to initial levels, whereas that of SPD was increased. The directions of the absolute change in inflammatory mediators varied at the day 90 and 180 time points; all values were different from those at initial assessment and are shown in figure 8.1.

As the course of change in CC-16 was similar to that of RelB during exacerbation, we assessed the relationship between these over time. A 1-unit (ng/mL) increase in RelB did not produce a statistically significant change in either CC-16 or SPD (β = -0.027, 95% confidence interval (CI) -0.143 to 0.088, p=0.64; and β= -5.967, 95% CI -13.617 to 1.683, p=0.125, respectively).

Associations between increased inflammatory mediator concentrations and cf-PWV over time

Figure 8.2 shows the relationships between the absolute course of change in inflammatory mediators and cf-PWV. We found statistically significant inverse associations between increases in the absolute course of change in CC-16 concentrations and the absolute course of change in cf-PWV over time, as well as between increases in the absolute course of change in RelB concentrations and the absolute course of change in cf-PWV over time. There was a trend for an inverse association between SPD and cf-PWV over time. Using multivariable analysis that included all 3 mediators, we found that increases in the absolute concentration of CC-16 and RelB resulted in approximately equal changes in cf-PWV (β = -0.320, 95% CI -0.569 to -0.071, p=0.013; and β= -0.363, 95% CI -0.514 to -0.212, $p<0.001$, respectively), while that of SPD remained non-significant ($p>0.05$).

Increased inflammatory mediator concentrations over time and risk of subsequent exacerbations

Table 8.2 shows the relative risks between increases in the absolute course of change in inflammatory mediator concentrations over time and subsequent exacerbations. Of the three mediators we evaluated, we found decreased relative risk for subsequent exacerbations with increases in CC-16 over time. There were no statistically significant relative risks for subsequent exacerbations with increases in SPD or RelB concentrations over time.

DISCUSSION

Finding biomarkers of patient-relevant outcomes is an emerging goal of COPD research, with lung-specific proteins being recognized as one the most useful in terms of identifying either disease-specific or disease activity-specific markers for COPD [368]. Despite the significant impact that CV comorbidity has on COPD, there have been no studies relating lung-specific inflammatory mediators to CV outcomes or CV risk in these patients. Moreover, the role of RelB, either in relation to the lung or to obstructive airway diseases such as COPD, is only just starting to become recognized [323, 324, 337, 376]. In this study, we show novel evidence that CC-16 may be a potential biomarker that links pulmonary inflammation to CV function in COPD. Additionally, we provide further evidence to support that RelB may mediate vascular outcomes in COPD.

RelB, a member of the NF-κB family, has been identified as an effective suppressor of cigarette smoke-induced inflammation [321-324, 337]. RelB is constitutively expressed in human lymphocytes and dendritic cells [346], suppresses cytokine production in lung epithelial cells [347] and is important for thymus development and T cell function [348, 349]. There is also growing evidence that RelB may be able to modulate endothelial function. Experimentally, RelB has been associated with balloon catheter injury in the rat carotid artery [350], and its expression can be modulated via treatment with DETA-NONOate-a nitric oxide donor [351]. Our group also showed that RelB is expressed in endothelial cells and such expression can suppress pulmonary ICAM-1 levels in response to smoke [343]. We were the first to show peripheral RelB expression in COPD subjects to provide novel evidence on its inversely relationship to systolic blood pressure at exacerbation [376]. In this current study, we showed that circulating RelB protein concentrations are inversely associated with cf-PWV over time. Taken together, it seems

plausible that RelB is an important modulator of endothelial and hence vascular function in COPD patients. As we found no association between the course of change in RelB and that of either CC-16 or SPD over time, it is likely that RelB does not modulate expression of these. This may suggest that different pathways are involved in mediating changes in cf-PWV that involve CC-16 and RelB, or it may suggest that both these mediators have a common upstream regulator. Further studies to better examine the mechanistic role of RelB in CVD and COPD could reveal a novel pathway for intervention.

To date, several pulmonary markers have been identified as potential biomarkers in COPD, notably CC-16 and SPD [123-125, 368, 377]. Of these, CC-16 has been emerged as a potential mediator of lung function in COPD, where reduced CC-16 levels are associated with accelerated decline in lung function over time as well as disease progression [123, 368]. CC-16 has also been described in other respiratory conditions, whereby decreased circulating levels are associated with obliterative bronchiolitis [378], asthma [379] and smoking [380]. The ECLIPSE data have shown that repeated measures of CC-16 are stable over time [125], and a recent randomized clinical trial showed that CC-16 levels can be modulated via treatment with salmeterol/fluticasone [381]. As a result, CC-16 represents an attractive and potentially modifiable biomarker of disease outcomes in COPD. In our study, we report for the first time a relationship between CC-16 and CV function in COPD patients, where increased circulating CC-16 is associated with decreased arterial stiffness over time. While our study did not allow us to examine the mechanisms responsible for this, we hypothesize that it may involve the ability of CC-16 to inhibit phospholipase A2 [382]. Phospholipase A2 can modify low-density lipoproteins, leading to increased uptake by macrophage, a feature in pre-atherosclerotic arterial wall that may lead to low-density lipoprotein modification, foam cell formation and inflammation to promote

atherogenesis [383]. It is also possible that CC-16 may act directly on the vascular endothelium or regulate other downstream effectors that lead to increased stiffening of the vessel walls.

We also found that increases in CC-16 could predict lower risk of subsequent exacerbations during follow-up, a finding not observed in the ECLIPSE study [123]. This discrepancy may be due to inherent differences between the populations studied. Our sample of subjects consisted of severely ill patients with advanced disease (GOLD stages 3-4) that have an elevated CV risk and likely reflect the frequent-exacerbator phenotype of COPD patients [117]. Similar to Patel *et al.* [31], we recently found that increased exacerbation frequency is associated with elevated cf-PWV (Labonté *et al*. see chapter 6). Thus, taken together, we hypothesize that decreased CC-16 may lead to increased exacerbation frequency, which in turn, could lead to increased arterial stiffness and increased CV susceptibility. This would present an interesting and highly clinically relevant mechanism, given that CC-16 expression can be modulated. While further study is needed to understand the role of CC-16 in COPD, our study provides interesting and novel data on CC-16 in COPD exacerbation and CV risk.

In addition to CC-16, SPD is another potential biomarker that has been positively associated with CV morbidity and mortality in ex and current smokers [384]. We were unable to detect a statistically significant association between SPD and cf-PWV over time. This may be a reflection of the relatively small sample size of our population or that changes in arterial stiffness occur independently of SPD. As such, continued assessment of the relationship between SPD and CVD may help elucidate the relevance of SPD to CVD in COPD. Although the ECLIPSE data previously showed that SPD could predict risk of exacerbation [124], our data did not show this. Despite this,

there remains strong evidence for a role for SPD as a biomarker in COPD [124, 125, 369, 370, 377, 385].

There are several strengths to our study, including that this project focused on a high-risk and clinically relevant COPD patient population. Frequent exacerbators are known to be at elevated CV risk [117], and as such, better understanding this association could improve health outcomes for these patients. Our measurement of inflammatory mediators over time provides new information on the course of their expression, thereby allowing better assessment of their relationship to patient-relevant outcomes, particularly exacerbation frequency and arterial stiffness. It is equally important to note the limitations of this study, including the relatively small sample size that may have resulted in insufficient power for our analyses due to high inter-individual variation. Second, we were not able to assess subjects before they received treatment with corticosteroids or systemic antibiotics. CC-16, SPD and RelB can be modulated by systemic corticosteroids [124, 363, 381], and as such, these treatments may have influenced the expression levels obtained in this study. Finally, while blood samples were collected at all of the same time point as our other clinical and physiological measurements, we were not able to measure inflammatory mediator concentrations for every time point due to the high cost of running assays. In the future, it would be useful to know the course of change in these during the first few days and even weeks of exacerbations, as this could provide important information on their course of change and relevance to exacerbation and allow for a more robust assessment. Finally, not having stable-state measurements on inflammatory mediators does not allow us to fully assess the significance of changes measured over time with exacerbation. Stable-state RelB protein measurements have not yet been reported in COPD patients, and so measuring these in future research is necessary.

Our study serves as an important step towards identifying biomarkers in frequent exacerbators that relate pulmonary inflammation to CV function, and CC-16 may represent such a marker. Moreover, with the potentially modifiable expression of CC-16 in COPD patients, further research should address whether modulation of CC-16 can lead to changes in arterial stiffness, which could ultimately decrease susceptibility to CV events. In this study we also show for the first time, systemic RelB expression in relation to arterial stiffness, which points to another potential pathway for the modulation of endothelial and vascular function in COPD. Mechanistic studies to elucidate the pathways linking CC-16 and RelB to these health outcomes would ultimately contribute to our understanding of their value as biomarkers of patient-relevant CV outcomes in COPD.

ACKNOWLEDGMENTS

We would like to thank all researchers, staff, in particular Katrina Metz and Meena Patel for their help with patient assessments, Pei Z. Li for her help with statistical analyses, and Angela Rico de Souza for her help with biomarker analysis, and to the COPD patients who participated in this study.

TABLES

Table 8.1: Characteristics and clinical measurements at initial assessment during exacerbation in subjects with COPD (n=38)

Characteristics	
Mean (± SD) age, years	71.7 (8.23)
Male (%)	55.3
Smoking status (%)	
Ex	81.6
Current	18.4
Mean (± SD) smoking pack-year history	55.6 (31.9)
Use of long-term oxygen therapy (%)	23.7
Mean number of exacerbations reported in previous year	2.55
Clinical measurements	
Mean (± SD) FEV1, % predicted	34.0 (14.9)
Mean (± SD) FEV1, (L)	0.777 (0.320)
Mean (± SD) FEV1/FVC	0.484 (0.142)
Mean (± SD) body mass index, kg/m2	25.7 (5.47)
Mean (± SD) carotid-femoral pulse wave velocity, m/s	11.6 (2.68)
Inflammatory mediator concentrations	
Mean (± SD) CC-16, ng/mL	7.29 (4.33)
Mean (± SD) SPD, ng/mL	234.69 (166.24)
Mean (± SD) RelB, ng/mL	1.87 (1.52)

SD: standard deviation, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, CC-16: club cell-16, SPD: surfactant protein D, RelB: V-rel avian reticuloendotheliosis viral oncogene homolog B.

Table 8.2: Relative risks of increasing inflammatory mediator concentrations over time and subsequent exacerbations

1 unit (ng/mL) increase in the absolute change in inflammatory mediators over time	Number of exacerbations during 6 months of F/U	
	RRs (95% CI)	P-value
CC-16	0.84 (0.75 -0.95)	0.004*
SPD	0.99 (0.99 - 1.01)	0.894
RelB	1.02 (0.93 -1.11)	0.717

Poisson regression models were used to estimate relative risks of exacerbations. RR adjusted for baseline age, sex, and FEV1 % predicted. *Denotes statistical significance of $p<0.05$. CC-16: club cell-16, SPD: surfactant protein-D, RelB: V-rel avian reticuloendotheliosis viral oncogene homolog B, CI: confidence interval, RR: relative risk.

FIGURE LEGENDS AND FIGURES

FIGURE LEGENDS

Figure 8.1. Mean absolute change in inflammatory mediators (ng/mL) (+/- standard error of the mean) over time. Panel A shows the course of change in CC-16 over time compared to CC-16 concentrations measured at initial assessment, panel B shows the course of change in SPD over time compared to SPD concentrations measured at initial assessment and panel C shows the course of change in RelB over time compared to RelB concentrations measured at initial assessment.

Figure 8.2. Relationship between the absolute course of change in inflammatory mediator concentrations (ng/mL) over time and that of cf-PWV (m/s). Panel A shows the relationship between CC-16 and cf-PWV over time, panel B shows the relationship between SPD and cf-PWV over time, and panel C shows the relationship between RelB and cf-PWV over time. Mixed-effects linear models adjusted for age, sex, forced expiratory volume in 1 second % predicted and days were used to estimate these relationships. β values show the resulting change in cf-PWV in m/s with a 1-unit (ng/mL) increase in an inflammatory mediator.

FIGURES

Figure 8.1

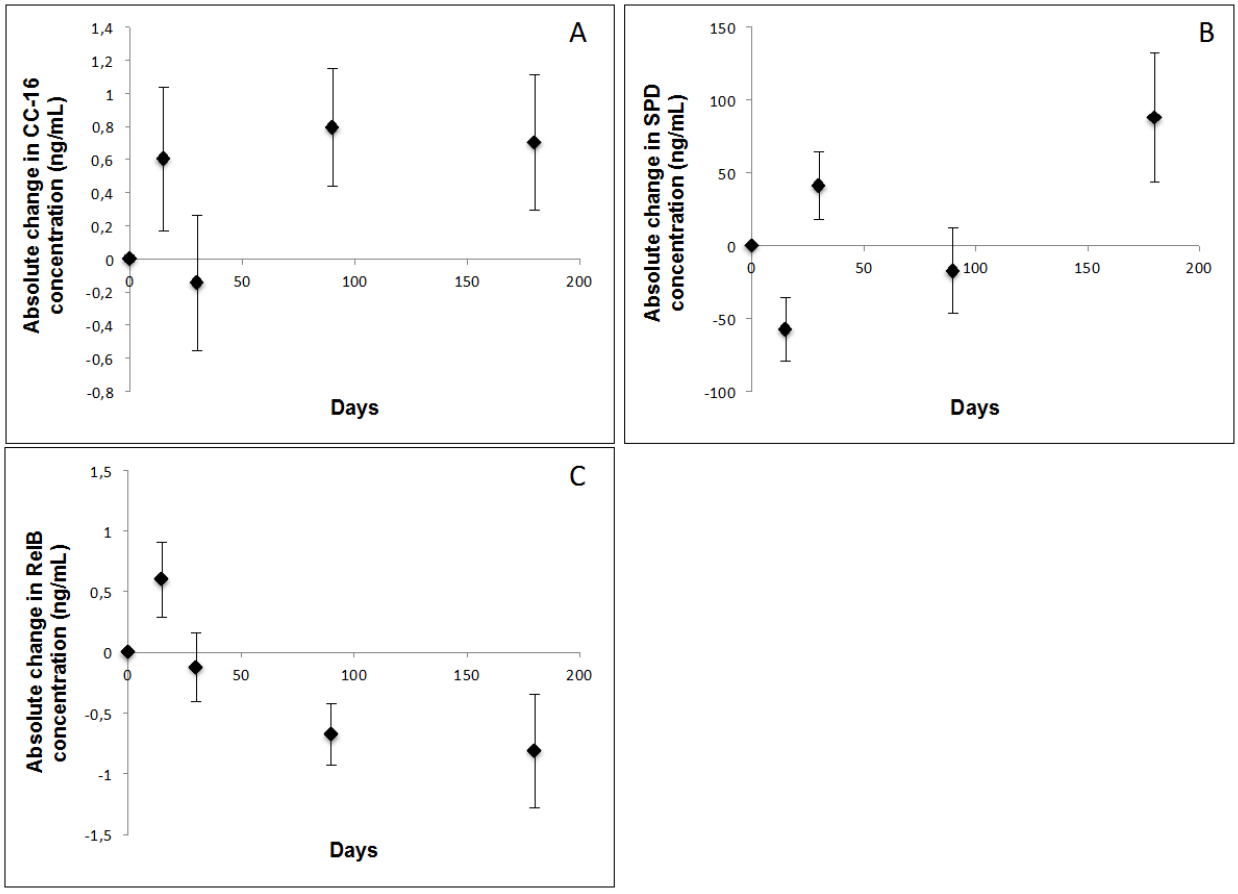
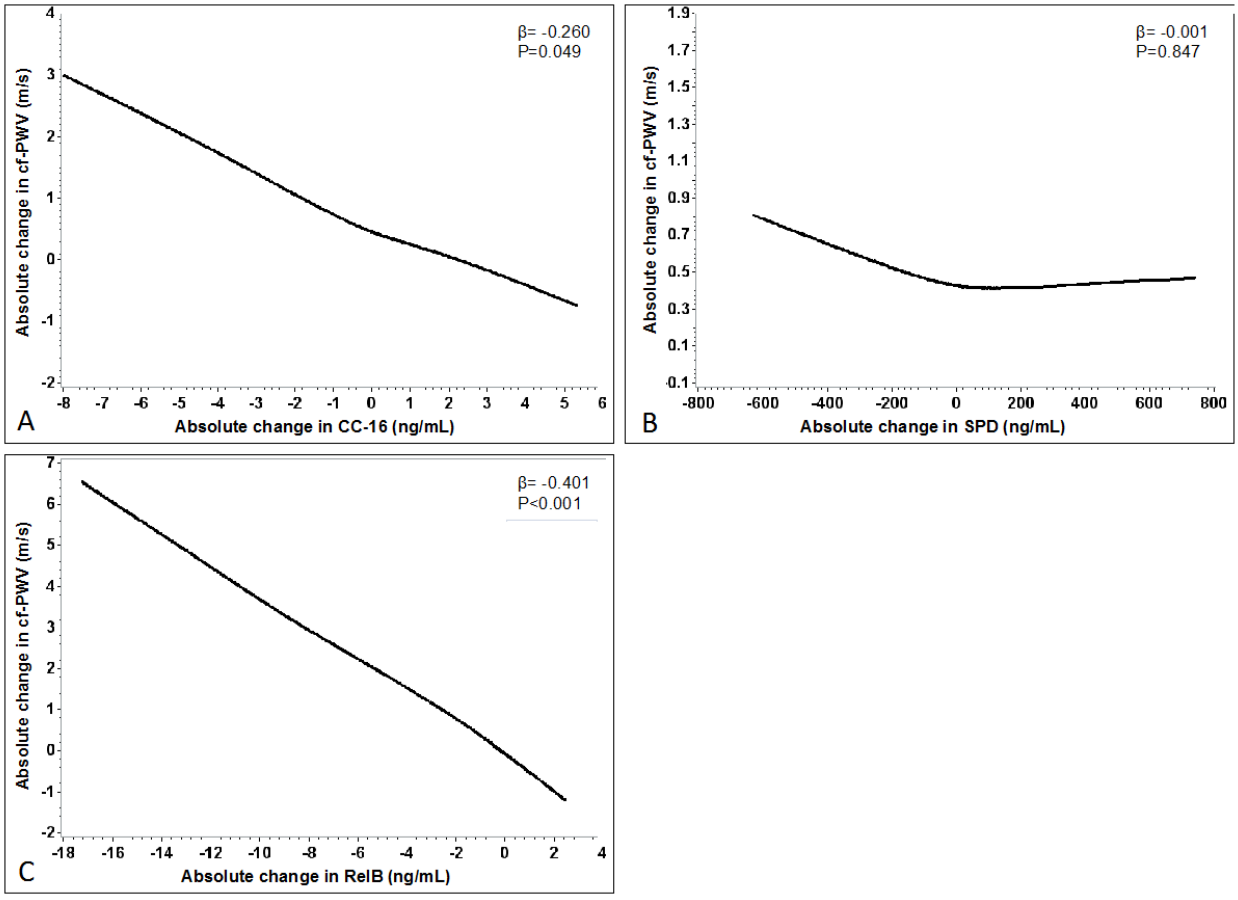


Figure 8.2


β= -0.260
P=0.049
Absolute change in cf-PWV (m/s)
Absolute change in CC-16 (ng/mL)
A
β= -0.001
P=0.847
Absolute change in cf-PWV (m/s)
Absolute change in SPD (ng/mL)
B
β= -0.401
P<0.001
Absolute change in cf-PWV (m/s)
Absolute change in RelB (ng/mL)
C

CHAPTER 9: SUMMARY OF FINDINGS, GENERAL DISCUSSION AND OVERALL CONCLUSIONS

9.1 Summary of findings

This thesis examined the range of impacts of COPD exacerbations, from populational burdens to changes in inflammatory mediator expression.

When considering the populational impacts assessed as part of this thesis, several key findings were reported. First, it was found that subjects sampled from the general population who never received a physician-diagnosis of COPD represent a significant proportion of individuals. These individuals have milder obstruction, are less symptomatic, and less functionally impaired than subjects who received a diagnosis, which may partly explain a lack of diagnosis. Remarkably however, despite experiencing fewer exacerbation-like events, these undiagnosed subjects use health services in a manner equivalent to that of subjects having received a previous physician diagnosis of COPD. This very important finding shows that subjects with undiagnosed COPD use health services much more than previously thought, and as a result, contribute seriously to the burden of exacerbations. If these patients were recognized as having COPD, then providing proper preventive therapies has the potential of reducing or preventing complications such as emergency room visits and hospital admissions, and thereby significantly impacting the overall burden of COPD.

Individuals who remain undiagnosed, will continue to experience recurrent exacerbations, and with no tools for proper disease and exacerbation management, will continue to experience progressive and not fully-reversible decline in lung function. They will also become susceptible to many comorbid conditions, the most predominant of which being CVD. Risk of CV morbidity

and mortality can be predicted using various methods, including the measurement of arterial stiffness, whereby increased stiffness as measure using cf-PWV, predicts increased CV risk. Identifying predictors of elevated arterial stiffness (such as inflammatory mediators, physiological or clinical factors, etc.) associated with COPD and its exacerbations could lead to the discovery of novel potentially modifiable targets that could mediate CV risk through proper intervention.

When considering the CV impacts assessed as part of this thesis, previous findings that there are significant changes in arterial stiffness at COPD exacerbation were validated and extended to the 6-month period following severe exacerbation that warranted hospital admission in subjects who frequently exacerbate and are at elevated CV risk. Several important predictors of increased arterial stiffness over time were identified including subsequent exacerbation, decreased lung function, LDL, cellular hemoglobin and increased circulating potassium. Clinical factors associated with increased odds of having the most elevated cf-PWV over time were also reported. These predictors can be assessed as part of routine clinical care, allowing for the identification of COPD patients who may be at risk of increased arterial stiffness. Moreover, they may represent potentially modifiable targets that could be used to regulate stiffness.

When considering the inflammatory impacts assessed as part of this thesis, several key findings were reported. RelB expression (both mRNA and protein) was assessed for the first time in COPD patients, and novel evidence was provided that RelB may be associated with vascular function in COPD patients. It was also found that CC-16, a lung-specific inflammatory mediator, may be a potential biomarker that links pulmonary inflammation to CV function in COPD. Both RelB and CC-16 are modifiable inflammatory targets, and as such they represent therapeutically-

relevant potential biomarkers of disease outcomes in COPD.

9.2 General discussion

9.2.1 General strengths and limitations

The two projects constituting this thesis had several important strengths and limitations. The greatest strength of the CanCOLD study includes that it is a population-based cohort, meaning that it reflects events that are occurring in the population at large that have significant impacts on the health and wellbeing of people, as well as on the healthcare system. A unique and novel aspect of the CanCOLD study is that it provides information on subjects from all COPD severity stages, including those with mild disease, and who are at-risk of developing COPD. These two groups tend to be under-represented in cohort studies, but represent important populations, as they include individuals who are most likely to benefit from early intervention. Moreover this cohort consists of men and women in almost equal proportion, something that has been a challenge in many other studies. With regards to the exacerbation analysis, the evaluation of both symptom-based and event-based exacerbation-like respiratory events allowed for the inclusion of milder events that may not require medication or health service utilization, but that may still impact the quality of life of patients [56], as well as more severe events that do require treatment. This is an important strength, as often, milder events are not considered in clinical and cohort studies. Furthermore having a follow-up of at least 12 months on these subjects provides stronger more robust data for analyses.

A limitation of the CanCOLD study is that this COPD-based cohort may include asthmatics. However, subjects included in our analysis did have a component of fixed airflow obstruction when performing spirometry at study entry, and were thus defined as having COPD.
To further address this, there is currently a substudy being undertaken to identify subjects with asthma or asthma-COPD overlap syndrome within the cohort. Once data has been analyzed for the entire cohort, it will be possible to assess the contribution of these subjects to the reported analysis on exacerbations. Although, there is still no definitive way of distinguishing between COPD and asthma with post-bronchodilator fixed airflow obstruction, and even in the absence of smoking, it remains difficult to differentiate between these two conditions, especially since there is evidence that COPD in never-smokers is much more prevalent than previously thought [305]. Other acute events that may manifest with exacerbation-like symptoms e.g. coronary events could have been included in the exacerbation analysis. However, it is very difficult to differentiate between these events when relying on patient report. Moreover, this limitation is inherent when using symptom-based definitions of exacerbations and is common to all work on COPD exacerbations. Finally, although the exacerbation questionnaire is administered every three months to ensure sufficient subject-recall, there are inaccuracies when relying on any symptom-based definition of exacerbations.

The most important strength of the local study entitled *"COPD exacerbations: understanding the course of change and recovery in extra-pulmonary outcomes"* is the inclusion of a particularly high-risk COPD population consisting of patients with a history of CV comorbidity and who are susceptible to frequent exacerbations. Patients with severe COPD exacerbations are particularly difficult to recruit, as they are very ill and usually refuse to participate in research studies. Moreover, being able to assess patients within the first 72 hours of hospitalization is very challenging, but

allowed for the detection of important physiological and clinical changes occurring systemically and locally within the lung due to exacerbation. Assessing these patients every three days during hospitalization, and then once a week up to one month, allowed for the collection of data showing changes in clinical outcomes and relationships between these through time, especially for several outcomes, which had not been previously measured longitudinally (e.g. lung-specific inflammatory mediators, RelB, cf-PWV). The three- and sixth-month measurement time points also provide additional longitudinal data on the changes detected at exacerbation.

A limitation to this study is that the sample size was relatively small and as a result, analyses may be underpowered. Additionally, it was not possible to assess patients upon hospital admission for exacerbation before receiving corticosteroid and antibiotic treatment. These likely mediated some of the systemic and CV effects that were measured. However, with regards to arterial stiffness, since the overall trend of change in cf-PWV observed during exacerbation mirrored that described by Patel *et al*. [31], who were able to evaluate patients before receiving antibiotic and corticosteroids, it is likely that their potential effects at least on cf-PWV, are negligible. Moreover, having stable-state measurements prior to exacerbation would have allowed for a better understanding of the severity of changes observed at and subsequent to exacerbation. Finally, despite best efforts and with study adjudication of exacerbations during study follow-up, the exact dates of all subsequent exacerbations during follow-up and whether subjects experienced other acute events may not be exact, and as a result, this could have influenced measurements taken at days 90- and 180.

9.2.2 Clinical relevance and future work

The results of the manuscripts within this thesis highlight the serious and wide ranging impacts of COPD exacerbations.

When considering the populational impacts, the finding that subjects with undiagnosed COPD contribute much more than previously thought to the burden of health service utilization highlights the urgent need of better diagnosis and management of COPD. Recognizing individuals who present with symptoms associated with acute exacerbation would allow for the prompt treatment of these patients, and could result in earlier diagnosis. Moreover, this would prevent further exacerbation, and undoubtedly reduce the overall burden of COPD on individuals and the healthcare system. This is analogous to identifying individuals who present with MI; these patients experience an acute event but are recognized as having CAD/CVD. The importance of making a diagnosis upon presentation is not only to treat the acute episode, but rather, to treat the underlying CVD and increase the likelihood of preventing future acute CV events, morbidity and mortality.

It is known that the best way of preventing exacerbations is through early intervention. This can be accomplished through greater public awareness about COPD, as well as by educating healthcare providers about accurate diagnosis, proper management of this disease and preventive therapies for its complications. Successfully targeting at risk individuals could result in decreased morbidity, mortality, better quality of life for patients, as well as significantly fewer healthcare expenditures related to COPD. Further research will provide new information that may help develop a phenotype for identifying early and/or milder disease susceptibility to exacerbations. This would be highly useful in at-risk subjects and those in the early stages of disease, who may

benefit most from preventive interventions. In CanCOLD, continued follow-up and analysis of exacerbation-like respiratory events will help in collecting such information. Furthermore, it will be important to conduct an economic cost analysis, to be able to compare the costs associated with treating exacerbation-like events in diagnosed and undiagnosed subjects and understand the associated economic burden.

With regards to the CV impacts of exacerbations, novel evidence on the predictors of increased arterial stiffness, as measured using cf-PWV is important. The clinical variables identified as being predictors in this thesis are routinely measured in COPD subjects whether in stable state or at exacerbation. As such, they may provide information that allow for the identification of patients at increased risk of elevated arterial stiffness overtime, and as a result, who are likely susceptible to increased CV risk as well. These patients could then be targeted for preventive measures or medications aimed at mediating CV risk. Future studies with a similar design should be carried out, with more frequent and longer longitudinal follow-up. This would help pinpoint and validate determinants of elevated arterial stiffness, and thus allow for the development of novel treatments that may target them.

With regards to the inflammatory impacts of exacerbations several important clinically relevant findings were reported. The data presented on RelB is highly novel, and is the first measurement of RelB protein expression and function in COPD patients. Moreover, the results of this study suggest for the first time that circulating RelB expression may be an important marker of CV function in COPD patients. Future longitudinal and mechanistic studies will undoubtedly help elucidate the functional significance of RelB in COPD pathogenesis and its potential for therapeutic modulation.

Prior to this study, lung-specific inflammatory mediators had not been measured sequentially during exacerbation, and as such, the data presented on CC-16 and SPD are quite novel. Moreover, given the elevated CV risk in frequent exacerbators, identifying biomarkers in these subjects that relate pulmonary inflammation to CV function is very clinically important. The fact that CC-16 may represent such a marker is promising. Further research should address whether modulation of CC-16 can lead to changes in arterial stiffness and susceptibility to CV events. Moreover it is important to measure CC-16 in a larger sample of subjects to validate its association with arterial stiffness, as well as assessing whether it may be related to other CV outcomes in COPD patients.

9.3 Overall conclusions

This thesis describes the scope of impacts of COPD exacerbations, across different populations and at different levels. There is significant interplay between these, which can best be appreciated and studied using translational research. As the course of COPD is aggravated by comorbidities manifesting in various systems from the lung to those that are extra-pulmonary, one may opt to take an "integrated body" approach to studying COPD. In doing so, it is possible to fully examine the amplified effects that these have on disease as well as their common mechanisms such as inflammation; and examine relationships starting at the molecular and cellular level, to the physiology and then progressing to the community and populational level. This, thus, may bridge important knowledge gaps in the field of COPD.

It is clear that the under-diagnosis of COPD has serious implications with regards to its healthcare burden. Untreated and unmanaged disease causes individuals to utilize many more health services than previously assumed. As subjects will continue to experience untreated

exacerbations, their COPD will progress, and subjects will become increasingly susceptible to comorbid conditions. CV comorbidity is tightly linked to COPD exacerbations. It seems that there are several potentially modifiable predictors of adverse CV outcomes that could be explored for therapeutic modulation, which could in the long run also susceptibility to CV morbidity. With regards to outcome-specific biomarkers, CC-16 may represent an important biomarker linking pulmonary function to CV function in COPD, while RelB, may be a novel biomarker of vascular function in COPD. These measurements may also help provide the therapeutic means to mediate arterial stiffness and other CV outcomes, which could have a significant impact on the morbidity and mortality of COPD patients.

Thus it is important to consider the translation of these findings. By implementing spirometry in at-risk subjects early in life, and identifying individuals susceptible to exacerbations, it may be possible to slow the progression of this disease while controlling for comorbidities and limiting the systemic consequences of COPD. The true future challenge with regards to COPD exacerbations remains promoting public health initiatives aimed at early recognition and diagnosis of this disease. Only through proper disease management will true beneficial changes for patients and the healthcare system come about.

BIBLIOGRAPHY

1. Fontanarosa, P.B. and C.D. DeAngelis, *Basic science and translational research: call for papers.* JAMA, 2001. **285**: p. 2246.
2. Drolet, B.C. and N.M. Lorenzi, *Translational research: understanding the continuum from bench to bedside.* Transl Res, 2011. **157**(1): p. 1-5.
3. Wolf, S., *Editorial: The real gap between bench and bedside.* N Engl J Med, 1974. **290**(14): p. 802-3.
4. GOLD, *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease*. 2015.
5. Lopez, A.D., et al., *Chronic obstructive pulmonary disease: current burden and future projections.* Eur Respir J, 2006. **27**(2): p. 397-412.
6. 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.
7. O'Donnell, D.E., et al., *Canadian Thoracic Society recommendations for management of chronic obstructive pulmonary disease--2003.* Can Respir J, 2003. **10 Suppl A**: p. 11A-65A.
8. Tan, W.C., et al., *Exacerbation-like respiratory symptoms in individuals without chronic obstructive pulmonary disease: results from a population-based study.* Thorax, 2014.
9. Tan, W.C., et al., *Can age and sex explain the variation in COPD rates across large urban cities? A population study in Canada.* Int J Tuberc Lung Dis, 2011. **15**(12): p. 1691-8.
10. Sullivan, S.D., S.D. Ramsey, and T.A. Lee, *The economic burden of COPD.* Chest, 2000. **117**(2 Suppl): p. 5S-9S.
11. Seemungal, T.A., et al., *Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 1998. **157**(5 Pt 1): p. 1418-22.
12. Donaldson, G.C., et al., *Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease.* Thorax, 2002. **57**(10): p. 847-52.
13. Soler-Cataluna, J.J., et al., *Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease.* Thorax, 2005. **60**(11): p. 925-31.
14. Zielinski, J., et al., *Causes of death in patients with COPD and chronic respiratory failure.* Monaldi Arch Chest Dis, 1997. **52**(1): p. 43-7.
15. Calverley, P.M., et al., *Cardiovascular events in patients with COPD: TORCH study results.* Thorax, 2010. **65**(8): p. 719-25.
16. Sin, D.D. and S.F. Man, *Chronic obstructive pulmonary disease as a risk factor for cardiovascular morbidity and mortality.* Proc Am Thorac Soc, 2005. **2**(1): p. 8-11.
17. Libby, P., et al., *Inflammation in atherosclerosis: transition from theory to practice.* Circ J, 2010. **74**(2): p. 213-20.
18. van Eeden, S.F. and D.D. Sin, *Chronic obstructive pulmonary disease: a chronic systemic inflammatory disease.* Respiration, 2008. **75**(2): p. 224-38.
19. Stockley, R.A., *Progression of chronic obstructive pulmonary disease: impact of inflammation, comorbidities and therapeutic intervention.* Curr Med Res Opin, 2009. **25**(5): p. 1235-45.

20. Sin, D.D. and S.F. Man, *Chronic obstructive pulmonary disease: a novel risk factor for cardiovascular disease.* Can J Physiol Pharmacol, 2005. **83**(1): p. 8-13.
21. Man, S.F., S. Van Eeden, and D.D. Sin, *Vascular risk in chronic obstructive pulmonary disease: role of inflammation and other mediators.* Can J Cardiol, 2012. **28**(6): p. 653-61.
22. Halpin, D.M., et al., *Risk of nonlower respiratory serious adverse events following COPD exacerbations in the 4-year UPLIFT(R) trial.* Lung, 2011. **189**(4): p. 261-8.
23. Donaldson, G.C., et al., *Increased risk of myocardial infarction and stroke following exacerbation of COPD.* Chest, 2010. **137**(5): p. 1091-7.
24. Vivodtzev, I., et al., *Arterial stiffness by pulse wave velocity in COPD: reliability and reproducibility.* Eur Respir J, 2013. **42**(4): p. 1140-2.
25. Stone, I.S., et al., *Reproducibility of arterial stiffness and wave reflections in chronic obstructive pulmonary disease: the contribution of lung hyperinflation and a comparison of techniques.* Respir Med, 2013. **107**(11): p. 1700-8.
26. Sabit, R., et al., *Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2007. **175**(12): p. 1259-65.
27. Mills, N.L., et al., *Increased arterial stiffness in patients with chronic obstructive pulmonary disease: a mechanism for increased cardiovascular risk.* Thorax, 2008. **63**(4): p. 306-11.
28. McAllister, D.A., et al., *Arterial stiffness is independently associated with emphysema severity in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2007. **176**(12): p. 1208-14.
29. Maclay, J.D., et al., *Systemic elastin degradation in chronic obstructive pulmonary disease.* Thorax, 2012. **67**(7): p. 606-12.
30. Maclay, J.D., et al., *Vascular dysfunction in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2009. **180**(6): p. 513-20.
31. Patel, A.R., et al., *Cardiovascular risk, myocardial injury, and exacerbations of chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2013. **188**(9): p. 1091-9.
32. Bhatt, S.P., et al., *Determinants of arterial stiffness in COPD.* BMC Pulm Med, 2014. **14**: p. 1.
33. 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.
34. Mathers, C.D. and D. Loncar, *Projections of global mortality and burden of disease from 2002 to 2030.* PLoS Med, 2006. **3**(11): p. e442.
35. Buist, A.S., et al., *International variation in the prevalence of COPD (The BOLD Study): a population-based prevalence study.* The Lancet, 2007. **370**(9589): p. 741-750.
36. Halbert, R.J., et al., *Interpreting COPD prevalence estimates: what is the true burden of disease?* Chest, 2003. **123**(5): p. 1684-92.
37. Halbert, R.J., et al., *Global burden of COPD: systematic review and meta-analysis.* Eur Respir J, 2006. **28**(3): p. 523-32.
38. Chapman, K.R., et al., *Epidemiology and costs of chronic obstructive pulmonary disease.* Eur Respir J, 2006. **27**(1): p. 188-207.
39. Buist, A.S., et al., *The Burden of Obstructive Lung Disease Initiative (BOLD): Rationale and Design.* COPD: Journal of Chronic Obstructive Pulmonary Disease, 2005. **2**(2): p. 277-283.
40. Menezes, A.M., et al., *Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study.* Lancet, 2005. **366**(9500): p. 1875-81.

41. Schirnhofer, L., et al., *COPD prevalence in Salzburg, Austria: results from the Burden of Obstructive Lung Disease (BOLD) Study.* Chest, 2007. **131**(1): p. 29-36.
42. Pena, V.S., et al., *Geographic variations in prevalence and underdiagnosis of COPD: results of the IBERPOC multicentre epidemiological study.* Chest, 2000. **118**(4): p. 981-9.
43. Talamo, C., et al., *Diagnostic labeling of COPD in five Latin American cities.* Chest, 2007. **131**(1): p. 60-7.
44. Jensen, H.H., et al., *Potential misclassification of causes of death from COPD.* Eur Respir J, 2006. **28**(4): p. 781-5.
45. McGarvey, L.P., et al., *Cause-specific mortality adjudication in the UPLIFT(R) COPD trial: findings and recommendations.* Respir Med, 2012. **106**(4): p. 515-21.
46. Pauwels, R.A., et al., *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary.* Am J Respir Crit Care Med, 2001. **163**(5): p. 1256-76.
47. *Health Indicators 2008*. 2008; Available from: http://secure.cihi.ca/cihiweb/products/Healthindicators2008_Engweb.pdf.
48. Mapel, D.W., et al., *A new method for examining the cost savings of reducing COPD exacerbations.* Pharmacoeconomics, 2010. **28**(9): p. 733-49.
49. Mittmann, N., et al., *The cost of moderate and severe COPD exacerbations to the Canadian healthcare system.* Respir Med, 2008. **102**(3): p. 413-21.
50. Maleki-Yazdi, M.R., et al., *The burden of illness in patients with moderate to severe chronic obstructive pulmonary disease in Canada.* Can Respir J, 2012. **19**(5): p. 319-24.
51. National Heart, L., and Blood Institute, *Morbidity and mortality chartbook on cardiovascular, lung and blood diseases* 2009, US Department of Health and Human Services, Public Health Service, National Institutes of Health: Bethesda, Maryland.
52. European Respiratory, S., *European Lung White Book*. 2003, European Respiratory Society Journals, Ltd
53. Burge, S. and J.A. Wedzicha, *COPD exacerbations: definitions and classifications.* Eur Respir J Suppl, 2003. **41**: p. 46s-53s.
54. Quint, J.K., et al., *Predictive accuracy of patient-reported exacerbation frequency in COPD.* Eur Respir J, 2011. **37**(3): p. 501-7.
55. Barnes, N., et al., *Chronic obstructive pulmonary disease and exacerbations: patient insights from the global Hidden Depths of COPD survey.* BMC Pulm Med, 2013. **13**: p. 54.
56. Langsetmo, L., et al., *Underreporting exacerbation of chronic obstructive pulmonary disease in a longitudinal cohort.* Am J Respir Crit Care Med, 2008. **177**(4): p. 396-401.
57. Seemungal, T.A., J.R. Hurst, and J.A. Wedzicha, *Exacerbation rate, health status and mortality in COPD--a review of potential interventions.* Int J Chron Obstruct Pulmon Dis, 2009. **4**: p. 203-23.
58. Haughney, J., et al., *Exacerbations of COPD: quantifying the patient's perspective using discrete choice modelling.* Eur Respir J, 2005. **26**(4): p. 623-9.
59. Gross, N.J., *Chronic obstructive pulmonary disease outcome measurements: what's important? What's useful?* Proc Am Thorac Soc, 2005. **2**(4): p. 267-71; discussion 290-1.
60. Anthonisen, N.R., et al., *Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease.* Ann Intern Med, 1987. **106**(2): p. 196-204.
61. Hurst, J.R. and J.A. Wedzicha, *The biology of a chronic obstructive pulmonary disease exacerbation.* Clin Chest Med, 2007. **28**(3): p. 525-36, v.

62. Bolger, N., A. Davis, and E. Rafaeli, *Diary methods: capturing life as it is lived.* Annu Rev Psychol, 2003. **54**: p. 579-616.
63. Donaldson, G.C., et al., *Exacerbations and time spent outdoors in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2005. **171**(5): p. 446-52.
64. Seemungal, T.A., et al., *Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2000. **161**(5): p. 1608-13.
65. Aaron, S.D., *Measuring and quantifying acute exacerbations of COPD: pitfalls and practicalities.* Eur Respir J, 2014. **43**(3): p. 662-4.
66. Leidy, N.K., et al., *Development of the EXAcerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT): a patient-reported outcome (PRO) measure.* Value Health, 2010. **13**(8): p. 965-75.
67. Mackay, A.J., et al., *Detection and severity grading of COPD exacerbations using the exacerbations of chronic pulmonary disease tool (EXACT).* Eur Respir J, 2014. **43**(3): p. 735-44.
68. Celli, B.R. and J. Vestbo, *The EXACT-Pro: measuring exacerbations of COPD.* Am J Respir Crit Care Med, 2011. **183**(3): p. 287-8.
69. Bischoff, E.W., et al., *Validity of an automated telephonic system to assess COPD exacerbation rates.* Eur Respir J, 2012. **39**(5): p. 1090-6.
70. Kocks, J.W., et al., *Health status measurement in COPD: the minimal clinically important difference of the clinical COPD questionnaire.* Respir Res, 2006. **7**: p. 62.
71. van der Molen, T., et al., *Development, validity and responsiveness of the Clinical COPD Questionnaire.* Health Qual Life Outcomes, 2003. **1**: p. 13.
72. Trappenburg, J.C., et al., *Detecting exacerbations using the Clinical COPD Questionnaire.* Health Qual Life Outcomes, 2010. **8**: p. 102.
73. Wedzicha, J.A., R. Singh, and A.J. Mackay, *Acute COPD exacerbations.* Clin Chest Med, 2014. **35**(1): p. 157-63.
74. GOLD, *Global Strategy for the Diagnosis, Management and Prevention of COPD: Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2014.* 2014.
75. Wedzicha, J.A., et al., *Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels.* Thromb Haemost, 2000. **84**(2): p. 210-5.
76. Pauwels, R., et al., *COPD exacerbations: the importance of a standard definition.* Respiratory Medicine, 2004. **98**(2): p. 99-107.
77. Rodriguez-Roisin, R., *Toward a consensus definition for COPD exacerbations.* Chest, 2000. **117**(5 Suppl 2): p. 398S-401S.
78. Trappenburg, J.C., et al., *The impact of using different symptom-based exacerbation algorithms in patients with COPD.* Eur Respir J, 2011. **37**(5): p. 1260-8.
79. Wedzicha, J.A. and T.A. Seemungal, *COPD exacerbations: defining their cause and prevention.* Lancet, 2007. **370**(9589): p. 786-96.
80. Wedzicha, J.A. and G.C. Donaldson, *Exacerbations of chronic obstructive pulmonary disease.* Respir Care, 2003. **48**(12): p. 1204-13; discussion 1213-5.
81. Celli, B.R. and P.J. Barnes, *Exacerbations of chronic obstructive pulmonary disease.* Eur Respir J, 2007. **29**(6): p. 1224-38.
82. Jones, P. and T. Higenbottam, *Quantifying of severity of exacerbations in chronic obstructive pulmonary disease: adaptations to the definition to allow quantification.* Proc Am Thorac Soc, 2007. **4**(8): p. 597-601.

83. Bourbeau, J., et al., *Impact on patients' health status following early identification of a COPD exacerbation.* Eur Respir J, 2007. **30**(5): p. 907-13.
84. Xu, W., et al., *Negative impacts of unreported COPD exacerbations on health-related quality of life at 1 year.* Eur Respir J, 2010. **35**(5): p. 1022-30.
85. Hurst, J.R., et al., *Susceptibility to exacerbation in chronic obstructive pulmonary disease.* N Engl J Med, 2010. **363**(12): p. 1128-1138.
86. Zvezdin, B., et al., *A postmortem analysis of major causes of early death in patients hospitalized with COPD exacerbation.* Chest, 2009. **136**(2): p. 376-80.
87. Hurst, J.R., et al., *Epidemiological relationships between the common cold and exacerbation frequency in COPD.* Eur Respir J, 2005. **26**(5): p. 846-52.
88. Seemungal, T.A., et al., *Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease.* Eur Respir J, 2000. **16**(4): p. 677-83.
89. Mallia, P., et al., *An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: a pilot study.* Respir Res, 2006. **7**: p. 116.
90. Falsey, A.R., et al., *Detection of respiratory syncytial virus in adults with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2006. **173**(6): p. 639-43.
91. Wilkinson, T.M., et al., *Respiratory syncytial virus, airway inflammation, and FEV1 decline in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2006. **173**(8): p. 871-6.
92. Retamales, I., et al., *Amplification of inflammation in emphysema and its association with latent adenoviral infection.* Am J Respir Crit Care Med, 2001. **164**(3): p. 469-73.
93. Sapey, E. and R.A. Stockley, *COPD exacerbations . 2: aetiology.* Thorax, 2006. **61**(3): p. 250-8.
94. Stockley, R.A., et al., *Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD.* Chest, 2000. **117**(6): p. 1638-45.
95. Sethi, S. and T.F. Murphy, *Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review.* Clin Microbiol Rev, 2001. **14**(2): p. 336-63.
96. Patel, I.S., et al., *Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations.* Thorax, 2002. **57**(9): p. 759-64.
97. Wilkinson, T.M.A., et al., *Effect of interactions between lower airway bacterial and rhinoviral infection in exacerbations of COPD.* Chest, 2006. **129**(2): p. 317-324.
98. Papi, A., et al., *Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations.* Am J Respir Crit Care Med, 2006. **173**(10): p. 1114-21.
99. Hurst, J.R., et al., *Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2006. **173**(1): p. 71-8.
100. Anderson, H.R., et al., *Health effects of an air pollution episode in London, December 1991.* Thorax, 1995. **50**(11): p. 1188-93.
101. Gouveia, N., et al., *[Respiratory and cardiovascular hospitalizations associated with air pollution in the city of Sao Paulo, Brazil].* Cad Saude Publica, 2006. **22**(12): p. 2669-77.
102. Ko, F.W., et al., *Temporal relationship between air pollutants and hospital admissions for chronic obstructive pulmonary disease in Hong Kong.* Thorax, 2007. **62**(9): p. 780-5.
103. Nordenhall, C., et al., *Airway inflammation following exposure to diesel exhaust: a study of time kinetics using induced sputum.* Eur Respir J, 2000. **15**(6): p. 1046-51.
104. Rudell, B., et al., *Bronchoalveolar inflammation after exposure to diesel exhaust: comparison between unfiltered and particle trap filtered exhaust.* Occup Environ Med, 1999. **56**(8): p. 527-34.

105. Finlay, G.A., et al., *Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients.* Thorax, 1997. **52**(6): p. 502-6.
106. Saetta, M., et al., *Inflammatory cells in the bronchial glands of smokers with chronic bronchitis.* Am J Respir Crit Care Med, 1997. **156**(5): p. 1633-9.
107. Traves, S.L., et al., *Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD.* Thorax, 2002. **57**(7): p. 590-5.
108. Hill, A.T., D. Bayley, and R.A. Stockley, *The interrelationship of sputum inflammatory markers in patients with chronic bronchitis.* Am J Respir Crit Care Med, 1999. **160**(3): p. 893-8.
109. Yamamoto, C., et al., *Airway inflammation in COPD assessed by sputum levels of interleukin-8.* Chest, 1997. **112**(2): p. 505-10.
110. Foxman, E.F., J.J. Campbell, and E.C. Butcher, *Multistep navigation and the combinatorial control of leukocyte chemotaxis.* J Cell Biol, 1997. **139**(5): p. 1349-60.
111. 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.
112. Gompertz, S., et al., *Changes in bronchial inflammation during acute exacerbations of chronic bronchitis.* Eur Respir J, 2001. **17**(6): p. 1112-9.
113. Sinden, N.J. and R.A. Stockley, *Systemic inflammation and comorbidity in COPD: a result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence.* Thorax, 2010. **65**(10): p. 930-6.
114. Qiu, Y., et al., *Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2003. **168**(8): p. 968-75.
115. Drost, E.M., et al., *Oxidative stress and airway inflammation in severe exacerbations of COPD.* Thorax, 2005. **60**(4): p. 293-300.
116. Agusti, A.G., et al., *Serial measurements of exhaled nitric oxide during exacerbations of chronic obstructive pulmonary disease.* Eur Respir J, 1999. **14**(3): p. 523-8.
117. Wedzicha, J.A., et al., *Mechanisms and impact of the frequent exacerbator phenotype in chronic obstructive pulmonary disease.* BMC Med, 2013. **11**: p. 181.
118. Saha, S. and C.E. Brightling, *Eosinophilic airway inflammation in COPD.* Int J Chron Obstruct Pulmon Dis, 2006. **1**(1): p. 39-47.
119. Donaldson, G.C., et al., *Airway and systemic inflammation and decline in lung function in patients with COPD.* Chest, 2005. **128**(4): p. 1995-2004.
120. Perera, W.R., et al., *Inflammatory changes, recovery and recurrence at COPD exacerbation.* Eur Respir J, 2007. **29**(3): p. 527-34.
121. De Gruttola, V.G., et al., *Considerations in the evaluation of surrogate endpoints in clinical trials. summary of a National Institutes of Health workshop.* Control Clin Trials, 2001. **22**(5): p. 485-502.
122. Rosenberg, S.R. and R. Kalhan, *Biomarkers in chronic obstructive pulmonary disease.* Transl Res, 2012. **159**(4): p. 228-37.
123. Lomas, D.A., et al., *Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort.* Thorax, 2008. **63**(12): p. 1058-63.
124. 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.
125. Dickens, J.A., et al., *COPD association and repeatability of blood biomarkers in the ECLIPSE cohort.* Respir Res, 2011. **12**: p. 146.

126. O'Donnell, D.E. and P. Laveneziana, *The clinical importance of dynamic lung hyperinflation in COPD.* COPD, 2006. **3**(4): p. 219-32.
127. O'Donnell, D.E. and C.M. Parker, *COPD exacerbations . 3: Pathophysiology.* Thorax, 2006. **61**(4): p. 354-61.
128. Wedzicha, J.A., M. Decramer, and T.A. Seemungal, *The role of bronchodilator treatment in the prevention of exacerbations of COPD.* Eur Respir J, 2012. **40**(6): p. 1545-54.
129. Kanner, R.E., et al., *Lower respiratory illnesses promote FEV(1) decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease: results from the lung health study.* Am J Respir Crit Care Med, 2001. **164**(3): p. 358-64.
130. Celli, B.R., et al., *Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study.* Am J Respir Crit Care Med, 2008. **178**(4): p. 332-8.
131. Jones, P.W., et al., *Correlating changes in lung function with patient outcomes in chronic obstructive pulmonary disease: a pooled analysis.* Respir Res, 2011. **12**: p. 161.
132. 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.
133. Halpin, D.M., et al., *Exacerbation frequency and course of COPD.* Int J Chron Obstruct Pulmon Dis, 2012. **7**: p. 653-61.
134. Spruit, M.A., et al., *Muscle force during an acute exacerbation in hospitalised patients with COPD and its relationship with CXCL8 and IGF-I.* Thorax, 2003. **58**(9): p. 752-6.
135. Pitta, F., et al., *Physical activity and hospitalization for exacerbation of COPD.* Chest, 2006. **129**(3): p. 536-44.
136. Miravitlles, M., et al., *Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study.* Thorax, 2004. **59**(5): p. 387-95.
137. Mackay, A.J., et al., *Usefulness of the Chronic Obstructive Pulmonary Disease Assessment Test to evaluate severity of COPD exacerbations.* Am J Respir Crit Care Med, 2012. **185**(11): p. 1218-24.
138. Spencer, S., P.W. Jones, and G.S. Group, *Time course of recovery of health status following an infective exacerbation of chronic bronchitis.* Thorax, 2003. **58**(7): p. 589-93.
139. Anzueto, A., S. Sethi, and F.J. Martinez, *Exacerbations of chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2007. **4**(7): p. 554-64.
140. Baghai-Ravary, R., et al., *Determinants and impact of fatigue in patients with chronic obstructive pulmonary disease.* Respir Med, 2009. **103**(2): p. 216-23.
141. Roberts, C.M., et al., *Clinical audit indicators of outcome following admission to hospital with acute exacerbation of chronic obstructive pulmonary disease.* Thorax, 2002. **57**(2): p. 137-41.
142. Connors, A.F., Jr., et al., *Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments).* Am J Respir Crit Care Med, 1996. **154**(4 Pt 1): p. 959-67.
143. Suissa, S., S. Dell'Aniello, and P. Ernst, *Long-term natural history of chronic obstructive pulmonary disease: severe exacerbations and mortality.* Thorax, 2012. **67**(11): p. 957-63.
144. Sin, D.D., et al., *Mortality in COPD: Role of comorbidities.* Eur Respir J, 2006. **28**(6): p. 1245-57.
145. Hanania, N.A., et al., *Determinants of depression in the ECLIPSE chronic obstructive pulmonary disease cohort.* Am J Respir Crit Care Med, 2011. **183**(5): p. 604-11.

146. Quint, J.K., et al., *Relationship between depression and exacerbations in COPD.* Eur Respir J, 2008. **32**(1): p. 53-60.
147. Abrams, T.E., M. Vaughan-Sarrazin, and M.W. Van der Weg, *Acute exacerbations of chronic obstructive pulmonary disease and the effect of existing psychiatric comorbidity on subsequent mortality.* Psychosomatics, 2011. **52**(5): p. 441-9.
148. Keating, A., A. Lee, and A.E. Holland, *What prevents people with chronic obstructive pulmonary disease from attending pulmonary rehabilitation? A systematic review.* Chron Respir Dis, 2011. **8**(2): p. 89-99.
149. Khdour, M.R., et al., *Potential risk factors for medication non-adherence in patients with chronic obstructive pulmonary disease (COPD).* Eur J Clin Pharmacol, 2012. **68**(10): p. 1365-73.
150. Dodd, J.W., et al., *Cognitive dysfunction in patients hospitalized with acute exacerbation of COPD.* Chest, 2013. **144**(1): p. 119-27.
151. 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.
152. Bolton, C.E., et al., *Insulin resistance and inflammation - A further systemic complication of COPD.* COPD, 2007. **4**(2): p. 121-6.
153. Chakrabarti, B., et al., *Hyperglycaemia as a predictor of outcome during non-invasive ventilation in decompensated COPD.* Thorax, 2009. **64**(10): p. 857-62.
154. Baker, E.H., et al., *Hyperglycaemia is associated with poor outcomes in patients admitted to hospital with acute exacerbations of chronic obstructive pulmonary disease.* Thorax, 2006. **61**(4): p. 284-9.
155. Patel, A.R. and J.R. Hurst, *Extrapulmonary comorbidities in chronic obstructive pulmonary disease: state of the art.* Expert Rev Respir Med, 2011. **5**(5): p. 647-62.
156. Seymour, J.M., et al., *The prevalence of quadriceps weakness in COPD and the relationship with disease severity.* Eur Respir J, 2010. **36**(1): p. 81-8.
157. Di Francia, M., et al., *Tumor necrosis factor-alpha levels and weight loss in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 1994. **150**(5 Pt 1): p. 1453-5.
158. Jorgensen, N.R., et al., *The prevalence of osteoporosis in patients with chronic obstructive pulmonary disease: a cross sectional study.* Respir Med, 2007. **101**(1): p. 177-85.
159. Ferguson, G.T., et al., *Prevalence and progression of osteoporosis in patients with COPD: results from the TOwards a Revolution in COPD Health study.* Chest, 2009. **136**(6): p. 1456-65.
160. Sin, D.D., J.P. Man, and S.F. Man, *The risk of osteoporosis in Caucasian men and women with obstructive airways disease.* Am J Med, 2003. **114**(1): p. 10-4.
161. Ohara, T., et al., *Relationship between pulmonary emphysema and osteoporosis assessed by CT in patients with COPD.* Chest, 2008. **134**(6): p. 1244-9.
162. Kiyokawa, H., et al., *Impact of COPD exacerbations on osteoporosis assessed by chest CT scan.* COPD, 2012. **9**(3): p. 235-42.
163. Terada, K., et al., *Impact of gastro-oesophageal reflux disease symptoms on COPD exacerbation.* Thorax, 2008. **63**(11): p. 951-5.
164. Rascon-Aguilar, I.E., et al., *Role of gastroesophageal reflux symptoms in exacerbations of COPD.* Chest, 2006. **130**(4): p. 1096-101.
165. Sasaki, T., et al., *A randomized, single-blind study of lansoprazole for the prevention of exacerbations of chronic obstructive pulmonary disease in older patients.* J Am Geriatr Soc, 2009. **57**(8): p. 1453-7.

166. Mayne, S.T., J. Buenconsejo, and D.T. Janerich, *Previous lung disease and risk of lung cancer among men and women nonsmokers.* Am J Epidemiol, 1999. **149**(1): p. 13-20.
167. Mannino, D.M., et al., *Low lung function and incident lung cancer in the United States: data From the First National Health and Nutrition Examination Survey follow-up.* Arch Intern Med, 2003. **163**(12): p. 1475-80.
168. O'Byrne, K.J. and A.G. Dalgleish, *Chronic immune activation and inflammation as the cause of malignancy.* Br J Cancer, 2001. **85**(4): p. 473-83.
169. Wasswa-Kintu, S., et al., *Relationship between reduced forced expiratory volume in one second and the risk of lung cancer: a systematic review and meta-analysis.* Thorax, 2005. **60**(7): p. 570-5.
170. Vestbo, J., S.S. Hurd, and R. Rodriguez-Roisin, *The 2011 revision of the global strategy for the diagnosis, management and prevention of COPD (GOLD)--why and what?* Clin Respir J, 2012. **6**(4): p. 208-14.
171. Bhatt, S.P. and M.T. Dransfield, *Chronic obstructive pulmonary disease and cardiovascular disease.* Transl Res, 2013. **162**(4): p. 237-51.
172. Camilli, A.E., D.R. Robbins, and M.D. Lebowitz, *Death certificate reporting of confirmed airways obstructive disease.* Am J Epidemiol, 1991. **133**(8): p. 795-800.
173. 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.
174. Anthonisen, N.R., et al., *Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. The Lung Health Study.* JAMA, 1994. **272**(19): p. 1497-505.
175. Hudson, L.D., et al., *Arrhythmias associated with acute respiratory failure in patients with chronic airway obstruction.* Chest, 1973. **63**(5): p. 661-5.
176. Salisbury, A.C., K.J. Reid, and J.A. Spertus, *Impact of chronic obstructive pulmonary disease on post-myocardial infarction outcomes.* Am J Cardiol, 2007. **99**(5): p. 636-41.
177. Bursi, F., et al., *Chronic obstructive pulmonary disease after myocardial infarction in the community.* Am Heart J, 2010. **160**(1): p. 95-101.
178. Wakabayashi, K., et al., *Impact of chronic obstructive pulmonary disease on acute-phase outcome of myocardial infarction.* Am J Cardiol, 2010. **106**(3): p. 305-9.
179. Hadi, H.A., et al., *Prevalence and prognosis of chronic obstructive pulmonary disease among 8167 Middle Eastern patients with acute coronary syndrome.* Clin Cardiol, 2010. **33**(4): p. 228-35.
180. Bertoletti, L., et al., *Clinical presentation and outcome of venous thromboembolism in COPD.* Eur Respir J, 2012. **39**(4): p. 862-8.
181. Rizkallah, J., S.F. Man, and D.D. Sin, *Prevalence of pulmonary embolism in acute exacerbations of COPD: a systematic review and metaanalysis.* Chest, 2009. **135**(3): p. 786-93.
182. Patel, A.R., et al., *The impact of ischemic heart disease on symptoms, health status, and exacerbations in patients with COPD.* Chest, 2012. **141**(4): p. 851-7.
183. Abusaid, G.H., et al., *Diastolic dysfunction and COPD exacerbation.* Postgrad Med, 2009. **121**(4): p. 76-81.
184. Abroug, F., et al., *Association of left-heart dysfunction with severe exacerbation of chronic obstructive pulmonary disease: diagnostic performance of cardiac biomarkers.* Am J Respir Crit Care Med, 2006. **174**(9): p. 990-6.
185. Ito, K. and P.J. Barnes, *COPD as a disease of accelerated lung aging.* Chest, 2009. **135**(1): p. 173-80.

186. Maclay, J.D. and W. MacNee, *Cardiovascular disease in COPD: mechanisms.* Chest, 2013. **143**(3): p. 798-807.
187. MacNee, W., *Accelerated lung aging: a novel pathogenic mechanism of chronic obstructive pulmonary disease (COPD).* Biochem Soc Trans, 2009. **37**(Pt 4): p. 819-23.
188. Savale, L., et al., *Shortened telomeres in circulating leukocytes of patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2009. **179**(7): p. 566-71.
189. Tsuji, T., K. Aoshiba, and A. Nagai, *Alveolar cell senescence in patients with pulmonary emphysema.* Am J Respir Crit Care Med, 2006. **174**(8): p. 886-93.
190. Yokohori, N., et al., *Increased levels of cell death and proliferation in alveolar wall cells in patients with pulmonary emphysema.* Chest, 2004. **125**(2): p. 626-32.
191. Centers for Disease, C. and Prevention, *Prevalence of coronary heart disease--United States, 2006-2010.* MMWR Morb Mortal Wkly Rep, 2011. **60**(40): p. 1377-81.
192. Ho, K.K., et al., *The epidemiology of heart failure: the Framingham Study.* J Am Coll Cardiol, 1993. **22**(4 Suppl A): p. 6A-13A.
193. Minamino, T., et al., *Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction.* Circulation, 2002. **105**(13): p. 1541-4.
194. Wang, Y.Y., et al., *Association of shorter mean telomere length with large artery stiffness in patients with coronary heart disease.* Aging Male, 2011. **14**(1): p. 27-32.
195. Anthonisen, N.R., et al., *Hospitalizations and mortality in the Lung Health Study.* Am J Respir Crit Care Med, 2002. **166**(3): p. 333-9.
196. Sorlie, P.D., W.B. Kannel, and G. O'Connor, *Mortality associated with respiratory function and symptoms in advanced age. The Framingham Study.* Am Rev Respir Dis, 1989. **140**(2): p. 379-84.
197. Truelsen, T., et al., *Lung function and risk of fatal and non-fatal stroke. The Copenhagen City Heart Study.* Int J Epidemiol, 2001. **30**(1): p. 145-51.
198. Ezzati, M. and A.D. Lopez, *Estimates of global mortality attributable to smoking in 2000.* Lancet, 2003. **362**(9387): p. 847-52.
199. Wilson, D., et al., *Difficulties identifying and targeting COPD and population-attributable risk of smoking for COPD: a population study.* Chest, 2005. **128**(4): p. 2035-42.
200. Prescott, E., et al., *Smoking and risk of myocardial infarction in women and men: longitudinal population study.* BMJ, 1998. **316**(7137): p. 1043-7.
201. Yusuf, S., et al., *Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study.* Lancet, 2004. **364**(9438): p. 937-52.
202. Sackett, D.L., et al., *Relation between aortic atherosclerosis and the use of cigarettes and alcohol. An autopsy study.* N Engl J Med, 1968. **279**(26): p. 1413-20.
203. Van Eeden, S., et al., *The relationship between lung inflammation and cardiovascular disease.* Am J Respir Crit Care Med, 2012. **186**(1): p. 11-6.
204. Garcia-Aymerich, J., et al., *Regular physical activity modifies smoking-related lung function decline and reduces risk of chronic obstructive pulmonary disease: a population-based cohort study.* Am J Respir Crit Care Med, 2007. **175**(5): p. 458-63.
205. Das, U.N., *Anti-inflammatory nature of exercise.* Nutrition, 2004. **20**(3): p. 323-6.
206. Decramer, M., et al., *COPD as a lung disease with systemic consequences--clinical impact, mechanisms, and potential for early intervention.* COPD, 2008. **5**(4): p. 235-56.
207. El-Sayed, M.S., P.G. Jones, and C. Sale, *Exercise induces a change in plasma fibrinogen concentration: fact or fiction?* Thromb Res, 1999. **96**(6): p. 467-72.

208. DeSouza, C.A., P.P. Jones, and D.R. Seals, *Physical activity status and adverse age-related differences in coagulation and fibrinolytic factors in women.* Arterioscler Thromb Vasc Biol, 1998. **18**(3): p. 362-8.
209. Dinenno, F.A., et al., *Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men.* J Physiol, 2001. **534**(Pt 1): p. 287-95.
210. Izquierdo-Porrera, A.M., et al., *Effects of exercise rehabilitation on cardiovascular risk factors in older patients with peripheral arterial occlusive disease.* J Vasc Surg, 2000. **31**(4): p. 670-7.
211. Rowland, T.W., *The role of physical activity and fitness in children in the prevention of adult cardiovascular disease.* Prog Pediatr Cardiol, 2001. **12**(2): p. 199-203.
212. Drygas, W., et al., *Long-term effects of different physical activity levels on coronary heart disease risk factors in middle-aged men.* Int J Sports Med, 2000. **21**(4): p. 235-41.
213. Vivodtzev, I., et al., *Arterial stiffness in COPD.* Chest, 2014. **145**(4): p. 861-75.
214. Benetos, A., et al., *Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries.* Arterioscler Thromb, 1993. **13**(1): p. 90-7.
215. Vasan, R.S., et al., *Impact of high-normal blood pressure on the risk of cardiovascular disease.* N Engl J Med, 2001. **345**(18): p. 1291-7.
216. Heindl, S., et al., *Marked sympathetic activation in patients with chronic respiratory failure.* Am J Respir Crit Care Med, 2001. **164**(4): p. 597-601.
217. van Gestel, A.J., M. Kohler, and C.F. Clarenbach, *Sympathetic overactivity and cardiovascular disease in patients with chronic obstructive pulmonary disease (COPD).* Discov Med, 2012. **14**(79): p. 359-68.
218. Miller, K.A., et al., *Long-term exposure to air pollution and incidence of cardiovascular events in women.* N Engl J Med, 2007. **356**(5): p. 447-58.
219. Pell, J.P., et al., *Smoke-free legislation and hospitalizations for acute coronary syndrome.* N Engl J Med, 2008. **359**(5): p. 482-91.
220. Mills, N.L., et al., *Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease.* N Engl J Med, 2007. **357**(11): p. 1075-82.
221. Brook, R.D., et al., *Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association.* Circulation, 2010. **121**(21): p. 2331-78.
222. Laratta, C.R. and S. van Eeden, *Acute Exacerbation of Chronic Obstructive Pulmonary Disease: Cardiovascular Links.* Biomed Res Int, 2014. **2014**: p. 528789.
223. Kido, T., et al., *Particulate matter induces translocation of IL-6 from the lung to the systemic circulation.* Am J Respir Cell Mol Biol, 2011. **44**(2): p. 197-204.
224. Kullo, I.J., W.D. Edwards, and R.S. Schwartz, *Vulnerable plaque: pathobiology and clinical implications.* Ann Intern Med, 1998. **129**(12): p. 1050-60.
225. Emerging Risk Factors, C., et al., *C-reactive protein, fibrinogen, and cardiovascular disease prediction.* N Engl J Med, 2012. **367**(14): p. 1310-20.
226. Macnee, W., J. Maclay, and D. McAllister, *Cardiovascular injury and repair in chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2008. **5**(8): p. 824-33.
227. Verma, S., et al., *Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein.* Circulation, 2002. **105**(16): p. 1890-6.
228. Vaughan, D.E., *PAI-1 and atherothrombosis.* J Thromb Haemost, 2005. **3**(8): p. 1879-83.
229. Vanfleteren, L.E., et al., *Clusters of comorbidities based on validated objective measurements and systemic inflammation in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2013. **187**(7): p. 728-35.

230. Campo, G., et al., *Coagulation factors and recurrence of ischemic and bleeding adverse events in patients with acute coronary syndromes.* Thromb Res, 2013. **132**(2): p. 151-7.
231. Aldonyte, R., et al., *Circulating monocytes from healthy individuals and COPD patients.* Respir Res, 2003. **4**: p. 11.
232. Libby, P., P.M. Ridker, and A. Maseri, *Inflammation and atherosclerosis.* Circulation, 2002. **105**(9): p. 1135-43.
233. Miyata, R., et al., *Statins reduce ambient particulate matter-induced lung inflammation by promoting the clearance of particulate matter, < 10 mum from lung tissues.* Chest, 2013. **143**(2): p. 452-60.
234. Miyata, R., et al., *Statins attenuate the development of atherosclerosis and endothelial dysfunction induced by exposure to urban particulate matter (PM10).* Toxicol Appl Pharmacol, 2013. **272**(1): p. 1-11.
235. Singh, R.B., et al., *Pathogenesis of atherosclerosis: A multifactorial process.* Exp Clin Cardiol, 2002. **7**(1): p. 40-53.
236. Griendling, K.K. and M. Ushio-Fukai, *Redox control of vascular smooth muscle proliferation.* J Lab Clin Med, 1998. **132**(1): p. 9-15.
237. Aoki, M., et al., *Endothelial apoptosis induced by oxidative stress through activation of NF-kappaB: antiapoptotic effect of antioxidant agents on endothelial cells.* Hypertension, 2001. **38**(1): p. 48-55.
238. Kameda, K., et al., *Correlation of oxidative stress with activity of matrix metalloproteinase in patients with coronary artery disease. Possible role for left ventricular remodelling.* Eur Heart J, 2003. **24**(24): p. 2180-5.
239. Lattimore, J.D., et al., *Repetitive hypoxia increases lipid loading in human macrophages-a potentially atherogenic effect.* Atherosclerosis, 2005. **179**(2): p. 255-9.
240. Ichikawa, H., et al., *Molecular mechanisms of anoxia/reoxygenation-induced neutrophil adherence to cultured endothelial cells.* Circ Res, 1997. **81**(6): p. 922-31.
241. Drexler, H. and B. Hornig, *Endothelial dysfunction in human disease.* J Mol Cell Cardiol, 1999. **31**(1): p. 51-60.
242. Lee, M.A., et al., *Tissue renin-angiotensin systems. Their role in cardiovascular disease.* Circulation, 1993. **87**(5 Suppl): p. IV7-13.
243. Flammer, A.J., et al., *The assessment of endothelial function: from research into clinical practice.* Circulation, 2012. **126**(6): p. 753-67.
244. Clarenbach, C.F., et al., *Determinants of endothelial function in patients with COPD.* Eur Respir J, 2013. **42**(5): p. 1194-204.
245. Wendt, T., et al., *RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes.* Atherosclerosis, 2006. **185**(1): p. 70-7.
246. Van Bortel, L.M., et al., *Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity.* J Hypertens, 2012. **30**(3): p. 445-8.
247. Laurent, S., et al., *Aortic stiffness as a tissue biomarker for predicting future cardiovascular events in asymptomatic hypertensive subjects.* Ann Med, 2012. **44 Suppl 1**: p. S93-7.
248. O'Leary, D.H., et al., *Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group.* N Engl J Med, 1999. **340**(1): p. 14-22.

249. Lahousse, L., et al., *Chronic obstructive pulmonary disease and lipid core carotid artery plaques in the elderly: the Rotterdam Study.* Am J Respir Crit Care Med, 2013. **187**(1): p. 58-64.
250. Wang, J.G., et al., *Carotid intima-media thickness and antihypertensive treatment: a meta-analysis of randomized controlled trials.* Stroke, 2006. **37**(7): p. 1933-40.
251. Barr, R.G., et al., *Impaired flow-mediated dilation is associated with low pulmonary function and emphysema in ex-smokers: the Emphysema and Cancer Action Project (EMCAP) Study.* Am J Respir Crit Care Med, 2007. **176**(12): p. 1200-7.
252. Eickhoff, P., et al., *Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2008. **178**(12): p. 1211-8.
253. Anderson, T.J., et al., *Close relation of endothelial function in the human coronary and peripheral circulations.* J Am Coll Cardiol, 1995. **26**(5): p. 1235-41.
254. Modena, M.G., et al., *Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women.* J Am Coll Cardiol, 2002. **40**(3): p. 505-10.
255. Perticone, F., et al., *Prognostic significance of endothelial dysfunction in hypertensive patients.* Circulation, 2001. **104**(2): p. 191-6.
256. Witte, D.R., et al., *Measurement of flow-mediated dilatation of the brachial artery is affected by local elastic vessel wall properties in high-risk patients.* Atherosclerosis, 2005. **182**(2): p. 323-30.
257. Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis, *Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis.* J Am Coll Cardiol, 2010. **55**(13): p. 1318-27.
258. Mattace-Raso, F.U., et al., *Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study.* Circulation, 2006. **113**(5): p. 657-63.
259. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications.* Eur Heart J, 2006. **27**(21): p. 2588-605.
260. Boutouyrie, P., et al., *Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study.* Hypertension, 2002. **39**(1): p. 10-5.
261. Safar, M.E., B.I. Levy, and H. Struijker-Boudier, *Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases.* Circulation, 2003. **107**(22): p. 2864-9.
262. Vivodtzev, I., et al., *Significant improvement in arterial stiffness after endurance training in patients with COPD.* Chest, 2010. **137**(3): p. 585-92.
263. Vanfleteren, L.E., et al., *Arterial stiffness in patients with COPD: the role of systemic inflammation and the effects of pulmonary rehabilitation.* Eur Respir J, 2014. **43**(5): p. 1306-15.
264. Stickland, M.K., et al., *Physical activity and arterial stiffness in chronic obstructive pulmonary disease.* Respir Physiol Neurobiol, 2013. **189**(1): p. 188-94.
265. Pepin, J.L., et al., *Long-Acting Bronchodilators and Arterial Stiffness in Patients With COPD: A Comparison of Fluticasone Furoate/Vilanterol With Tiotropium.* Chest, 2014. **146**(6): p. 1521-30.
266. Cinarka, H., et al., *Arterial stiffness measured via carotid femoral pulse wave velocity is associated with disease severity in COPD.* Respir Care, 2014. **59**(2): p. 274-80.
267. Zureik, M., et al., *Reduced pulmonary function is associated with central arterial stiffness in men.* Am J Respir Crit Care Med, 2001. **164**(12): p. 2181-5.

268. Sabit, R., et al., *Sub-clinical left and right ventricular dysfunction in patients with COPD.* Respir Med, 2010. **104**(8): p. 1171-8.
269. Albu, A., et al., *Carotid arterial stiffness in patients with chronic obstructive pulmonary disease.* Acta Physiol Hung, 2011. **98**(2): p. 117-27.
270. Aykan, A.C., et al., *Assessment of arterial stiffness in chronic obstructive pulmonary disease by a novel method: cardio-ankle vascular index.* Herz, 2014. **39**(7): p. 822-7.
271. Gale, N.S., et al., *Does pulmonary rehabilitation address cardiovascular risk factors in patients with COPD?* BMC Pulm Med, 2011. **11**: p. 20.
272. Dransfield, M.T., et al., *Effect of fluticasone propionate/salmeterol on arterial stiffness in patients with COPD.* Respir Med, 2011. **105**(9): p. 1322-30.
273. Bartels, M.N., et al., *Supplemental oxygen increases arterial stiffness in chronic obstructive pulmonary disease.* Respir Med, 2004. **98**(1): p. 84-9.
274. Mancia, G., et al., *2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).* Eur Heart J, 2007. **28**(12): p. 1462-536.
275. Tsai, P.S. and C.B. Yucha, *Noninvasive measurements of central arterial pressure and distensibility by arterial applanation tonometry with a generalized transfer function: implications for nursing.* Heart Lung, 2001. **30**(6): p. 437-44.
276. Lehmann, E.D., et al., *Non-invasive assessment of cardiovascular disease in diabetes mellitus.* Lancet, 1997. **350 Suppl 1**: p. SI14-9.
277. Mancia, G., et al., *2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).* J Hypertens, 2007. **25**(6): p. 1105-87.
278. Blacher, J., et al., *Impact of aortic stiffness on survival in end-stage renal disease.* Circulation, 1999. **99**(18): p. 2434-9.
279. Blacher, J., et al., *Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients.* Hypertension, 1999. **33**(5): p. 1111-7.
280. Cruickshank, K., et al., *Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function?* Circulation, 2002. **106**(16): p. 2085-90.
281. Laurent, S., et al., *Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients.* Hypertension, 2001. **37**(5): p. 1236-41.
282. Willum-Hansen, T., et al., *Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population.* Circulation, 2006. **113**(5): p. 664-70.
283. Safar, M.E., et al., *Central pulse pressure and mortality in end-stage renal disease.* Hypertension, 2002. **39**(3): p. 735-8.
284. Meaume, S., et al., *Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age.* Arterioscler Thromb Vasc Biol, 2001. **21**(12): p. 2046-50.
285. Waddell, T.K., et al., *Carotid pressure is a better predictor of coronary artery disease severity than brachial pressure.* Hypertension, 2001. **38**(4): p. 927-31.
286. Shoji, T., et al., *Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease.* J Am Soc Nephrol, 2001. **12**(10): p. 2117-24.
287. Sutton-Tyrrell, K., et al., *Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults.* Circulation, 2005. **111**(25): p. 3384-90.

288. Laurent, S., et al., *Aortic stiffness is an independent predictor of fatal stroke in essential hypertension.* Stroke, 2003. **34**(5): p. 1203-6.
289. Stefanadis, C., et al., *Aortic stiffness as a risk factor for recurrent acute coronary events in patients with ischaemic heart disease.* Eur Heart J, 2000. **21**(5): p. 390-6.
290. Hill, K., et al., *Prevalence and underdiagnosis of chronic obstructive pulmonary disease among patients at risk in primary care.* CMAJ, 2010. **182**(7): p. 673-8.
291. Spencer, S., et al., *Impact of preventing exacerbations on deterioration of health status in COPD.* Eur Respir J, 2004. **23**(5): p. 698-702.
292. Wouters, E.F., *The burden of COPD in The Netherlands: results from the Confronting COPD survey.* Respir Med, 2003. **97** (Suppl C): p. S51-S59.
293. Burge, P.S., et al., *Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial.* BMJ, 2000. **320**(7245): p. 1297-303.
294. Miravitlles, M., et al., *Factors associated with increased risk of exacerbation and hospital admission in a cohort of ambulatory COPD patients: a multiple logistic regression analysis. The EOLO Study Group.* Respiration, 2000. **67**(5): p. 495-501.
295. Boulet, L.P., et al., *Major care gaps in asthma, sleep and chronic obstructive pulmonary disease: a road map for knowledge translation.* Can Respir J, 2013. **20**(4): p. 265-9.
296. Bourbeau, J., et al., *Canadian Cohort Obstructive Lung Disease (CanCOLD): Fulfilling the need for longitudinal observational studies in COPD.* COPD, 2014. **11**(2): p. 125-32.
297. Bischoff, E.W., et al., *Effects of written action plan adherence on COPD exacerbation recovery.* Thorax, 2011. **66**(1): p. 26-31.
298. Vestbo, J., et al., *Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE).* Eur Respir J, 2008. **31**(4): p. 869-73.
299. Balcells, E., et al., *Characterisation and prognosis of undiagnosed chronic obstructive pulmonary disease patients at their first hospitalisation.* BMC Pulm Med, 2015. **15**: p. 4.
300. Lindberg, A., et al., *Prevalence and underdiagnosis of COPD by disease severity and the attributable fraction of smoking Report from the Obstructive Lung Disease in Northern Sweden Studies.* Respir Med, 2006. **100**(2): p. 264-72.
301. Miravitlles, M., et al., *Prevalence of COPD in Spain: impact of undiagnosed COPD on quality of life and daily life activities.* Thorax, 2009. **64**(10): p. 863-8.
302. Jordan, R.E., et al., *Case finding for chronic obstructive pulmonary disease: a model for optimising a targeted approach.* Thorax, 2010. **65**(6): p. 492-8.
303. Ramsey, S.D. and F.D. Hobbs, *Chronic obstructive pulmonary disease, risk factors, and outcome trials: comparisons with cardiovascular disease.* Proc Am Thorac Soc, 2006. **3**(7): p. 635-40.
304. O'Donnell, D.E., et al., *Canadian Thoracic Society recommendations for management of chronic obstructive pulmonary disease - 2008 update - highlights for primary care.* Can Respir J, 2008. **15 Suppl A**: p. 1A-8A.
305. Lamprecht, B., et al., *COPD in never smokers: results from the population-based burden of obstructive lung disease study.* Chest, 2011. **139**(4): p. 752-63.
306. Sin, D.D., *Is COPD really a cardiovascular disease?* Chest, 2009. **136**(2): p. 329-30.
307. Pasquale, M.K., et al., *Impact of exacerbations on health care cost and resource utilization in chronic obstructive pulmonary disease patients with chronic bronchitis from a predominantly Medicare population.* Int J Chron Obstruct Pulmon Dis, 2012. **7**: p. 757-64.
308. Anzueto, A., *Impact of exacerbations on COPD.* Eur Respir Rev, 2010. **19**(116): p. 113-8.

309. Dal Negro, R., *Optimizing economic outcomes in the management of COPD.* Int J Chron Obstruct Pulmon Dis, 2008. **3**(1): p. 1-10.
310. Blasi, F., et al., *The clinical and economic impact of exacerbations of chronic obstructive pulmonary disease: a cohort of hospitalized patients.* PLoS One, 2014. **9**(6): p. e101228.
311. Asmar, R., et al., *Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies.* Hypertension, 1995. **26**(3): p. 485-90.
312. Tabara, Y., et al., *Airflow limitation in smokers is associated with arterial stiffness: the Nagahama Study.* Atherosclerosis, 2014. **232**(1): p. 59-64.
313. Wang, F., et al., *Association of serum lipids with arterial stiffness in a population-based study in Beijing.* Eur J Clin Invest, 2011. **41**(9): p. 929-36.
314. Lemogoum, D., et al., *Arterial stiffness and wave reflections in patients with sickle cell disease.* Hypertension, 2004. **44**(6): p. 924-9.
315. Cheung, Y.F., G.C. Chan, and S.Y. Ha, *Arterial stiffness and endothelial function in patients with beta-thalassemia major.* Circulation, 2002. **106**(20): p. 2561-6.
316. Shimizu, Y., et al., *Association between hemoglobin levels and arterial stiffness for general Japanese population in relation to body mass index status: The Nagasaki Islands study.* Geriatr Gerontol Int, 2014. **14**(4): p. 811-8.
317. Anderson, J., et al., *The impact of renal insufficiency and anaemia on survival in patients with cardiovascular disease: a cohort study.* BMC Cardiovasc Disord, 2009. **9**: p. 51.
318. Eckardt, K.U., *Cardiovascular consequences of renal anaemia and erythropoietin therapy.* Nephrol Dial Transplant, 1999. **14**(5): p. 1317-23.
319. Perez, G.O., J.R. Oster, and C.A. Vaamonde, *Serum potassium concentration in acidemic states.* Nephron, 1981. **27**(4-5): p. 233-43.
320. Mahmud, A. and J. Feely, *Arterial stiffness and the renin-angiotensin-aldosterone system.* J Renin Angiotensin Aldosterone Syst, 2004. **5**(3): p. 102-8.
321. Zago, M., et al., *The NF-kappaB family member RelB regulates microRNA miR-146a to suppress cigarette smoke-induced COX-2 protein expression in lung fibroblasts.* Toxicol Lett, 2014. **226**(2): p. 107-16.
322. Thatcher, T.H., et al., *Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB.* Am J Pathol, 2007. **170**(3): p. 855-64.
323. McMillan, D.H., et al., *Lung-targeted overexpression of the NF-kappaB member RelB inhibits cigarette smoke-induced inflammation.* Am J Pathol, 2011. **179**(1): p. 125-33.
324. 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.
325. GOLD, *Global Strategy for the Diagnosis, Management and Prevention of COPD: Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2014.* http://www.goldcopd.org/, 2014.
326. Tam, A. and D.D. Sin, *Pathobiologic mechanisms of chronic obstructive pulmonary disease.* Med Clin North Am, 2012. **96**(4): p. 681-98.
327. Hurst, J.R., et al., *Susceptibility to exacerbation in chronic obstructive pulmonary disease.* N Engl J Med, 2010. **363**(12): p. 1128-38.
328. Aaron, S.D., et al., *Time course and pattern of COPD exacerbation onset.* Thorax, 2012. **67**(3): p. 238-43.

329. Barnes, P.J. and M. Karin, *Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases.* N Engl J Med, 1997. **336**(15): p. 1066-71.
330. Barnes, P.J., *Transcription factors in airway diseases.* Lab Invest, 2006. **86**(9): p. 867-72.
331. Barnes, P.J., *The Cytokine Network in Chronic Obstructive Pulmonary Disease* . Am J Respir Cell Mol Biol, 2009. **41**: p. 631-638.
332. Grossmann, M., et al., *New insights into the roles of ReL/NF-kappa B transcription factors in immune function, hemopoiesis and human disease.* Int J Biochem Cell Biol, 1999. **31**(10): p. 1209-19.
333. Hayden, M.S. and S. Ghosh, *Shared principles in NF-kappaB signaling.* Cell, 2008. **132**(3): p. 344-62.
334. Barnes, P.J., S.D. Shapiro, and R.A. Pauwels, *Chronic obstructive pulmonary disease: molecular and cellularmechanisms.* European Respiratory Journal, 2003. **22**(4): p. 672-688.
335. Sarir, H., et al., *Cells, mediators and Toll-like receptors in COPD.* Eur J Pharmacol, 2008. **585**(2-3): p. 346-53.
336. Barnes, P.J., *Mediators of chronic obstructive pulmonary disease.* Pharmacol Rev, 2004. **56**(4): p. 515-48.
337. de Souza, A.R., et al., *Aryl Hydrocarbon Receptor (AhR) Attenuation of Subchronic Cigarette Smoke-induced Pulmonary Neutrophilia is Associated with Retention of Nuclear RelB and Suppression of Intercellular Adhesion Molecule-1 (ICAM-1).* Toxicol Sci, 2014. **140**(1): p. 204-223.
338. Weih, F., et al., *Multifocal defects in immune responses in RelB-deficient mice.* J Immunol, 1997. **158**(11): p. 5211-8.
339. Weih, F., et al., *Both multiorgan inflammation and myeloid hyperplasia in RelB-deficient mice are T cell dependent.* J Immunol, 1996. **157**(9): p. 3974-9.
340. Oliver, K.M., et al., *Hypercapnia induces cleavage and nuclear localization of RelB protein, giving insight into CO2 sensing and signaling.* J Biol Chem, 2012. **287**(17): p. 14004-11.
341. Bruno, C.M. and M. Valenti, *Acid-base disorders in patients with chronic obstructive pulmonary disease: a pathophysiological review.* J Biomed Biotechnol, 2012. **2012**: p. 915150.
342. McAllister, D.A., et al., *Diagnosis of myocardial infarction following hospitalisation for exacerbation of COPD.* Eur Respir J, 2012. **39**(5): p. 1097-103.
343. de Souza, A.R., et al., *Aryl Hydrocarbon Receptor (AhR) Attenuation of Subchronic Cigarette Smoke-induced Pulmonary Neutrophilia is Associated with Retention of Nuclear RelB and Suppression of Intercellular Adhesion Molecule-1 (ICAM-1).* Toxicol Sci, 2014.
344. Lo, D., et al., *A recessive defect in lymphocyte or granulocyte function caused by an integrated transgene.* Am J Pathol, 1992. **141**(5): p. 1237-46.
345. Jones, P.W. and A.G.N. Agusti, *Outcomes and markers in the assessment of chronic obstructive pulmonary disease.* Eur Respir J, 2006. **27**: p. 822-832.
346. Ammon, C., et al., *Differential expression of the transcription factor NF-kappaB during human mononuclear phagocyte differentiation to macrophages and dendritic cells.* Biochem Biophys Res Commun, 2000. **268**(1): p. 99-105.
347. Tully, J.E., et al., *Cooperation between classical and alternative NF-kappaB pathways regulates proinflammatory responses in epithelial cells.* Am J Respir Cell Mol Biol, 2012. **47**(4): p. 497-508.

348. Millet, P., C. McCall, and B. Yoza, *RelB: an outlier in leukocyte biology.* J Leukoc Biol, 2013. **94**(5): p. 941-51.
349. Carrasco, D., R.P. Ryseck, and R. Bravo, *Expression of relB transcripts during lymphoid organ development: specific expression in dendritic antigen-presenting cells.* Development, 1993. **118**(4): p. 1221-31.
350. Lindner, V., *The NF-kappaB and IkappaB system in injured arteries.* Pathobiology, 1998. **66**(6): p. 311-20.
351. Braam, B., et al., *Nitric oxide donor induces temporal and dose-dependent reduction of gene expression in human endothelial cells.* Am J Physiol Heart Circ Physiol, 2004. **287**(5): p. H1977-86.
352. Kraut, J.A. and N.E. Madias, *Serum anion gap: its uses and limitations in clinical medicine.* Clin J Am Soc Nephrol, 2007. **2**(1): p. 162-74.
353. Xiong, S.L., X. Liu, and G.H. Yi, *High-density lipoprotein induces cyclooxygenase-2 expression and prostaglandin I-2 release in endothelial cells through sphingosine kinase-2.* Mol Cell Biochem, 2014. **389**(1-2): p. 197-207.
354. Taketa, K., et al., *Oxidized low density lipoprotein activates peroxisome proliferator-activated receptor-alpha (PPARalpha) and PPARgamma through MAPK-dependent COX-2 expression in macrophages.* J Biol Chem, 2008. **283**(15): p. 9852-62.
355. Hocherl, K., et al., *Cyclo-oxygenase-2 inhibition increases blood pressure in rats.* Br J Pharmacol, 2002. **136**(8): p. 1117-26.
356. Cathcart, M.C., et al., *Cyclooxygenase-2-linked attenuation of hypoxia-induced pulmonary hypertension and intravascular thrombosis.* J Pharmacol Exp Ther, 2008. **326**(1): p. 51-8.
357. Sincer, I., et al., *Relationship between red cell distribution width and right ventricular dysfunction in patients with chronic obstructive pulmonary disease.* Heart Lung, 2012. **41**(3): p. 238-43.
358. 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.
359. Testelmans, D., et al., *Profile of circulating cytokines: impact of OSA, obesity and acute cardiovascular events.* Cytokine, 2013. **62**(2): p. 210-6.
360. Coakley, R.J., et al., *Ambient pCO2 modulates intracellular pH, intracellular oxidant generation, and interleukin-8 secretion in human neutrophils.* J Leukoc Biol, 2002. **71**(4): p. 603-10.
361. Jancic, C.C., et al., *Low extracellular pH stimulates the production of IL-1beta by human monocytes.* Cytokine, 2012. **57**(2): p. 258-68.
362. Sethi, S., *Pathogenesis and treatment of acute exacerbations of chronic obstructive pulmonary disease.* Semin Respir Crit Care Med, 2005. **26**(2): p. 192-203.
363. Barnes, P.J., *Role of HDAC2 in the pathophysiology of COPD.* Annu Rev Physiol, 2009. **71**: p. 451-64.
364. Cejas, P.J., et al., *Regulation of RelB expression during the initiation of dendritic cell differentiation.* Mol Cell Biol, 2005. **25**(17): p. 7900-16.
365. Tully, J.E., et al., *Epithelial NF-kappaB orchestrates house dust mite-induced airway inflammation, hyperresponsiveness, and fibrotic remodeling.* J Immunol, 2013. **191**(12): p. 5811-21.
366. Sin, D.D. and J. Vestbo, *Biomarkers in chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2009. **6**(6): p. 543-5.

367. Sin, D.D. and S.F. Man, *Biomarkers in COPD: are we there yet?* Chest, 2008. **133**(6): p. 1296-8.
368. Park, H.Y., et al., *Club cell protein 16 and disease progression in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2013. **188**(12): p. 1413-9.
369. Sin, D.D., P.S. Pahlavan, and S.F. Man, *Surfactant protein D: a lung specific biomarker in COPD?* Ther Adv Respir Dis, 2008. **2**(2): p. 65-74.
370. Sin, D.D., et al., *Circulating surfactant protein D as a potential lung-specific biomarker of health outcomes in COPD: a pilot study.* BMC Pulm Med, 2007. **7**: p. 13.
371. Moreno, D., et al., *Surfactant protein D in chornic obstructive pulmonary disease (COPD).* Recent Pat Endocr Metab Immune Drug Discov, 2014. **8**(1): p. 42-7.
372. Lakind, J.S., et al., *A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects.* Biomarkers, 2007. **12**(5): p. 445-67.
373. Broeckaert, F. and A. Bernard, *Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker.* Clin Exp Allergy, 2000. **30**(4): p. 469-75.
374. Mason, R.J., K. Greene, and D.R. Voelker, *Surfactant protein A and surfactant protein D in health and disease.* Am J Physiol, 1998. **275**(1 Pt 1): p. L1-13.
375. Korfhagen, T.R., et al., *Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo.* J Biol Chem, 1998. **273**(43): p. 28438-43.
376. Labonte, L., et al., *Alterations in the Expression of the NF-kappaB Family Member RelB as a Novel Marker of Cardiovascular Outcomes during Acute Exacerbations of Chronic Obstructive Pulmonary Disease.* PLoS One, 2014. **9**(11): p. e112965.
377. 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.
378. Mattsson, J., et al., *Decreased serum levels of clara cell secretory protein (CC16) are associated with bronchiolitis obliterans and may permit early diagnosis in patients after allogeneic stem-cell transplantation.* Transplantation, 2005. **79**(10): p. 1411-6.
379. Shijubo, N., et al., *Clara cell protein-positive epithelial cells are reduced in small airways of asthmatics.* Am J Respir Crit Care Med, 1999. **160**(3): p. 930-3.
380. Bernard, A., et al., *Clara cell protein in serum and bronchoalveolar lavage.* Eur Respir J, 1992. **5**(10): p. 1231-8.
381. Lomas, D.A., et al., *An oral inhibitor of p38 MAP kinase reduces plasma fibrinogen in patients with chronic obstructive pulmonary disease.* J Clin Pharmacol, 2012. **52**(3): p. 416-24.
382. Lesur, O., et al., *Clara cell protein (CC-16) induces a phospholipase A2-mediated inhibition of fibroblast migration in vitro.* Am J Respir Crit Care Med, 1995. **152**(1): p. 290-7.
383. Elinder, L.S., et al., *Expression of phospholipase A2 isoforms in human normal and atherosclerotic arterial wall.* Arterioscler Thromb Vasc Biol, 1997. **17**(10): p. 2257-63.
384. 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.
385. Shakoori, T.A., et al., *Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease.* Dis Markers, 2009. **27**(6): p. 287-94.

APPENDIX I
CanCOLD CONSENT FORMS

English consent form

Centre universitaire de santé McGill
McGill University Health Centre
Les meilleurs soins pour la vie
The Best Care for Life

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Pour l'amour des enfants

Université de Montréal

SUBJECT INFORMATION AND INFORMED CONSENT FORM

RESEARCH STUDY: *The Canadian Cohort Obstructive Lung Disease Study (CanCOLD)*

PRINCIPAL INVESTIGATOR: Dr. Jean Bourbeau, Montreal Chest Institute, Montréal

FUNDING AGENCY: CIHR/Rx&D Collaborative Research Program (IRO- 93326)
In partnership with Astra Zeneca, GlaxoSmithKline, Pfizer and Boehringer Ingelheim and the Respiratory Health Network of the FRSQ

PURPOSE OF THE SUBJECT INFORMATION AND CONSENT FORM:

The purpose of this form is to give you information on this research study and if you sign it, it means you agree to take part in the study. It is very important that you read and understand the following patient information. The form describes the purpose, procedures, benefits, and risks of the research study. It may contain words you do not understand. Please ask the study doctor or personnel to explain any words or procedures that you do not clearly understand. You may take this information home and discuss it with a family member or your family doctor. You may refuse to take part or withdraw from this study at any time.

INTRODUCTION:

You are being invited to take part in this research study because you were a participant in a previous study called Chronic Obstructive Lung Disease (COLD). The COLD study was designed to measure chronic obstructive pulmonary disease [COPD] in the community. You are being asked to participate because you indicated you would participate in future studies of COPD such as CanCOLD.

The present study, i.e., CanCOLD, is funded by the Canadian Institutes of Health Research (CIHR), a governmental body, as part of a collaborative research program with partners from the pharmaceutical industry. All the funds available for this study are being used for research coordination and assistant salaries, services of data management and analysis, and purchase of research equipment.

COPD stands for "Chronic Obstructive Pulmonary Disease"

- Chronic means it won't go away.
- Obstructive means partly blocked.
- Pulmonary means in the lungs.
- Disease means sickness.

COPD is the fourth leading cause of death in Canada. Our current knowledge of COPD and its consequences on patients' health and society is not complete.

This is a longitudinal study, involving men and women who are 40 years of age or older, who may be at risk of developing COPD or who have COPD. A longitudinal study allows researchers to study participants at regular intervals over time. The researchers want to characterize the disease and how the disease progresses over time. We will ask for one additional blood sample (75 mls or 5 tablespoons) if you agree to participate in the related genetic research study. The period of time for CanCOLD is 3 years or beyond.

There are 9 sites participating. Besides Montreal, the other research sites include: Calgary, Saskatoon, Toronto, Kingston, Ottawa, Vancouver, Québec (Ste Foy), and Halifax.

COPD is a common lung disease that obstructs the airways, making breathing difficult.

- COPD is usually caused by smoking or exposure to fumes or very dusty places.
- COPD can be prevented.
- When COPD develops, it can be treated, although it cannot be cured.
- The earlier it is detected, the better the results of treatment.

COPD is a major and growing world-wide public health problem:

- The World Health Organization [WHO] estimates that it caused 2.74 million deaths in the year 2000.
- In 1990, COPD was ranked 12th as a burden of disease; by 2020 it is projected to rank 3rd globally.

PURPOSE OF THE STUDY:
The purpose of this research study is to develop a framework to combat this major health problem (COPD), by better characterization of the population of men and women at risk and patients with early disease, by better understanding which factors modifiable through health interventions are related to health perception (health-related quality of life) and disease evolution.

PARTICIPANTS:
We hope to include 2000 persons from across Canada which will include 200-250 people from the Montreal center. You have been selected because you indicated in a previous study called COLD that you would be interested in receiving information on future research such as this study.

Subjects included in this study are in one of four groups:
1) Healthy subjects (no respiratory disease)
2) Subjects at risk (smoker without respiratory disease)
3) Subjects with mild COPD
4) Subjects with moderate-severe COPD

STUDY PROCEDURES:
The study staff or the doctor in charge of the study will carefully explain all procedures to you, and you should ask whenever you need more information. No procedure will be initiated before you provide consent to participate in this study. Your participation in this study will require visits to the Montreal Chest Institute, to the Hospital Ste-Justine (or at a radiology clinic) and to respond to a telephone or online questionnaire..

Visit to the Montreal Chest Institute (1 visit every 18 months, over the next 3 years)

- **Questionnaires:** You will be asked questions concerning your family medical history, the presence of respiratory symptoms (cough, sputum, wheezing, shortness of breath), exposure to potential risk factors, respiratory diagnoses (asthma, emphysema, COPD, chronic bronchitis, etc.), and other illnesses. You will also be asked about your occupation, health care utilization, your medication use, activity limitation, state of health and nutrition. These questionnaires take about 3 hours to be completed. To reduce your time spent at the Montreal Chest Institute, we will give you the choice of answering some of the questionnaires at a later date over the phone with the Coordinator.
- **Tests**: we will ask you to do some tests
  - a pulmonary lung function test (PFT). This test takes approximately 20 min, and will be done at the initial visit and at 3 years.
  - a cardiopulmonary exercise test (CPET). This test takes approximately 45 min, and will be done at the initial visit and at 3 years.
  - a blood draw, if you agree to participate in the related genetic research study. This test takes approximately 10 min, and will be done at the initial visit, 18 months and at 3 years.
  - a simple breathing test (spirometry). This test takes approximately 15 min, and will be done at the initial visit, 18 months and at 3 years.

- an exercise test called the 6-minute walk. This test takes approximately 18 min, and will be done at 18 months.
- Grip strength measure. This measure takes about 5 min and will be performed in every visit.

Visit to the Hospital Ste-Justine (or at a radiology clinic) for a CT Scan

We will ask you to complete a multi-detector computer tomography scan (MDCT Scan) of your lungs and abdomen. This test will only take place at the initial visit if you are a healthy participant with no respiratory disease and never smoked; otherwise, at the initial visit and at year 3.

Blood draw and bank

At every visit and fasting, blood from your vein will be collected to measure proteins (e.g. Surfactant Protein-D (SPD) and C Reactive Protein (CRP)) and to measure other inflammatory substances (e.g. Interleukin-6 (IL-6) and Clara cell secretory protein (CC16)). The amount of blood that will be collected from a vein at a given visit is 75mls or 5 tablespoons.

Also, we will ask that you consent to deposit your blood and personal data in a "blood bank" which will serve for future research on COPD disease only. If you are interested, you will be asked to sign a separate consent form for this specific matter. The samples will be stored for additional future work to discover genetic aspects and potential new targets of therapy for people with COPD. Two genetic components, the DNA and the RNA, will be extracted from your blood cells and saved in the 'blood bank'. The DNA is the molecule that contains the genetic code of all organisms. The RNA is the nucleic acid that is used in key metabolic processes for all steps of protein synthesis in all living cells. **It is important to understand that you can take part in the main study and not take part in this option of long-term banking.**

Telephone or Online Questionnaire: You will be asked to answer a telephone or online questionnaire every 3 months about your respiratory symptoms, medication, health care visits, changes at work and any change in your health status. This will take approximately 5 to 10 minutes to complete.

Administrative data base from provincial health care administrative databases

You will be asked to provide permission to follow your health care utilisation (including use of medications) and health outcomes by allowing the researchers to obtain data from the provincial health care data banks. The Province of Quebec collects data for everyone who has a medicare card. Every time you receive medical service or obtain prescription medication, the details of these contacts are stored by the provincial health system (Régie de l'assurance maladie du Québec or RAMQ) in computer files. With Your permission, this information collected on how you used the health care system in the last five years, and the information to be collected on how you will use the health care system in the next 15 years – all of this information will be sent by the RAMQ to the study doctor. This information would be used to see how your health is affected by COPD or by other illnesses you may develop in the future. This information about you will be coded so that you cannot be identified. If you agree, you will be asked to sign a

separate consent form for this specific matter. **It is important to understand that you can take part in the main study and not take part in this optional administrative database.**

PARTICIPANT RESPONSIBILITIES

If you take any respiratory medication, we will ask you to withhold them before some of the tests (pulmonary function test, spirometry, cardiorespiratory pulmonary exercise test and 6-minute walk test). 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 a table that explains how many hours before each test you should withhold your respiratory medications. You will be able to take them once the tests are completed.

In addition, we will ask you to fast for your blood test that will be performed as soon as you arrive at the hospital.

DESCRIPTION OF THE QUESTIONNAIRES AND TESTS:

Questionnaires:

The CanCOLD research study involves the administration of 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.

Grip Strength Measure:

The hand grip strength will be obtained by a maximal proof of grip in which we shall ask you to close the hand around a hydraulic dynamometer of hand.

Breathing Tests (Spirometry and full pulmonary function tests "PFT"):

- **Spirometry** is the name for lung function testing and is carried out both before and after administration of an inhaled bronchodilating drug, which are standard procedures for lung function testing. You will perform the breathing test twice, before and 15 minutes after inhaling 2 puffs of a bronchodilator called salbutamol [Ventolin®]. This inhaled drug is the standard drug used by asthmatic and COPD patients to relax the airways and relieve symptoms. In this case, the use of this drug is part of the routine procedure for spirometry testing in all subjects, as it allows us to measure your personal best lung function, when the airways are fully relaxed.

- You will be asked to perform a **Full Pulmonary Function Test (PFT)** in addition to the breathing test described above. This involves breathing on a mouthpiece while sitting inside a glass body box. This will give us complete information on the state of health of

the lungs, including the presence of airway obstruction or changes in lung volumes, and how well the lungs are able to exchange oxygen and carbon dioxide.

- **Measure of maximum inspiratory and expiratory pressure (visit 1 and 3): Your inspiratory and expiratory pressure will be measured according to the maximum pressure you will apply against the quasi-occlusion of your airways.**

Exercise Tests:

There are two tests that we will use to measure your ability to exercise. The tests are:

1. **6-Minute Walk:** The object of this test is to walk for as far as possible for 6 minutes. You will walk back and forth on a flat surface such as a hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will repeat this test 2 or 3 times.
2. **Cardiopulmonary Exercise Test (CPET):** This test will measure the oxygen use (O_2) and carbon dioxide production (CO_2) of the lungs during a period of exercise. In exceptional cases and if approved by the Principle Investigator of the study, the test may performed on a treadmill. Exercise will be performed on a stationary bicycle. In addition, your heart function will be assessed using the Physioflow device. For this evaluation, we will place 6 electrodes, 2 on your neck and 4 on your body which will allow the machine to monitor your heart response continuously during exercise. Diseases that affect the heart, lungs, circulation, or blood, will cause an abnormal response to exercise. Exercise testing is useful to help evaluate the cause of shortness of breath that otherwise cannot be determined at rest (heart vs. lungs). It also evaluates the physical fitness of the heart and lungs. The test is also more sensitive for detecting early disease than are less comprehensive tests that are done at rest.

Multi-Detector Computed Tomography (or CT scan):

The MDCT test will take place at the Ste-Justine Hospital (or at a radiology clinic). For the CT scan you will be required to remove your upper clothing and wear a hospital gown. You will then lie on a table with your arms extended above your head. The table will then be moved through a "doughnut" shaped apparatus to obtain the CT scan. The whole CT procedure including the change of clothing, positioning on the table, the scan and the re-dressing will take approximately half an hour. The actual CT scan will take approximately 5 minutes. A registered CT technologist will perform all of the CT scans. Participants who have no respiratory illness (healthy subjects who have never smoked) will have only one CT scan.

POTENTIAL RISKS AND DISCOMFORTS:

Questionnaires: 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.

Symptoms: 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. 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.

Exercise Tests:

1. **The 6-Minute Walk:** If you become fatigued or tired, you will be allowed to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.
2. **Cardiopulmonary Exercise Test (CPET):** Exercise may make your muscles tired and possibly sore in the days following the exercise. This is generally temporary and is not harmful. As with any type of strenuous physical activity there is a very slight risk of a serious event (e.g. heart attack) during the exercise tests that you will perform, but this risk is no different than if such exercise were performed at home or local gym. In fact, it is probably safer in that you are being closely watched and exercise will be stopped immediately if there are any signs of a problem. Patients without heart disease should not experience chest pain, dizziness, or irregular heart rhythm during the exercise tests. Your heart rate, heart rhythm tracing (ECG) and amount of oxygen in your blood will be closely monitored during all tests. If, at any time during the exercise tests, you do not wish to continue for any reason, you may stop exercising. A study physician will always be available during the exercise tests to monitor you and will be available should there be any complication or if you have any questions. Emergency equipment will always be available in case of a serious event. The supervising physician may decide to terminate the exercise test at any time.

Multi-Detector Computed Tomography (or CT scan): The CT scans will be used to measure the structure of your lungs and of your abdomen (only one image) will be x-rayed. 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 lung function box and the CT scan uncomfortable. The lung function box is constructed of clear plastic so you can always see out and the technician will allow you to enter and leave the box as you need. 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).

POTENTIAL BENEFITS:

There are no direct benefits to you from participating in this study.

The indirect benefits associated with participation in the study include:

- You may have undiagnosed COPD, 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 lung 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.

What are the benefits to society?

The results of this study will for the first time provide precise information about:

- The prevalence and the evolution of all grades of COPD in the community.
- The role of smoking and non-smoking risk factors for development of COPD.
- The role of modifiable factors (exercise, nutrition) on COPD evolution and the potential for new health interventions.
- The extent of the impact of COPD on the subjects' quality of life.
- Both the direct [hospitalization, clinic visits, medications] and indirect [time loss from work by subject and caregiver, and disability compensation] economic burden of COPD in the community, and projection for the future.

FINANCIAL COMPENSATION:

As compensation towards your time, effort and travel costs you will receive $25 per yearly visit at the Montreal Chest Institute. You will also receive $20 for your visit to the Ste-Justine Hospital for your MDCT Scan or at a radiology clinic.

CONFIDENTIALITY:

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate, a representative of Health Canada and/or the MUHC Research Ethics Board and by the Research Ethics Board of Sainte-Justine University Health Center (CHU Sainte-Justine) for the purpose of monitoring the research. We will also ask you to provide us with a

second phone number of a relative who doesn't live with you (parent, sister or brother) where we will be able to reach you for the time of the study.

On papers, questionnaires, files and tests for the study, you will be identified only by a research number.

For this study, the study doctor may need to access your personal health records for health information such as past medical history and test results. He/she may also need to contact your family physician and your other health care providers to obtain additional medical information. The health information collected as part of this study will be kept confidential unless release is required by law, and will be used only for the purpose of the research study. By signing the consent form you give permission to the study staff to access any personally identifiable health information which is under the custody of other health care professionals as deemed necessary for the conduct of the research. Information furnished from the RAMQ and MedECHO data bases will be coded to ensure confidentiality.

The questionnaire(s) and the sample(s)/data collected will be used for this study only and will be kept for 25 years after completion of the study as required by Health Canada. Computer information will be password protected.

The results of your lung function tests will only be sent to your family doctor upon your request because the tests are strictly performed for research purposes.

VOLUNTARY PARTICIPATION AND WITHDRAWAL:

Your participation in the study is voluntary. You may refuse to take part or you may decide to stop at any time. If you decide to withdraw from the study, it is important that you inform the study doctor or coordinator. The doctor may also withdraw you from the study if you do not follow the study instructions, or in the unlikely event that you have a serious side effect from the study procedures. This will not affect your relationship with the study doctor, or with your regular doctor, who will continue to give you the best treatment he/she can offer. Your data will be kept but no new data will be collected.

If new information arises that may affect your willingness to remain in this study, you will be advised of this information promptly.

LEGAL RIGHTS:

You are not waiving any of your legal rights by participating in this research study, or by signing this consent form, including, for example, the right to seek damages under civil law for any research related injury.

If you suffer any injury due to a study procedure required as part of this research, you will receive all care and services needed to treat you covered by the Quebec Medicare health care system and by your drug insurance plan.

PRINCIPAL INVESTIGATOR AND RESOURCE PERSONS

Principal Investigator:
Dr. Jean Bourbeau. Telephone: 514 934-1934, ext. 32185

Research Coordinators:
Myriam Costa. Telephone: 514-934-1934, ext.32463
Palmina Mancino. Telephone: 514 934-1934, ext. 32116.

PATIENT REPRESENTATIVE OF THE HOSPITAL:

For all other questions concerning your rights pertaining to your participation in a research project, you can contact the ombudsman of the McGill University Health Center at 514 934-1934 ext. 35655. If you believe that you have been injured while participating in this study, you can contact the Director of Professional Services at 514 934-1934 ext. 34329.

SUBJECT INFORMED CONSENT- Signature Page

RESEARCH STUDY: *The **Can**adian **C**ohort **O**bstructive **L**ung **D**isease Study*

PRINCIPAL INVESTIGATOR: Dr. Jean Bourbeau, Montreal Chest Institute, Montréal

1. I understand that this is a research study.
2. I have read all the pages of the consent form. The research personnel have explained the information and procedures involved in the study. I have had the opportunity to ask questions and my questions have been answered satisfactorily. I have been given time to consider the information carefully and to decide whether or not to participate in this study.
3. I have been informed that my participation in this study is entirely voluntary and that I may refuse to participate, or withdraw at any time, without any consequences to my ongoing and future medical care in this institution.
4. I authorize the release of my medical records to the regulatory authorities and the ethics committee of this institution for purposes of this study only. This authorization will be valid for a period of 25 years.
5. I understand that I will be given a copy of this informed consent to keep for my own information, once it is signed.
6. I understand that I do not give up any of my legal rights by signing this form nor am I freeing the investigators, sponsors, or the health establishment where the study takes place from their civil and professional responsibilities.
7. My signature below indicates that I voluntarily agree to take part in this study.

Subject's signature	Name (in block letters)	Date
Signature of Person Administering Informed Consent	Name (in block letters)	Date
Investigator's Signature	Name (in block letters)	Date

SUBJECT INFORMED CONSENT- Signature Page

PROVINCIAL ADMINISTRATIVE DATABASE

RESEARCH STUDY: *The Canadian Cohort Obstructive Lung Disease Study*

PRINCIPAL INVESTIGATOR: Dr. Jean Bourbeau, Montreal Chest Institute, Montréal

The researchers from the CanCOLD study would like to have access to your RAMQ and MED ECHO data to validate their research data. Therefore, you are being asked to give the CanCOLD researchers access to your RAMQ and MED ECHO of the previous 5 years and of next 15 years from the start of the study.

Yes, I agree that you may access my RAMQ and MED ECHO of the previous 5 years and of the 15 years from the start of the study. ☐

Medicare Number: _____

No, I **do not** agree that you may access my RAMQ and MED ECHO. ☐

_____	_____	_____
Subject's signature	Name (in block letters)	Date

_____	_____	_____
Signature of Person Administering Informed Consent	Name (in block letters)	Date

_____	_____	_____
Investigator's Signature	Name (in block letters)	Date

French consent form

Centre universitaire de santé McGill
McGill University Health Centre
Les meilleurs soins pour la vie
The Best Care for Life

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Pour l'amour des enfants
Université de Montréal

INFORMATION ET FORMULAIRE DE CONSENTEMENT ÉCLAIRÉ

PROJET DE RECHERCHE : *L'étude de la cohorte canadienne de maladie pulmonaire obstructive chronique (The Canadian Cohort Obstructive Lung Disease Study)*

CHERCHEUR PRINCIPAL : Dr Jean Bourbeau, Institut Thoracique de Montréal, Montréal

ORGANISME DE FINANCEMENT : Programme de recherche en collaboration Rx&D-IRSC (IRO- 93326) en partenariat avec Astra Zeneca, GlaxoSmithKline, Pfizer et Boehringer Ingelheim et le réseau en santé respiratoire du FRSQ

BUT DU FORMULAIRE D'INFORMATION ET DE CONSENTEMENT ÉCLAIRÉ :

Le but de ce formulaire est de vous donner de l'information sur de cette étude de recherche et si vous le signez, cela signifie que vous acceptez de prendre part à l'étude. Il est très important que vous lisiez et compreniez l'information au participant ci-dessous. Ce formulaire décrit le but, les procédures, les bénéfices, et les risques inhérents à l'étude de recherche. Il peut contenir des mots que vous ne comprenez pas. S'il vous plaît, demandez au médecin ou au personnel de l'étude de vous expliquer les mots ou les procédures qui ne sont pas clairs. Vous pouvez apporter cette information à la maison et en discuter avec un membre de votre famille ou votre médecin de famille. Vous pouvez refuser de prendre part ou de vous retirer l'étude à n'importe quel moment.

INTRODUCTION:

Vous êtes invité à prendre part à cette étude de recherche parce que vous étiez un participant dans une étude antérieure intitulée « Chronic Obstructive Lung Disease » (COLD). L'étude COLD était conçue pour mesurer la maladie pulmonaire obstructive chronique (MPOC) dans la communauté. Vous êtes invité à participer parce que vous aviez indiqué que vous participeriez à des études futures sur la MPOC telles que CanCOLD.

L'étude présente, c'est-à-dire CanCOLD, est financée par les Instituts de recherche en santé du Canada (IRSC), un organisme gouvernemental, dans le cadre d'un programme de recherche en collaboration avec des partenaires de l'industrie pharmaceutique. Tous les fonds disponibles pour

L'INSTITUT THORACIQUE DE MONTRÉAL DE L'HÔPITAL ROYAL VICTORIA
MONTREAL CHEST INSTITUTE OF THE ROYAL VICTORIA HOSPITAL
3650 St. Urbain, Montréal (Québec) H2X 2P4, Tél.: (514) 934-1934

cette étude sont utilisés pour coordonner la recherche, les salaires aux assistants, les services de gestion et d'analyse des données et l'achat d'équipement de recherche.

MPOC signifie "Maladie Pulmonaire Obstructive Chronique"

- Maladie signifie une affection.
- Pulmonaire signifie dans les poumons.
- Obstructive signifie blocage partiel.
- Chronique signifie qui ne partira pas.

La MPOC est la quatrième cause principale de décès au Canada. Nos connaissances actuelles de la MPOC et de ses conséquences sur la santé des patients et sur la société sont incomplètes.

Ceci est une étude longitudinale, impliquant des hommes et des femmes qui sont âgés de 40 ans ou plus, qui pourraient être à risque de développer la MPOC ou qui ont une MPOC.
Une étude longitudinale permet aux chercheurs d'étudier les participants à intervalles réguliers au fil du temps. Les chercheurs veulent caractériser la maladie et sa progression au fil du temps. Nous vous demanderons un prélèvement sanguin additionnel (75 ml ou 5 cuillères à soupe) si vous acceptez de participer à l'étude portant sur la génétique. La période de temps pour CanCOLD est de 3 ans ou plus.

Il y a 9 sites participants. À part Montréal, les autres sites de recherche sont : Calgary, Saskatoon, Toronto, Kingston, Ottawa, Vancouver, Québec (Ste-Foy), et Halifax.

La MPOC est une maladie commune des poumons qui obstrue les voies respiratoires, rendant la respiration difficile.

- La MPOC est habituellement causée par le tabagisme ou l'exposition à des émanations ou à des endroits très poussiéreux.
- La MPOC peut être prévenue.
- Quand la MPOC se développe, elle peut être traitée, mais elle ne peut pas se guérir.
- Le plus tôt elle est détectée, meilleurs seront les résultats du traitement.

La MPOC est un problème mondial majeur et grandissant de santé publique:

- L'Organisation mondiale de la santé [OMS] estime qu'elle a causé 2.74 millions de décès en l'an 2000.
- En 1990, la MPOC était classée 12^{e} comme fardeau de maladie; d'ici 2020 il est prévu qu'elle sera classée 3^{e} globalement.

BUT DE L'ÉTUDE:

Le but de cette étude de recherche est de développer un cadre de travail pour combattre ce problème de santé majeur (MPOC), par une meilleure caractérisation de la population d'hommes et de femmes à risque et des patients en début de maladie, par une meilleure compréhension des facteurs modifiables qui sont reliés à la perception de la santé (qualité de vie reliée à la santé) et à l'évolution de la maladie via une intervention sur la santé.

PARTICIPANTS:

Nous espérons inclure 2000 personnes à travers le Canada dont 200-250 personnes du Centre universitaire de santé McGill (CUSM) de Montréal. Vous avez été sélectionné parce que vous aviez indiqué dans une étude précédente intitulée COLD que vous seriez intéressé à recevoir de l'information sur des études futures comme celle-ci.

Les sujets inclus dans cette étude font partie d'un des quatre groupes suivants :

1) Sujets en santé (sans maladie pulmonaire)
2) Sujets à risque (fumeur sans maladie pulmonaire)
3) Sujets avec MPOC légère
4) Sujets avec MPOC modérée-sévère

PROCÉDURES DE L'ÉTUDE:

Le personnel de l'étude ou le médecin en charge de l'étude vont vous expliquer avec soin toutes les procédures et à tout moment vous pourrez demander plus d'informations. Aucune procédure ne sera entamée avant que vous ne consentiez à participer à cette étude. Votre participation à cette étude va nécessiter des visites à l'Institut Thoracique de Montréal, à l'Hôpital Ste-Justine (ou dans une clinique de radiologie) et que vous répondiez à un questionnaire par téléphone ou par internet.

Visites à l'Institute thoracique de Montréal (1 visite à chaque 18 mois, pour les prochains 3 ans).

- **Questionnaires :** Des questions vous seront posées concernant vos antécédents médicaux, et familiaux; la présence de symptômes respiratoires (toux, crachats, sifflements, essoufflements), l'exposition à des facteurs de risques potentiels, les diagnostics respiratoires (asthme, emphysème, MPOC, bronchite chronique, etc.), et autres maladies. 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. Ces questionnaires prennent environ 3 heures à compléter. Dans le but de réduire le temps que vous passerez a l'Institut thoracique, vous aurez le choix de compléter certains questionnaires plus tard, par téléphone, à l'aide de la coordonatrice.

- **Tests :** Nous vous demanderons de vous soumettre à certains tests
  - un test de fonction pulmonaire (TFP). Ce test prend environ 20 min et sera effectué à la visite initiale et à la visite de 3 ans.
  - un test d'exercice cardio-pulmonaire (TECP). Ce test prend environ 45 min et sera effectué à la visite initiale et à la visite de 3 ans.
  - un prélèvement sanguin, si vous acceptez de participer à l'étude reliée, portant sur la génétique. Ce prélèvement prend environ 10 min et sera effectué à la visite initiale, à 18 mois et à 3 ans.
  - un simple test respiratoire (spirométrie). Ce test prend environ 15 min et sera effectué à chaque visite.

- le test de marche de 6 minutes. Ce test prend environ 18 min et sera effectué à la visite de 18 mois.
- Mesure de la force de préhension. Cette mesure prend environ 5 min et sera effectuée à chaque visite.

Visite à l hôpital Ste-Justine (ou dans une clinique de radiologie) pour une tomodensitométrie

Nous vous demanderons de compléter une tomodensitométrie, calculée par ordinateur, de vos poumons et abdomen. Cela aura lieu à la visite initiale si vous êtes un sujet en santé, sans maladie respiratoire et que vous n'avez jamais fumé. Sinon ce test sera effectué à la visite initiale et à la visite de 3 ans.

Prises de sang et banque de sang

À chaque visite et à jeun, du sang de votre veine sera prélevée pour mesurer des protéines (par exemple : agent tensioactif protéine-D (SDP) et protéine réactive-C (CRP)) et pour mesurer d'autres substances inflammatoires (par exemple : interleukine-6 (IL-6) et protéine sécrétoire des cellules Clara (CC16)). La quantité de sang qui sera prélevé de votre veine à chaque occasion est de 75 ml ou 5 cuillères à soupe.

De plus, nous vous demanderons de consentir à déposer votre sang et vos données personnelles dans une "banque de sang" qui servira pour des études futures sur la MPOC seulement. Si vous êtes intéressé, vous devrez signer un formulaire de consentement distinct pour cet aspect en particulier. Les prélèvements seront conservés pour des travaux futurs additionnels pour découvrir des aspects génétiques et des cibles potentielles de thérapie pour les personnes ayant une MPOC. Deux composantes génétiques, l'ADN et l'ARN, seront extraites de votre sang et conservées dans la "banque de sang". L'ADN est la molécule qui contient le code génétique de tous les organismes. L'ARN est l'acide nucléique qui est utilisé dans les procédures métaboliques clés de toutes les étapes de la synthèse de protéines dans toutes les cellules vivantes. **Il est important de comprendre que vous pouvez prendre part à l'étude principale sans prendre part à cette option de banque à long terme.**

Questionnaire par téléphone ou par internet: On va vous demander de répondre à un questionnaire par téléphone ou par internet à tous les 3 mois à propos de vos symptômes respiratoires, vos médicaments, vos visites médicales, votre travail et votre état de santé en général. Ceci vous prendra approximativement 5 à 10 minutes à compléter.

Base de données administrative des soins de santé provinciaux

Nous vous demandons votre permission pour suivre votre utilisation des soins de santé (incluant l'utilisation de médication) et les conséquences sur votre santé en permettant aux chercheurs d'obtenir des données à partir de la base de données provinciale des soins de santé. La province du Québec amasse des données pour toutes les personnes qui ont une carte d'assurance maladie. Chaque fois que vous recevez un service médical ou obtenez des médicaments par une prescription, les détails des ces contacts sont conservés par le système provincial de santé (Régie de l'assurance maladie du Québec ou RAMQ) dans des fichiers informatiques. Si vous nous

donnez la permission, les informations amassées sur la façon dont vous avez utilisé le système de santé au cours des cinq dernières années, et les informations qui seront amassées dans les 15 prochaines années seront envoyées par la RAMQ au médecin de l'étude. Ces informations seront utilisées pour voir si votre santé est affectée par la MPOC or par d'autres maladies que vous pourriez développer dans le futur. Toute information à propos de vous sera encodée de sorte que vous ne puissiez être identifié. Si vous acceptez, vous devrez signer un formulaire de consentement distinct pour cet aspect en particulier. **Il est important de comprendre que vous pouvez prendre part à l'étude principale sans prendre part à cette base de données administrative optionnelle.**

RESPONSABILITÉS DU SUJET

Si vous prenez des médicaments pour votre respiration, on vous demandera de ne pas les prendre avant certains tests (tests de fonction pulmonaire, spirométrie, test d'exercice cardio-pulmonaire et test de marche de 6 minutes). 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 un tableau qui explique combien d'heures avant les tests vous devez arrêter vos médicaments respiratoires. Vous pourrez les prendre une fois les tests complétés.

De plus, on vous demandera d'être à jeun pour vos prélèvements sanguins qui seront faits dès votre arrivée à l'hôpital.

DESCRIPTION DES QUESTIONNAIRES ET DES TESTS:

Questionnaires:
L'étude de recherche CanCOLD implique de compléter des questionnaires.
Nous vous poserons des questions concernant vos antécédents médicaux et familiaux, les médicaments que vous prenez, votre exposition professionnelle, vos antécédents tabagiques, votre qualité de vie, votre nutrition, comment vous vous sentez dans votre vie au quotidien et vos activités physiques.

Mesure de la force de préhension :
La force de préhension de la main sera obtenue par une épreuve de préhension maximale au cours de laquelle nous vous demanderons de fermer la main autour d'un dynamomètre hydraulique de main.

Tests respiratoires (Spirométrie et tests de fonction pulmonaire (TFP):

- **Spirométrie** est le nom donné au test de fonction pulmonaire et il est fait avant et après l'administration d'un agent bronchodilatateur inhalé, qui est une procédure standard pour tester la fonction pulmonaire. Vous effectuerez le test respiratoire deux fois, avant et 15 minutes après l'inhalation de 2 bouffées d'un bronchodilatateur nommé salbutamol [Ventolin®]. Ce médicament inhalé est l'agent standard utilisé par les asthmatiques et les patients ayant une MPOC pour détendre les voies aériennes et soulager les symptômes. Dans ce cas, l'utilisation de ce médicament fait partie de la procédure de routine pour le test de spirométrie chez tous les sujets, parce qu'il nous permet de mesurer votre

fonction pulmonaire à son meilleur, quand les voies aériennes sont complètement détendues.

- Nous vous demanderons d'effectuer des **tests de fonction pulmonaire complets (TFP)** en plus du test respiratoire décrit ci-haut. Ces tests impliquent que vous respiriez dans un embout buccal pendant que vous êtes assis dans une cabine de verre. Ceci nous donnera des informations complètes sur l'état de santé de vos poumons, incluant la présence d'obstruction des voies aériennes ou des changements de volume pulmonaire, et sur la capacité de vos poumons d'échanger l'oxygène et le monoxyde de carbone.

Mesure des pressions inspiratoire et expiratoire maximales (PImax et PEmax) (Visites 1 et 3) : Vos forces inspiratoire et expiratoire sont mesurées par la pression inspiratoire maximale (P_{Imax}) et la pression expiratoire maximale (PE_{max}) que vous exercerez contre une quasi-occlusion des voies aériennes

Tests d'exercice:

Nous utiliserons deux tests pour mesurer votre habileté à faire de l'exercice :

1. **Test de marche de 6minutes:** Le but de ce test est de marcher aussi loin que possible pour 6 minutes. Vous marcherez aller retour sur une surface plane tel un couloir. Six minutes est une longue durée de marche, alors cela vous demandera un effort. Vous répéterez ce test à deux ou trois reprises.
2. **Test d'exercice cardio-pulmonaire (TECP):** Ce test mesurera l'utilisation oxygène (O_2) et la production de dioxyde de carbone (CO_2) des poumons durant une période d'exercice. L'exercice sera effectué sur une bicyclette stationnaire. Dans des cas exceptionnels, et si approuvé par le chercheur principal de l'étude, le test pourrait se faire sur un tapis roulant. De plus, votre débit cardiaque sera mesuré à l'aide du logiciel Physio Flow. Pour ce faire, nous utiliserons 6 électrodes : 2 au cou et 4 sur votre corps, ce qui permettra à l'appareil d'enregistrer les réactions de votre cœur de façon continue pendant l'exercice. Les maladies qui affectent le coeur, les poumons, la circulation, le sang, entraineront une réponse anormale à l'exercice. Le test d'exercice est utile pour aider à évaluer la cause de l'essoufflement qui ne pourrait être déterminé au repos. (cœur vs. poumons). Il évalue aussi la condition physique du cœur et des poumons. Ce test est aussi plus sensible pour détecter une maladie à son stade initial que d'autres tests moins approfondis effectué au repos.

Tomographie axiale calculée par ordinateur TACO (ou tomodensitométrie ou TDM):

La tomodensitométrie sera effectuée à l'hôpital Ste-Justine (ou dans une clinique de radiologie). Pour la TDM, vous devrez enlever vos vêtements jusqu'à la taille et porter une jaquette d'hôpital. Vous vous coucherez ensuite sur une table avec les bras étendus au-dessus de votre tête. La table se déplacera ensuite à travers un appareil en forme de "beigne" pour obtenir la tomographie. La durée totale de la TDM, incluant le déshabillage, le positionnement sur la table, la TDM et le rhabillage, sera d'environ une demi-heure. La TDM comme telle prendra approximativement 5 minutes. Un technicien accrédité effectuera toutes les TDM. Les sujets ne

souffrant pas de maladie respiratoire (sujets en santé qui n'ont jamais fumé) ne seront soumis qu'à une seule TDM.

RISQUES POTENTIELS ET INCONFORT :

Questionnaires: 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.

Symptômes : tel que mentionné, on vous demandera d'arrêter vos médicaments respiratoires (si vous en avez) avant de 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. 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 (TFP): 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 à 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.

Tests en exercice:

1. Test de marche de 6minutes: Si vous devenez fatigué ou épuisé, vous pourrez ralentir, arrêter et vous reposer au besoin. Vous pourrez vous appuyer sur le mur pendant que vous vous reposez, mais reprendre la marche aussitôt que vous en serez capable.

2. Test en exercice cardio-pulmonaire (TECP): L'exercice peut rendre vos muscles fatigués et possiblement endoloris au cours des jours suivant l'exercice. Ceci est généralement temporaire et sans conséquence. Comme avec n'importe quel type d'activité physique intense, il y a un très léger risque d'évènement sérieux (par exemple une crise cardiaque) pendant le test d'exercice que vous aller exécuter, mais ce risque n'est pas différent de celui d'effectuer un tel exercice à la maison ou au gymnase local. En fait, il est probablement plus sécuritaire étant donné que vous êtes surveillé de près et que l'exercice sera arrêté immédiatement s'il y a signes d'un problème.

Les patients sans problème cardiaque ne devraient pas ressentir de douleur à la poitrine, d'étourdissement, ou de rythme cardiaque irrégulier pendant le test en exercice. Votre pouls, le tracé de votre rythme cardiaque (ECG), et la quantité d'oxygène dans votre sang seront surveillés de près durant tous les tests. Si, à n'importe quel moment durant les tests en exercice, vous ne voulez plus continuer pour n'importe quelle raison, vous pourrez arrêter l'exercice. Un médecin de l'étude sera toujours disponible pendant les tests en exercice pour vous surveiller et en cas de complication ou pour répondre à vos questions. L'équipement d'urgence sera toujours disponible en cas d'évènement sérieux. Le médecin qui supervise pourra décider d'interrompe le test en exercice à tout moment.

Tomographie axiale calculée par ordinateur TACO (ou 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 inconfortable.

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 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).

BÉNÉFICES POTENTIELS:

Il n'y a pas de bénéfices directs liés à votre participation à cette étude.

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

- Vous avez peut-être une MPOC 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 pulmonaire 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.

Quels sont les bénéfices pour la société?

Les résultats de cette étude donneront pour la première fois des informations précises sur :

- La prévalence et l'évolution de tous les stades de la MPOC dans la communauté.
- Le rôle des facteurs de risque tabagiques et non-tabagiques dans le développement de la MPOC.
- Le rôle des facteurs de risque modifiables (exercice, alimentation) sur l'évolution de la MPOC et la possibilité de nouvelles interventions en santé.
- L'étendue de l'impact MPOC de la sur la qualité de vie des sujets.
- Le fardeau économique direct [hospitalisation, visites à la clinique, médication] et indirect [absentéisme par les sujets et leurs soignants, et compensation d'invalidité] de la MPOC dans la communauté, et projections pour le futur.

COMPENSATION FINANCIÈRE:

Vous recevrez 25$ par visite annuelle à l'Institut thoracique de Montréal à titre de compensation pour votre temps, efforts et dépenses de déplacement. Vous recevrez une compensation additionnelle de 20$ pour la visite à l'hôpital Ste-Justine (ou dans une clinique de radiologie) pour votre TDM.

CONFIDENTIALITÉ:

La confidentialité de vos informations sera respectée. Aucune information qui divulgue votre identité ne sera communiquée ou publiée sans votre consentement spécifique. Cependant, les dossiers de recherche et votre dossier médical vous identifiant pourraient être inspectés en présence du chercheur ou de son représentant, d'un représentant de Santé Canada et/ou du comité d'éthique de la recherche du CUSM et par le comité d'éthique de la recherche du Centre hospitalier universitaire Sainte-Justine (**CHU** Sainte-Justine) dans le but de surveiller la recherche. Nous vous demanderons aussi de nous donner un deuxième numéro de téléphone d'un proche qui n'habite pas avec vous (parents, frère ou sœur) où nous pourrons vous joindre tout au long de l'étude.

Tous les questionnaires, dossiers, tests, et formulaires reliés à cette étude seront identifiés uniquement par un numéro de recherche.

Pour cette étude, le médecin de l'étude pourrait avoir besoin de consulter votre dossier médical concernant vos antécédents médicaux et résultats de tests. Il/elle pourrait aussi avoir besoin de contacter votre médecin de famille et autres fournisseurs de soins de santé pour obtenir des informations médicales additionnelles. L'information sur la santé amassée pour cette étude sera gardée confidentielle à moins que la divulgation ne soit requise par la loi, et sera utilisée seulement pour le cadre de cette étude de recherche. En signant ce formulaire de consentement vous donnez la permission au personnel de l'étude d'accéder à n'importe quelle information de santé identifiable qui est sous la garde d'autres professionnels de la santé et jugée nécessaire pour mener cette étude. L'information fournie par la RAMQ et les bases de données MED-ÉCHO seront codées pour assurer la confidentialité.

Les questionnaires et les échantillons/données amassés seront utilisés pour cette étude seulement et seront gardés 25 ans après l'achèvement de l'étude, tel que requis par Santé Canada. Les données informatiques seront protégées par mot de passe.

Les résultats de vos fonctions pulmonaires seront envoyés à votre médecin de famille seulement sur demande parce que ces tests sont faits pour des raisons de recherche seulement.

PARTICIPATION VOLONTAIRE ET RETRAIT:

Votre participation à l'étude est volontaire. Vous pouvez refuser de prendre part ou vous pouvez décider d'arrêter à n'importe quel moment. Si vous décidez de vous retirer de l'étude, il est important d'en informer le médecin de l'étude ou le coordonnateur. Le médecin peut aussi vous retirer de l'étude si vous ne suivez pas les instructions, ou dans l'éventualité peu probable que vous ayez un effet secondaire sérieux suite à l'une des procédures de l'étude. Ceci n'affectera pas votre relation avec le médecin de l'étude, ou avec votre médecin régulier, qui continuera de vous donner le meilleur traitement possible. Vos données sont conservées mais aucune nouvelle information ne sera alors recueillie.

Si de nouvelles informations survenaient et que celles-ci pourraient affecter votre volonté de participer à l'étude, vous en seriez informé promptement.

DROITS LÉGAUX:

Vous ne renoncez à aucun de vos droits légaux en participant à cette étude de recherche, ou en signant ce formulaire de consentement, incluant par exemple, le droit de réclamer des dommages selon le code civil pour une blessure occasionnée en lien avec l'étude.

Si vous deviez subir quelque préjudice que ce soit dû à votre participation au projet de recherche, vous recevrez tous les soins requis par votre état de santé couverts par la Régie d'assurance-maladie du Québec et par votre régime d'assurance-médicaments.

CHERCHEUR PRINCIPAL ET PERSONNES RESSOURCES

Chercheur principal: Dr Jean Bourbeau
Téléphone: 514 934-1934, poste 32185

Coordonnatrices de recherche: Myriam Costa
Téléphone : 514-934-1934 poste 32463
Palmina Mancino
Téléphone: 514 934-1934, poste 32116

REPRÉSENTANT DES PATIENTS DE L'HÔPITAL:

Pour toute autre question concernant vos droits en regard votre participation à un projet de recherche, vous pouvez contacter l'ombudsman du Centre Universitaire de Santé McGill au 514 934-1934 poste 35655. Si vous pensez avoir été blessé en participant à cette étude, vous pouvez contacter le directeur des services professionnels au 514 934-1934 poste 34329.

CONSENTEMENT ÉCLAIRÉ DU SUJET- Page de signature

PROJET DE RECHERCHE: L'étude *de la cohorte canadienne de maladie pulmonaire obstructive chronique (The Canadian Cohort Obstructive Lung Disease Study*

CHERCHEUR PRINCIPAL: Dr Jean Bourbeau, Institut thoracique de Montréal

1. Je comprends qu'il s'agit d'un projet de recherche.
2. J'ai lu toutes les pages du formulaire de consentement. Le personnel de recherche m'a donné les informations et m'a expliqué les procédures utilisées dans cette étude. J'ai eu l'occasion de poser des questions et j'ai obtenu des réponses satisfaisantes. J'ai eu le temps de réfléchir tranquillement à ces informations et de décider de participer ou non à cette étude.
3. J'ai été informé que ma participation à cette étude est entièrement volontaire et que je peux refuser d'y participer ou m'en retirer à tout moment sans aucune conséquence sur mes soins médicaux en cours ou à venir dans cette institution.
4. J'autorise les autorités réglementaires et le comité d'éthique de cet établissement à consulter mon dossier médical aux fins de cette étude seulement. Cette autorisation est valide pour une période de 25 ans.
5. Je comprends que je recevrai une copie de ce formulaire de consentement pour mes dossiers personnels, lors qu'il sera signé.
6. Je comprends que je ne renonce à aucun de mes droits légaux en signant ce formulaire et que je ne libère aucun des chercheurs, commanditaires ni l'établissement de santé où a lieu l'étude, de leurs responsabilités civiles et professionnelles.
7. Ma signature ci-dessous indique que j'accepte volontairement de participer à cette étude.

Signature du sujet	Nom (lettres moulées)	Date
Signature de la personne obtenant le consentement	Nom (lettres moulées)	Date
Signature du chercheur	Nom (lettres moulées)	Date

CONSENTEMENT ÉCLAIRÉ DU SUJET- Page de signature

BASE DE DONNÉES ADMINISTRATIVES PROVINCIALE

PROJET DE RECHERCHE : *L'étude de la cohorte canadienne de maladie pulmonaire obstructive chronique (The Canadian Cohort Obstructive Lung Disease Study)*

CHERCHEUR PRINCIPAL : Dr Jean Bourbeau, Institut thoracique de Montréal

Les chercheurs de l'étude CanCOLD aimeraient avoir accès à vos données RAMQ et MED-ÉCHO pour valider leurs données de recherche. Pour cette raison, nous vous demandons de donner accès aux chercheurs de CanCOLD à vos données RAMQ et MED-ÉCHO des 5 années précédentes et des 15 prochaines années à partir du début de l'étude.

Oui, j'accepte que vous ayez accès à mes données RAMQ et MED-ÉCHO des 5 années précédentes et des 15 années suivant le début de l'étude. ☐

Numéro d'assurance maladie: _____

Non, je **n'**accepte **pas** que vous accédiez à mes données RAMQ et MED-ÉCHO. ☐

_____	_____	_____
Signature du sujet	Nom (lettres moulées)	Date
_____	_____	_____
Signature de la personne obtenant le consentement	Nom (lettres moulées)	Date
_____	_____	_____
Signature du chercheur	Nom (lettres moulées)	Date

COPD EXACERBATIONS: UNDERSTANDING THE COURSE OF CHANGE AND RECOVERY IN EXTRA-PULMONARY OUTCOMES CONSENT FORMS

English consent form-hospitalized patients

HOSPITALIZED PATIENT INFORMATION AND CONSENT FORM

Title: COPD exacerbations: Understanding the course of change and recovery in extra-pulmonary outcomes

Principal investigator: J. Bourbeau MD
Co-investigators: S. Daskalopoulou MD/PhD, C. Baglole PhD
Student: L. Labonté (PhD candidate under the supervision of Dr. Bourbeau)
Collaborator: P. Ernst MD
Other study staff: K. Metz, M. Patel
Institutions: Montreal Chest Institute/ Montreal General Hospital/ Jewish General Hospital

The pharmaceutical company funding this study is GlaxoSmithKline.

1. PURPOSE OF THE SUBJECT INFORMATION AND CONSENT FORM

You are being invited to take part in a research study involving patients with chronic obstructive pulmonary disease (COPD). Before you decide to participate in this study, it is important for you to understand why the research is being done and what it will involve. The purpose of this form is to provide you with information about this research study and if you sign it, it means that you have agreed to take part in the study. Please take the time to read the following information carefully and discuss it with another person or your family doctor if you wish. You may also request that another person be given this information and a copy of this consent form. The form describes the purpose, procedures, benefits and risks of the research study. It may contain words you do not understand. Please ask the study doctor or study staff to explain any words or procedures that you do not clearly understand. You may refuse to take part or withdraw from this study at any time, without affecting your medical care at this institution.

You should only sign the consent form to participate if you have fully read and understood how the study will work, what the study doctor and the study coordinator expect you to do, and any possible benefits and risks that may result from your participation. Participation is voluntary.

2. INTRODUCTION

You are asked to participate in a research project at the Montreal Chest Institute/Montreal General Hospital/Jewish General Hospital because you have an established COPD and are currently hospitalized due to a severe COPD exacerbation.

The purpose of this study is to examine how a COPD exacerbation affects certain chemicals and cells that are part of your immune system, and how in turn, these may affect the health of your blood vessels. This information will be essential in planning a clinical trial that will test a new therapy for exacerbations that will be beneficial for the lungs and potentially for other parts of the body such as blood vessels and the heart.

3. STUDY DESIGN

A maximum of 40 subjects will be enrolled in the study from the Montreal Chest Institute (MCI), Montreal General hospital (MGH) and Jewish General Hospital (JGH). Of the hospitalized patients from the MCI, a maximum of 30 patients will be recruited prior to hospitalization at the day-hospital based on a known history of frequent-exacerbations.

This study will last a total of 6 months and if you accept to participate in this study, you will be required to participate in a total of about 9 clinical assessments (depending on the length of your hospitalization, where if you are hospitalized for longer you will have more assessments. These are described in more detail in the next section).

This is an observational study, which means that we will not be modifying the treatment you normally require to treat either your COPD or COPD exacerbations. If you agree to participate, we will assess you as you are having an exacerbation and throughout your recovery after your exacerbation. These assessments are relatively non-invasive. Each assessment will include an interview, lung function test, blood sample collection, a very basic physical examination, and an assessment of the health of your blood vessels. The length of time and procedures to be done at each assessment are described in more detail in the next section.

4. STUDY PROCEDURES

Evaluation during an exacerbation (assessments 1, 2 and 3):

You will be assessed during your hospitalization due to a severe exacerbation at the Montreal Chest Institute/Montreal General Hospital/Jewish General Hospital. You will first be assessed within 48 (±24) hours of your hospitalization. You will then be assessed every 72 (±24) hours during the duration of your hospitalization (assessments 2 a, b and possibly more depending on the length of your hospitalization), at 1 week after the beginning on your exacerbation and every week thereafter for 1 month since your first assessment (assessments 3 a, b, c, d, assuming you are hospitalized for less than a week. If your hospitalization is longer you will only come once a week during the weeks after you are discharged up to 1 month since your first assessment). These assessments will last about 1-1.5 hours and the following procedures will be done:

- An interview will be conducted, where you will be asked about the history of your COPD and to review your complete medical history including medication that you are taking, and any medications you have taken during the last 3 months prior to this assessment. It is important to mention all the medications you are taking, including herbal or "natural" remedies.

- A lung function test will be performed. This measures how much air is in your lungs and how well the air moves in and out of your lungs when you breathe. You will be asked to blow into the mouthpiece of a measuring device called a spirometer. The procedure will be explained in detail before the test (assessment 1). If you smoke, you will be asked about the time of your last cigarette.

- Blood samples will be collected. Approximately 35 mL (equal to about 7 teaspoons) of blood will be drawn from a vein in your arm and used to measure chemicals and cells produced by your immune system.

- A physical examination will be conducted. This will include assessing your vital signs (blood pressure and heart rate) and measuring the oxygen content in your blood (using a probe that will be placed on your index finger).

- The health of your arteries will be assessed. This will be done after you have been lying down and resting. You will be asked not to sleep or speak while measurements are being taken. Electrodes will be placed on you so that an ECG can be performed. This will measure your heart rate. A pressure sensor that can measure blood flow will be applied to the surface of your skin and gently pressed against it near an artery. This measurement will be taken at 3 places: near your wrist, on your neck and at the inner thigh.

Follow-up during recovery (assessments 4 and 5)

You will be re-assessed at 3 and 6 months after the beginning of your exacerbation (assessment 1). During these assessments, the study staff will review your health and medical information since your last assessment and exacerbation.

This will last about 1-1.5 hours and will be scheduled following your last assessment. The same procedures as in your other assessments will be repeated.

Assessment timeline and summary

Assessment	Duration	Procedures
1: beginning of exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries

2 (a & b and possibly more depending on the duration of your hospitalization): every 72 (±24) hours	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries
3 (a, b, c, d, possibly less depending on the duration of your hospitalization): every week up to 1 month since assessment 1	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries
4: 3 months post-exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries
5: 6 months post-exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries

5. PATIENT RESPONSIBILITIES

- You must refrain from strenuous activity for at least 12 hours prior to any assessment.
- You must refrain from smoking for at least 12 hours prior to any assessment. You will be asked to record the time of your last cigarette before you have the lung function tests.
- You must refrain from consuming coffee, tea, chocolate, cola and other caffeine-containing beverages and foods the morning of, or during any assessment.

Decaffeinated beverages are acceptable.

- You should avoid exposure to cold temperatures, environmental smoke, dust or areas with strong odours (such as perfumes) before any assessment.

6. Potential Risks and Discomforts

Lung function test:
Discomfort is unusual; however, some people experience headache and/or a sense of dizziness when performing these tests; these symptoms are usually temporary. Should this occur, you may receive treatment.

Blood sample collection:
Blood sampling from a vein in the arm is routine. It may cause some minor pain, bleeding and/or bruising at the puncture site. Other risks include a temporary feeling of light-headedness and rarely, infection at the site of puncture.

7. Potential Benefits

You may not personally benefit from participating in this study, but you may contribute new information that may benefit other people and provide the medical community with information about the treatment of COPD. This research may provide valuable information in preparing for a larger study that will assess the effectiveness of a new treatment that could improve the lungs and other parts of the body affected by COPD exacerbations. You may benefit from having a physical examination and laboratory tests during the study.

8. Compensation and Costs

For every assessment **after** you have been discharged from the hospital, you will receive 50$ to cover parking/travel costs.

9. CONFIDENTIALITY

The study coordinator and the principal investigators will have access to your medical file to verify or complete heath information such as past medical history and test results. All the records identifying you will be kept confidential at all times except where required by law and will be kept at the MCI in a locked room in a locked cabinet. Only the principal investigator (Dr. Jean Bourbeau) and his PhD student (Laura Labonté) will have access to this cabinet. On documents, your initials and assigned patient number will identify you and only the principal investigator (Dr. Jean Bourbeau) will keep a record of your name. All information contained in reports or publications issued as a result of this study will be coded and presented in such a way that your identity will not be revealed. Study data will be kept for 25 years and may be accessible to a third party. Biological samples will be kept up to 5 years after analyses. If required, auditors and representatives of the hospital or McGill research ethics board may access the study data to ensure the ethical conduct of this study.

10. VOLUNTARY PARTICIPATION AND WITHDRAWAL

It is entirely up to you whether or not to participate in this research study. If you decide not to participate, your current and future medical care at this institution will not be affected by this choice. You may decide to withdraw from this study at any time. Your participation in this study may be terminated by you or your study doctor if need be.

11. INCIDENTAL HEALTH RELATED FINDINGS

You will be informed of any findings that come up during an assessment or analysis of biological samples that indicate a potential health problem. You will be able to obtain a copy of these results to take to your family doctor for further discussion.

12. PERSONS TO CONTACT

You have the right at any time to request information from the study physician about your condition. You may also request that your personal doctor be given this information, findings from this study and a copy of this form.
If you have any questions during the study please contact:

Primary investigator: Dr. Jean Bourbeau: 514-934-1934 ext. 32185
Research technician: Katrina Metz 514-934-1934 ext. 32489
PhD student: Laura Labonté: 514-882-1074

If you have any questions about your rights as a research subject you may contact the Ombudsman of the:
Montreal Chest Institute (514) 934-1934 ext. 35655
Montreal General Hospital (514) 934-8306
Jewish General Hospital (514) 340-8222 ext. 5833

PATIENT INFORMED CONSENT

SIGNATURE PAGE

COPD exacerbations: Understanding the course of change and recovery in extra-pulmonary outcomes

Principal Investigator: Dr. Jean Bourbeau

1. I am aware that this is a research study.
2. I have read all the pages of the consent form. The research personnel have explained the information and procedures involved in the study. I have had the opportunity to ask questions and my questions have been answered satisfactorily. I have been given time to consider the information carefully and to decide whether or not to participate in this study.
3. I have been informed that my participation in this study is entirely voluntary and that I may refuse to participate, or withdraw at any time, without any consequences to my ongoing medical care at this institution.
4. I authorize access to my medical records by study investigators, as well as the regulatory authorities and the ethics committee of this institution for purposes of this study only. This authorization will be valid for a period of 25 years.
5. I am aware that I will be given a copy of this informed consent to keep for my own information, once it is signed. I have been informed that a copy of this consent form will be placed in my medical chart so that health care providers at this institution will know that I am participating in a study and what is involved.
6. I have been informed that I do not give up any of my legal rights by signing this form nor am I freeing the investigators, sponsors, or the health establishment where the study takes place from their civil and professional responsibilities.
7. My signature below indicates that I voluntarily agree to take part in this study.

_____	_____	_____
Subject's signature	Name (in block letters)	Date
_____	_____	_____
Signature of Person Administering Informed Consent	Name (in block letters)	Date

I confirm having met with the subject at the time of the enrolment and that I have answered his/her questions about this study.

_____	_____	_____
Investigator's signature	Name (in block letters)	Date

English consent form- day-hospital patients

PATIENTS ADMITTED TO THE DAY HOSPITAL INFORMATION AND CONSENT FORM

COPD exacerbations: Understanding the course of change and recovery in extra-pulmonary outcomes

Principal investigator: J. Bourbeau MD
Co-investigators: S. Daskalopoulou MD/PhD, C. Baglole PhD
Student: L. Labonté (PhD candidate under the supervision of Dr. Bourbeau)
Collaborator: P. Ernst
Other study staff: K. Metz, M. Patel
Institution: Montreal Chest Institute

The pharmaceutical company funding this study is GlaxoSmithKline.

1. PURPOSE OF THE SUBJECT INFORMATION AND CONSENT FORM

You are being invited to take part in a research study involving patients with chronic obstructive pulmonary disease (COPD). Before you decide to participate in this study, it is important for you to understand why the research is being done and what it will involve. The purpose of this form is to provide you with information about this research study and if you sign it, it means that you have agreed to take part in the study. Please take the time to read the following information carefully and discuss it with another person or your family doctor if you wish. You may also request that another person be given this information and a copy of this consent form. The form describes the purpose, procedures, benefits and risks of the research study. It may contain words you do not understand. Please ask the study doctor or study staff to explain any words or procedures that you do not clearly understand. You may refuse to take part or withdraw from this study at any time, without affecting your medical care at this institution.

You should only sign the consent form to participate if you have fully read and understood how the study will work, what the study doctor and the study coordinator expect you to do, and any possible benefits and risks that may result from your participation. Participation is voluntary.

2. INTRODUCTION

You are being asked to participate in a research project at the Montreal Chest Institute because you have an established COPD.

The purpose of this study is to examine how a COPD exacerbation affects certain chemicals and cells that are part of your immune system, and how in turn, these may affect the health of your blood vessels. This information will be essential in planning a clinical trial that will test a new therapy for exacerbations that will be beneficial for the lungs and potentially for other parts of the body such as blood vessels and the heart.

3. STUDY DESIGN

A maximum of 40 subjects will be enrolled in the study from the Montreal Chest Institute (MCI), Montreal General hospital (MGH) and Jewish General Hospital (JGH). Of the hospitalized patients from the MCI, a maximum of 30 patients will be recruited prior to hospitalization at the day-hospital based on a known history of frequent-exacerbations.

This study will last a total of 6 months and if you accept to participate in this study, you will be required to come for a total of about 10 clinical assessments (depending on the length of your hospitalization, where if you are hospitalized for longer you will have more assessments. These are described in more detail in the next section).

This is an observational study, which means that we will not be modifying the treatment you normally require to treat either your COPD or COPD exacerbations. If you agree to participate, we will assess you as you are having an exacerbation and throughout your recovery after your exacerbation. These assessments are relatively non-invasive. Each assessment will include an interview, lung function test, blood sample collection, a very basic physical examination, and an assessment of the health of your blood vessels. You will also be required to perform a very basic exercise test for some assessments. The length of time and procedures to be done at each assessment are described in more detail in the next section.

4. STUDY PROCEDURES

Initial evaluation in stable disease state (assessment 1):

You will be required to come to the Montreal Chest Institute for a baseline assessment that will be scheduled after you have provided informed consent, if you decide to participate in this study.

The first assessment will last about 2 hours and the following procedures will be done:

- An interview will be conducted, where you will be asked about the history of your COPD and to review your complete medical history including medication that you are taking, and any medications you have taken during the last 3 months prior to this assessment. It is important to mention all the medications you are taking, including herbal or "natural" remedies.

- A lung function test will be performed. This measures how much air is in your lungs and how well the air moves in and out of your lungs when you breathe. You will be asked to blow into the mouthpiece of a measuring device called a spirometer. The procedure will be explained in detail before the test (assessment 1). If you smoke, you will be asked about the time of your last cigarette.

- Blood samples will be collected. Approximately 35 mL (equal to about 7 teaspoons) of blood will be drawn from a vein in your arm and used to measure chemicals and cells produced by your immune system.

- A physical examination will be conducted. This will include assessing your vital signs (blood pressure and heart rate) and measuring the oxygen content in your blood (using a probe that will be placed on your index finger).

- The health of your arteries will be assessed. This will be done after you have been lying down and resting. You will be asked not to sleep or speak while measurements are being taken. Electrodes will be placed on you so that an ECG can be performed to record your heart rate. A pressure sensor that can measure blood flow will be applied to the surface of your skin and gently pressed against it near an artery. This measurement will be taken at 3 places: near your wrist, on your neck and at the inner thigh.

- You will be asked to perform 1 relatively short standard exercise test supervised by members of the study staff. You will be asked to pedal on a stationary bicycle at **increasing** intensities (incremental exercise test) for as long as you possibly can. While you exercise, you will have to wear a mouthpiece to allow us to measure the air you are breathing in and out of your lungs. We will also assess your vital signs (heart rate and blood pressure) and the oxygen in your blood (using a probe that will be placed on your index finger) throughout the exercise test. When you are done, you will be able to rest and lie down for 35 minutes while we assess your blood vessels and vital signs.

Follow-up during an exacerbation (assessments 2, 3 and 4)

You will be required to come to the Montreal Chest Institute within 72 hours of the beginning of your exacerbation (assessment 2), and you will be assessed every 72 (±24) hours during the duration of your hospitalization (assessments 3 a, b and possibly more depending on the length of your hospitalization), and then 1 week after the beginning of your exacerbation and every week thereafter for 1 month since your first assessment (assessments 4 a, b, c, d, assuming you are hospitalized for less than a week. If your hospitalization is longer you will only come once a week during the weeks after you are discharged up to 1 month since your first assessment). You will be required to perform the incremental exercise test as you performed at your baseline assessment (described above) one month after the beginning of your exacerbation (assessment 4 d), this assessment will take 2 hours and will follow the same procedure as assessment 1.

Assessments 4a, 4b and 4c will last about 1-1.5 hours and will follow the same procedures as assessment 1 except for the exercise tests.

Follow-up during recovery (assessments 5, and 6)

You will be re-assessed at 3, and 6 months after the beginning of your exacerbation (assessment 2). During these assessments, the study staff will review your health and medical information since your last assessment and exacerbation. Assessment 5, at 3 months will also require you to perform an incremental exercise test. This assessment will take 2 hours and will follow the same procedure as assessment 1.

Assessments 6 will last about 1-1.5 hours and will be scheduled following your last assessment. The same procedures as in your other assessments will be repeated, except for the exercise tests, which you will not have to repeat.

Assessment timeline and summary

Assessment	Duration	Procedures
1: baseline	2 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries - Exercise test
2: beginning of exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries
3 (a & b and possibly more depending on the duration of your hospitalization): every	1-1.5 hours	- Interview - Lung function test - Blood sample collection

72 (±24) hours		- Physical examination - Assessment of arteries
4 (a, b & c): 1, 2 & 3 weeks post-exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries
4 (d): 1 month post-exacerbation	2 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries - Exercise test
5: 3 months post-exacerbation	2 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries - Exercise test
6: 6 months post-exacerbation	1-1.5 hours	- Interview - Lung function test - Blood sample collection - Physical examination - Assessment of arteries

5. Patient Responsibilities

- You must refrain from strenuous activity for at least 12 hours prior to any assessment.
- You must refrain from smoking for at least 12 hours prior to any assessment. You will be asked to record the time of your last cigarette before you have the lung function tests.
- You must refrain from consuming coffee, tea, chocolate, cola and other caffeine-containing beverages and foods the morning of, or during any assessment. Decaffeinated beverages are acceptable.
- You should avoid exposure to cold temperatures, environmental smoke, dust or areas with strong odours (such as perfumes) before any assessment.

6. Potential Risks and Discomforts

Lung function test:
Discomfort is unusual; however, some people experience headache and/or a sense of dizziness when performing these tests; these symptoms are usually temporary. Should this occur, you may receive treatment.

Blood sample collection:
Blood sampling from a vein in the arm is routine. It may cause some minor pain, bleeding and/or bruising at the puncture site. Other risks include a temporary feeling of light-headedness and rarely, infection at the site of puncture.

7. Potential Benefits

You may not personally benefit from participating in this study, but you may contribute new information that may benefit other people and provide the medical community with information about the treatment of COPD. This research may provide valuable information in

preparing for a larger study that will assess the effectiveness of a new treatment that could improve the lungs and other parts of the body that may be affected by COPD exacerbations. You may benefit from having a physical examination and laboratory tests during the study.

8. COMPENSATION AND COSTS

You will be reimbursed 50$ per assessment to cover your parking/travel costs, except for the duration of your hospitalization.

9. CONFIDENTIALITY

The study coordinator and the principal investigators will have access to your medical file to verify or complete heath information such as past medical history and test results. All the records identifying you will be confidential at all times except where required by law and will be kept at the MCI in a locked room in a locked cabinet. Only the principal investigator (Dr. Jean Bourbeau) and his PhD student (Laura Labonté) will have access to this cabinet. On documents, your initials and assigned patient number will identify you and only the principal investigator (Dr. Jean Bourbeau) will keep a record of your name. All information contained in reports or publications issued as a result of this study will be coded and presented in such a way that your identity will not be revealed. Study data will be kept for 25 years and may be accessible to a third party. Biological samples will be kept up to 5 years after analyses. If required, auditors and representatives of the hospital or McGill research ethics board may access the study data to ensure the ethical conduct of this study.

10. VOLUNTARY PARTICIPATION AND WITHDRAWAL

It is entirely up to you whether or not to participate in this research study. If you decide not to participate, your current and future medical care at this institution will not be affected by this choice. You may decide to withdraw from this study at any time. Your participation in this study may be terminated by you or your study doctor if need be.

11. INCIDENTAL HEALTH RELATED FINDINGS

You will be informed of any findings that come up during an assessment or analysis of biological samples that indicate a potential health problem. You will be able to obtain a copy of these results to take to your family doctor for further discussion.

12. PERSONS TO CONTACT

You have the right at any time to request information from the study physician about your condition. You may also request that your personal doctor be given this information, findings from this study and a copy of this form.
If you have any questions during the study please contact:

Primary investigator: Dr. Jean Bourbeau: 514-934-1934 ext. 32185
Research technician: Katrina Metz 514-934-1934 ext. 32489
PhD student: Laura Labonté: 514-882-1074

If you have any questions about your rights as a research subject you may contact the Ombudsman of the **Montreal Chest Institute** (514) 934-1934 ext. 35655.

PATIENT INFORMED CONSENT

SIGNATURE PAGE

COPD exacerbations: Understanding the course of change and recovery in extra-pulmonary outcomes

Principal Investigator: Dr. Jean Bourbeau

1. I am aware that this is a research study.
2. I have read all the pages of the consent form. The research personnel have explained the information and procedures involved in the study. I have had the opportunity to ask questions and my questions have been answered satisfactorily. I have been given time to consider the information carefully and to decide whether or not to participate in this study.
3. I have been informed that my participation in this study is entirely voluntary and that I may refuse to participate, or withdraw at any time, without any consequences to my ongoing medical care at this institution.
4. I authorize access to my medical records by study investigators, as well as the regulatory authorities and the ethics committee of this institution for purposes of this study only. This authorization will be valid for a period of 25 years.
5. I am aware that I will be given a copy of this informed consent to keep for my own information, once it is signed. I have been informed that a copy of this consent form will be placed in my medical chart so that health care providers at this institution will know that I am participating in a study and what is involved.
6. I have been informed that I do not give up any of my legal rights by signing this form nor am I freeing the investigators, sponsors, or the health establishment where the study takes place from their civil and professional responsibilities.
7. My signature below indicates that I voluntarily agree to take part in this study.

_____	_____	_____
Subject's signature	Name (in block letters)	Date

_____	_____	_____
Signature of Person Administering Informed Consent	Name (in block letters)	Date

I confirm having met with the subject at the time of the enrolment and that I have answered his/her questions about this study.

_____	_____	_____
Investigator's signature	Name (in block letters)	Date

French consent form- hospitalized patients

FORMULAIRE D'INFORMATION ET DE CONSENTEMENT DES PATIENTS HOSPITALISÉS

Titre: Exacerbation de MPOC: Comprendre le cours des changements et du rétablissement des issues extra-pulmonaires

Investigateur Principal: J. Bourbeau MD
Co-investigateurs: S. Daskalopoulou MD/PhD, C. Baglole PhD
Etudiante: L. Labonté (candidate au doctorat sous la supervision du Dr. Bourbeau)
Collaborateur: P. Ernst MD
Autres: K. Metz, M. Patel
Institutions: Institut Thoracique de Montréal/ Hôpital Général de Montréal / Hôpital Général Juif

GlaxoSmithKline est la compagnie pharmaceutique qui finance cette étude.

1. **BUT DU FORMULAIRE D'INFORMATION ET DE CONSENTEMENT**

Vous êtes invité à participer à une étude de recherche sur les patients qui ont la maladie pulmonaire obstructive chronique (MPOC). Avant que vous décidiez de participer à cette étude, il est important que vous compreniez pourquoi cette étude est entreprise et qu'est ce qu'elle implique. pour vous, si vous y participez. Le but de ce formulaire est de vous offrir l'information à propos de cette étude, et si vous le signez, cela signifie que vous acceptez de participez à l'étude. Nous vous prions de prendre le temps de bien lire l'information qui suit et d'en parler avec une autre personne ou votre médecin de famille si vous le désirez. Vous pouvez aussi demander que cette information et une copie de ce consentement soient données à quelqu'un d'autre. Le formulaire décrit le but, les procédures, les bénéfices et risques de cette étude de recherche. Il se peut qu'il contienne des mots que vous ne comprenez pas. Nous vous prions de demander au personnel de l'étude d'expliquer les mots ou procédures que vous ne comprenez pas clairement. Vous pouvez refuser de participer ou vous retirez de l'étude n'importe quand, sans que cela n'affecte les soins de santé que vous recevez à cette institution. Vous ne devriez signer ce formulaire de consentement pour participer à cette étude que si vous avez tout lu et compris le déroulement de l'étude, les attentes du médecin et du personnel de cette étude envers vous, et tous bénéfices et risques potentiels qui peuvent résulter de votre participation. La participation est volontaire.

2. INTRODUCTION

Vous êtes invité à participer à cette étude de recherche parce que vous avez une MPOC reconnu et que vous êtes présentement hospitalisé à cause d'une exacerbation sévère de la MPOC.

Le but de cette étude est d'examiner comment les exacerbations de la MPOC affectent certains produits chimiques et certaines cellules qui font partie de votre système immunitaire, et comment ceux-ci peuvent conséquemment affecter la santé de vos vaisseaux sanguins. Cette information sera essentielle pour la planification d'une étude clinique qui évaluera une nouvelle thérapie contre les exacerbations qui sera bénéfique pour les poumons et potentiellement pour d'autres parties du corps comme les vaisseaux sanguins et le cœur.

3. DESIGN DE L'ÉTUDE

Un maximum de 40 patients de l'Institut Thoracique de Montréal (ITM), de l'Hôpital Général de Montréal (HGM) et de l'Hôpital Général Juif (HGJ) participeront à cette étude. Parmi les patients hospitalisés à l'ITM, un maximum de 30 patients ayant des exacerbations fréquentes seront recrutés avant leur hospitalisation à l'hôpital de jour.

Cette étude durera un total de 6 mois, et si vous acceptez d'y participer, vous devrez participer à un total d'à peu prêt 9 évaluations cliniques (dépendant de la durée de votre hospitalisation, où si vous êtes hospitalisés pendant plus longtemps, vous aurez plus d'évaluations. Les évaluations sont décrites en plus de détails dans la section suivante).

Ceci est une étude observationnelle, ce qui veut dire qu'on ne modifiera pas le traitement que vous recevez normalement pour votre MPOC ou vos exacerbations de la MPOC. Si vous acceptez de participer, nous vous évaluerons lorsque vous avez une exacerbation et lors de votre rétablissement suite à l'exacerbation. Ces évaluations sont relativement non-invasives. Chaque évaluation inclut une entrevue, un test de fonction pulmonaire, la collection d'échantillons de sang, un examen physique sommaire de base, et une évaluation de la santé de vos vaisseaux sanguins. La durée et la nature des procédures qui seront faites sont décrites

plus en détails dans la prochaine section.

4. PROCÉDURES DE L'ÉTUDE

Évaluation durant une exacerbation (évaluations 1, 2 et 3):

Vous serez évalué lors de votre hospitalisation à cause d'une exacerbation sévère de MPOC à l'Institut Thoracique de Montréal/ Hôpital Général de Montréal / Hôpital Général Juif. Vous serez évalué une première fois, dans les premières 48 (±24) heures de votre hospitalisation. Vous serez réévalué tous les 72 (±24) heures durant votre hospitalisation (évaluations 2 a, b, et possiblement plus, dépendant de la durée de votre hospitalisation), à 1 semaine après le début de votre exacerbation et toutes les semaines suivantes, pendant 1 mois depuis votre première évaluation clinique (évaluations 3 a, b, c, d, en prenant pour acquis que vous êtes hospitalisé pendant moins qu'une semaine. Si votre hospitalisation est plus longue, vous viendrez seulement une fois par semaine pendant les semaines après que vous avez quitté l'hôpital jusqu'à ce que ça fasse 1 mois depuis votre première évaluation clinique). Ces évaluations dureront environ 1-1.5 heures et les procédures suivantes seront effectuées :

- Une entrevue, qui couvrira votre historique de MPOC et révisera votre historique médicale complète incluant les médicaments que vous prenez présentement, et tous médicaments que vous avez pris lors des 3 derniers mois. Il est important de mentionner tous les médicaments que vous prenez incluant les médicaments à base d'herbes ou naturels.

- Test de fonction pulmonaire. Ceci mesure combien d'air est dans vos poumons et nous dit avec quel aisance cet air circule dans vos poumons quand vous respirez. On vous demandera de souffler dans une pièce buccale qui mesure les volumes d'air, appelée un spiromètre. Cette procédure vous sera expliquée en détail avant qu'elle soit exécutée (évaluation 1). Si vous fumez, on vous demandera l'heure de votre dernière cigarette.

- Collecte d'échantillons de sang. Environ 35 mL (c'est à dire environ 7 cuillères à thé) de sang seront recueillis d'une veine de votre bras et utilisés pour évaluer les produits chimiques et cellules produits par votre système immunitaire.

- Un examen physique. Celui-ci inclura l'évaluation de vos signes vitaux (pression sanguine, rythme cardiaque) et la mesure du taux d'oxygène dans votre sang (en utilisant une sonde placée sur votre doigt).

- La santé de vos artères sera évaluée. Ceci sera accompli après que vous ayez été couché et que vous êtes reposé. On vous demande de ne pas dormir ou parler lorsque les mesures sont prises. Des électrodes seront placées sur vous pour qu'un électrocardiogramme puisse être fait. Ceci mesurera votre rythme cardiaque. Un senseur de pression qui peut mesurer l'écoulement sanguin sera appliqué à la surface de votre peau et pressé doucement près d'une artère. Cette mesure sera répétée à 3 endroits : proche de votre poignet, sur votre cou et à l'intérieur de votre cuisse.

Suivi durant le rétablissement (évaluations 4 et 5)

Vous serez ré-évalué à 3 mois et 6 mois après le début de votre exacerbation (évaluation 1). Durant ces évaluations le personnel de l'étude révisera votre état de santé et vos informations médicales depuis votre dernière évaluation et exacerbation.

Ces évaluations dureront environ 1-1.5 heures à des dates déterminées après votre dernière évaluation. Les mêmes procédures qui ont été effectuées lors de vos autres évaluations seront répétées.

Horaire et résumé des évaluations

Évaluations	Durée	Procédures
1: début de l'exacerbation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
2 (a & b et possiblement plus, dépendant de la durée de votre hospitalisation): tous les 72 (±24) heures	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
3 (a, b, c, d, possiblement moins dépendant de la durée de votre hospitalisation): chaque semaine pendant 1 mois depuis votre 1ère évaluation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
4: 3 mois post-exacerbation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
5: 6 mois post-exacerbation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères

5. RESPONSABILITÉS DU PATIENT

- Vous ne devez pas faire d'activité physique vigoureuse pendant au moins 12 heures avant une évaluation.

- Vous ne devez pas fumer au moins 12 heures avant une évaluation. On vous demandera d'enregistrer l'heure de votre dernière cigarette avant votre test de fonction pulmonaire.
- Vous ne devez pas consommer de café, thé, chocolat, cola et autres breuvages ou nourriture contenant de la caféine les matins d'une évaluation ni durant une évaluation. Les breuvages décaféinés sont acceptables.

- Vous devriez éviter d'être exposé à des températures froides, un environnement où il y a de la fumée, de la poussière ou des endroits avec des odeurs fortes (comme des parfums) avant une évaluation.

6. RISQUES ET INCONFORTS POTENTIELS

Test de fonction pulmonaire:
L'inconfort est inhabituel; par contre certaines personnes ressentent des maux de tête et/ou des étourdissements quand ces tests sont effectués; ces symptômes sont habituellement temporaires. Si vous les éprouvez, vous pourrez recevoir un traitement.

Collecte d'échantillons de sang:
La collecte de sang d'une veine du bras est routinière. Ceci peut vous causer une douleur minime, un saignement et/ou des meurtrissures au site de la piqûre. D'autres risques incluent une sensation temporaire d'étourdissement et rarement, une infection au site de la piqûre.

7. BÉNÉFICES POTENTIELS

Il est possible que vous ne bénéficiiez pas vous-même de votre participation à cette étude, mais vous pourriez contribuer de l'information nouvelle qui pourrait bénéficier à d'autres personnes

et à la communauté médicale pour le traitement de la MPOC. Cette recherche peut offrir de l'information valable pour la préparation d'une plus grande étude qui évaluera l'efficacité d'un nouveau traitement qui pourrait améliorer les poumons et d'autres parties du corps affectés par les exacerbations de la MPOC. Il est possible que vous bénéficiiez du fait d'avoir des examens physiques ainsi que des tests de laboratoires durant l'étude.

8. COMPENSATION ET COÛTS

Pour chaque évaluation **après** votre sortie de l'hôpital, vous recevrez 50$ pour couvrir les frais de stationnement/voyage.

9. CONFIDENTIALITÉ

Le coordonnateur de l'étude ainsi que l'investigateur principal auront accès à vos dossiers médicaux pour vérifier ou compléter l'information sur votre santé telle que votre historique médicale et des résultats de tests. Tous les documents vous identifiant seront gardés confidentiels en tout temps sauf lorsque requis par la loi, et seront gardés à l'Institut Thoracique de Montréal dans une chambre verrouillée dans un cabinet verrouillé. Seul l'investigateur principal (Dr. Jean Bourbeau) et son étudiante au doctorat (Laura Labonté) auront accès à ce cabinet. Sur les documents, seuls vos initiales et numéro de patient vous identifieront et seul l'investigateur principal (Dr. Jean Bourbeau) gardera un dossier avec votre nom. Toute information contenue dans des rapports ou publications résultant de cette étude sera codée et présentée d'une façon à ce que votre identité ne soit pas révélée. Les résultats de l'étude seront gardés pendant 25 ans et pourront être accédés par un tiers parti. Les échantillons biologiques seront gardés jusqu'à 5 ans après l'analyse. Si requis, les auditeurs et représentants de l'hôpital ou du comité d'éthique de l'Université McGill pourront avoir accès aux données de l'étude pour s'assurer de la conduite éthique de celle-ci.

10. Participation Volontaire et Retrait

Vous êtes complètement libre de participer ou non à cette étude de recherche. Si vous décidez de ne pas y participer, vos soins de santés présents et futurs à cette institution ne seront aucunement affectés par votre choix. Vous pouvez décider de vous retirer de cette étude n'importe quand. Votre participation à cette étude pourra être terminée par vous ou votre médecin de l'étude s'il le faut.

11. Résultats Médicaux fortuits

Vous serez informé de résultats qui sont obtenus durant une évaluation ou analyse d'échantillons biologiques qui pourraient indiquer un problème de santé potentiel. Vous pourrez obtenir une copie de ces résultats pour votre médecin de famille afin de pouvoir en discuter plus en détails.

12. Personnes à Contacter

Vous avez le droit à n'importe quel moment de demander de l'information au médecin de l'étude sur votre condition. Vous pouvez aussi demander que cette information, que des résultats de l'étude ou que ce formulaire soient fournis à votre médecin.

Si vous avez des questions à propos de l'étude, on vous prie de contacter :

Investigateur principal: Dr. Jean Bourbeau: 514-934-1934 poste 32185
Technicienne de recherche: Katrina Metz 514-934-1934 poste 32489
Candidate au doctorat: Laura Labonté: 514-882-1074

Si vous avez des questions à propos de vos droits en tant que sujet de recherche, vous pouvez contacter l'Ombudsman de:
L'Institut Thoracique de Montréal (514) 934-1934 poste 35655

L'Hôpital Général de Montréal	(514) 934-8306
L'Hôpital Général Juif	(514) 340-8222 poste 5833

CONSENTEMENT ÉCLAIRÉ DU PATIENT

PAGE DE SIGNATURE

Exacerbation de MPOC: Comprendre le cours des changements et du rétablissement des issues extra-pulmonaires

Investigateur Principal: Dr. Jean Bourbeau

1. Je suis conscient que ceci est une étude de recherche.
2. J'ai lu toutes les pages de ce formulaire de consentement. Le personnel de l'étude m'a expliqué l'information et les procédures de l'étude. J'ai eu l'occasion de poser des questions et on m'a répondu d'une manière satisfaisante. On m'a donné le temps de considérer cette information attentivement et de décider de participer ou non à cette étude.
3. J'ai été informé que ma participation à cette étude est entièrement volontaire et que je peux refuser de participer, ou me retirer de l'étude n'importe quand, sans conséquences pour mes soins médicaux en cours à cette institution.
4. J'autorise l'accès à mes dossiers médicaux, pour les investigateurs de l'étude, pour les autorités réglementaires et pour le comité d'éthique de cette institution pour les buts de l'étude seulement. Cette autorisation est valide pour une période de 25 ans.
5. Je suis conscient qu'on me fournira une copie de ce consentement que je pourrai garder pour ma propre information, une fois signée. J'ai été informé qu'une copie de ce formulaire de consentement sera placée dans mon dossier médical pour que les fournisseurs de soins de santé à cette institution sachent que je participe à cette étude et sachent de quoi il s'agit.
6. J'ai été informé que je n'abandonne aucun de mes droits légaux en signant ce formulaire, ni ne libère les investigateurs, commanditaires, ni l'établissement où l'étude aura lieu de leurs responsabilités civiles et professionnelles.
7. Ma signature ci-dessous indique que j'accepte volontairement de participer à cette étude.

_____	_____	_____
Signature du sujet	Nom (en lettres moulées)	Date
_____	_____	_____
Signature de la personne Administrant le consentement	Nom (en lettres moulées)	Date

Je confirme avoir rencontré le sujet au moment de l'inscription à l'étude et d'avoir répondu à ses questions à propos de cette étude.

_____	_____	_____
Signature de l'investigateur	Nom (en lettres moulées)	Date

French consent form- day-hospital patients

FORMULAIRE D'INFORMATION ET DE CONSENTEMENT DES PATIENTS ADMIS À L'HÔPITAL DE JOUR

Exacerbation de MPOC: Comprendre le cours des changements et du rétablissement des issues extra-pulmonaires

Investigateur Principal: J. Bourbeau MD
Co-investigateurs: S. Daskalopoulou MD/PhD, C. Baglole PhD
Etudiante: L. Labonté (candidate au doctorat sous la supervision du Dr. Bourbeau)
Collaborateur: P. Ernst MD
Autres: K. Metz, M. Patel
Institutions: Institut Thoracique de Montréal

GlaxoSmithKline est la compagnie pharmaceutique qui finance cette étude.

1. **BUT DU FORMULAIRE D'INFORMATION ET DE CONSENTEMENT**

Vous êtes invité à participer à une étude de recherche sur les patients qui ont la maladie pulmonaire obstructive chronique (MPOC). Avant que vous décidiez de participer à cette étude, il est important que vous compreniez pourquoi cette étude est entreprise et qu'est ce qu'elle implique pour vous, si vous y participez. Le but de ce formulaire est de vous offrir l'information à propos de cette étude, et si vous le signez, cela signifie que vous acceptez de participez à l'étude. Nous vous prions de prendre le temps de bien lire l'information qui suit et d'en parler avec une autre personne ou votre médecin de famille si vous le désirez. Vous pouvez aussi demander que cette information et une copie de ce consentement soient données à quelqu'un d'autre. Le formulaire décrit le but, les procédures, les bénéfices et risques de cette étude de recherche. Il se peut qu'il contienne des mots que vous ne comprenez pas. Nous vous prions de demander au personnel de l'étude d'expliquer les mots ou procédures que vous ne comprenez pas clairement. Vous pouvez refuser de participer ou vous retirer de l'étude n'importe quand, sans que cela n'affecte pas les soins de santé que vous recevez à cette institution. Vous ne devriez signer ce formulaire de consentement pour participer à cette étude que si vous avez tout lu et compris le déroulement de l'étude, les attentes du médecin et du personnel de cette étude envers vous, et tous bénéfices et risques potentiels qui peuvent résulter de votre participation. La participation est volontaire.

2. INTRODUCTION

Vous êtes invité à participer à cette étude de recherche à l'Institut Thoracique de Montréal parce que vous avez une MPOC reconnu et que vous êtes présentement hospitalisé à cause d'une exacerbation sévère de la MPOC.

Le but de cette étude est d'examiner comment les exacerbations de la MPOC affectent certains produits chimiques et certaines cellules qui font partie de votre système immunitaire, et comment ceux-ci peuvent conséquemment affecter la santé de vos vaisseaux sanguins. Cette information sera essentielle pour la planification d'une étude clinique qui évaluera une nouvelle thérapie contre les exacerbations qui sera bénéfique pour les poumons et potentiellement pour d'autres parties du corps comme les vaisseaux sanguins et le cœur.

3. DESIGN DE L'ÉTUDE

Un maximum de 40 patients de l'Institut Thoracique de Montréal (ITM), de l'Hôpital Général de Montréal (HGM) et de l'Hôpital Général Juif (HGJ) participeront à cette étude. Parmi les patients hospitalisés à l'ITM, un maximum de 30 patients ayant des exacerbations fréquentes seront recrutés avant leur hospitalisation à l'hôpital de jour.

Cette étude durera un total de 6 mois, et si vous acceptez d'y participer, vous devrez participer à un total d'à peu prêt 10 évaluations cliniques (dépendant de la durée de votre hospitalisation, où si vous êtes hospitalisés pendant plus longtemps, vous aurez plus d'évaluations. Les évaluations sont décrites en plus de détails dans la section suivante).

Ceci est une étude observationnelle, ce qui veut dire qu'on ne modifiera pas le traitement que vous recevez normalement pour votre MPOC ou vos exacerbations de la MPOC. Si vous acceptez de participer, nous vous évaluerons lorsque vous avez une exacerbation et lors de votre rétablissement suite à l'exacerbation. Ces évaluations sont relativement non-invasives. Chaque évaluation inclut une entrevue, un test de fonction pulmonaire, la collection d'échantillons de sang, un examen physique sommaire de base, et une évaluation de la santé de vos vaisseaux sanguins. Vous devrez faire un test d'exercice de base pour certaines

évaluations. La durée et la nature des procédures qui seront faites sont décrites plus en détails dans la prochaine section.

4. PROCÉDURES DE L'ÉTUDE

Évaluation initiale durant l'état de maladie stable (évaluation 1):

Vous devrez venir à l'Institut Thoracique de Montréal pour une évaluation initiale qui sera planifiée après que vous aurez donné votre consentement, si vous décidez de participer à l'étude.
La première évaluation durera environ 2 heures et les procédures suivantes seront effectuées:

- Une entrevue, qui couvrira votre historique de MPOC et révisera votre historique médicale complète incluant les médicaments que vous prenez présentement, et tous médicaments que vous avez pris lors des 3 derniers mois. Il est important de mentionner tous les médicaments que vous prenez incluant les remèdes herbeux ou naturels.

- Test de fonction pulmonaire. Ceci mesure combien d'air est dans vos poumons et nous dit avec quel aisance cet air circule dans vos poumons quand vous respirez. On vous demandera de souffler dans une pièce buccale qui mesure les volumes d'air, appelée un spiromètre. Cette procédure vous sera expliquée en détail avant qu'elle soit exécutée (évaluation 1). Si vous fumez, on vous demandera l'heure de votre dernière cigarette.

- Collecte d'échantillons de sang. Environ 35 mL (c'est à dire environ 7 cuillères à thé) de sang seront recueillis d'une veine de votre bras et utilisés pour évaluer les produits chimiques et cellules produits par votre système immunitaire.

- Un examen physique. Celui-ci inclura l'évaluation de vos signes vitaux (pression sanguine, rythme cardiaque) et la mesure du taux d'oxygène dans votre sang (en utilisant une sonde placée sur votre doigt).

- La santé de vos artères sera évaluée. Ceci sera accompli après que vous ayez été couché et que vous êtes reposé. On vous demande de ne pas dormir ou parler lorsque les mesures sont prises. Des électrodes seront placées sur vous pour qu'un électrocardiogramme puisse être fait. Ceci mesurera votre rythme cardiaque. Un senseur de pression qui peut mesurer l'écoulement sanguin sera appliqué à la surface de votre peau et pressé doucement près d'une artère. Cette mesure sera répétée à 3 endroits : proche de votre poignet, sur votre cou et à l'intérieur de votre cuisse.

- On vous demandera de faire 1 test d'exercice relativement court et standard sous la supervision du personnel de l'étude. On vous demandera de pédaler sur une bicyclette stationnaire à des intensités **croissantes** pour aussi longtemps que vous le pourrez. Pendant que vous faites l'exercice, vous devrez porter une pièce buccale qui nous permettra de mesurer l'air que vous respirer. Nous évaluerons aussi vos signes vitaux (pouls et pression sanguine) et le taux d'oxygène dans votre sang (en utilisant une sonde placée sur votre doigt) durant votre exercice. Quand vous aurez fini, vous pourrez vous reposer et vous étendre pendant 35 minutes, pendant que nous évaluons vos vaisseaux sanguins et signes vitaux.

Suivi durant une exacerbation (évaluations 2, 3 et 4)

Vous devrez venir à l'Institut Thoracique de Montréal durant les premières 72 heures de votre exacerbation (évaluation 2), et vous serez évalué chaque 72 (±24) heures durant votre hospitalisation (évaluations 3 a, b et possiblement plus dépendant de la durée de votre hospitalisation), et ensuite à 1 semaine après le début de votre exacerbation et à chaque semaine par après pendant 1 mois suite au début de votre exacerbation (évaluations 4 a, b, c, d, en prenant pour acquis que vous êtes hospitalisé pendant moins qu'une semaine. Si votre hospitalisation est plus longue, vous viendrez seulement une fois par semaine pendant les

semaines après que vous avez quitté l'hôpital jusqu'à ce que ça fasse 1 mois depuis votre première évaluation clinique). Vous devrez répéter le même test d'exercice que durant votre visite initiale (décrits ci-haut) 1 mois après le début de votre exacerbation (évaluations 4 d), cette évaluation prendra 2 heures et suivront les mêmes procédures qu'à votre évaluation de base.

Les évaluations 4a, 4b et 4c dureront environ 1-1.5 heures et suivront les mêmes procédures qu'à votre évaluation de base sauf les tests d'exercice.

Suivi durant le rétablissement (évaluations 5 et 6)

Vous serez ré-évalué à 3 mois et 6 mois après le début de votre exacerbation (évaluation 1). Durant ces évaluations le personnel de l'étude révisera votre état de santé et vos informations médicales depuis votre dernière évaluation et exacerbation. Lors de l'évaluation 5, à 3 mois, vous devrez faire le même test d'exercice qu'à l'évaluation1. Cette évaluation durera 2 heures et suivra les mêmes procédures qu'à votre évaluation de base.

La 6^{ième} évaluation durera environ 1-1.5 heures à des dates déterminées après votre dernière évaluation. Les mêmes procédures qui ont été effectuées lors de vos autres évaluations seront répétées, sauf les tests d'exercice, que vous ne devrez pas répéter.

Horaire et résumé des évaluations

Évaluations	Durée	Procédures
1: visite initiale	2 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères - Test d'exercice
2: début de l'exacerbation	1-1.5 heures	- Entrevue

		- Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
3 (a, b et possiblement plus, dépendant de la durée de votre hospitalisation): tous les 72 (±24) heures)	1-1.5 heure	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
4 (a, b, c): 1, 2 & 3 semaines post-exacerbation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères
4 (d): 1 mois post-exacerbation	2 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères - Test d'exercice
5: 3 mois post-exacerbation	2 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang - Examen physique - Évaluation de la santé des artères - Test d'exercice
6: 6 mois post-exacerbation	1-1.5 heures	- Entrevue - Test de fonction pulmonaire - Collecte d'échantillons de sang

		- Examen physique - Évaluation de la santé des artères

5. RESPONSABILITÉS DU PATIENT

- Vous ne devez pas faire d'activité physique vigoureuse pendant au moins 12 heures avant une évaluation.
- Vous ne devez pas fumer au moins 12 heures avant une évaluation. On vous demandera d'enregistrer l'heure de votre dernière cigarette avant votre test de fonction pulmonaire.
- Vous ne devez pas consommer de café, thé, chocolat, cola et autre breuvages ou nourriture contenant de la caféine les matins d'une évaluation ni durant une évaluation. Les breuvages décaféinés sont acceptables.
- Vous devriez éviter d'être exposé à des températures froides, un environnement où il y a de la fumée, de la poussière ou des endroits avec des odeurs fortes (comme des parfums) avant une évaluation.

6. RISQUES ET INCONFORTS POTENTIELS

Test de fonction pulmonaire:
L'inconfort est inhabituel; par contre certaines personnes ressentent des maux de tête et/ou des étourdissements quand ces tests sont effectués; ces symptômes sont habituellement temporaires. Si vous les éprouvez, vous pourrez recevoir un traitement.

Collecte d'échantillons de sang:
La collecte de sang d'une veine du bras est routinière. Ceci peut vous causer une douleur minime, un saignement et/ou de meurtrissures au site de la piqûre. D'autres risques incluent une sensation temporaire d'étourdissement et rarement, une infection au site de la piqûre.

7. BÉNÉFICES POTENTIELS

Il est possible que vous ne bénéficiiez pas vous-même de votre participation à cette étude, mais vous pourriez contribuer de l'information nouvelle qui pourrait bénéficier à d'autres personnes et à la communauté médicale pour le traitement de la MPOC. Cette recherche peut offrir de l'information valable pour la préparation d'une plus grande étude qui évaluera l'efficacité d'un nouveau traitement qui pourrait améliorer les poumons et d'autres parties du corps affectés par les exacerbations de la MPOC. Il est possible que vous bénéficiiez du fait d'avoir des examens physiques ainsi que des tests de laboratoires durant l'étude.

8. COMPENSATION ET COÛTS

Vous recevrez 50$ pour couvrir les frais de stationnement/voyage, sauf durant votre hospitalisation.

9. CONFIDENTIALITÉ

Le coordonnateur de l'étude ainsi que l'investigateur principal auront accès à vos dossiers médicaux pour vérifier ou compléter l'information sur votre santé telle que votre historique médicale et des résultats de tests. Tous les documents vous identifiant seront gardés confidentiels à tout temps sauf lorsque requis par la loi, et seront gardés à l'Institut Thoracique de Montréal dans une chambre verrouillée dans un cabinet verrouillé. Seul l'investigateur principal (Dr. Jean Bourbeau) et son étudiante au doctorat (Laura Labonté) auront accès à ce cabinet. Sur les documents, seuls vos initiales et numéro de patient vous identifieront et seul l'investigateur principal (Dr. Jean Bourbeau) gardera un dossier avec votre nom. Toute information contenue dans des rapports ou publications résultant de cette étude sera codée et présentée d'une façon à ce que votre identité ne soit pas révélée. Les résultats de l'étude seront gardés pendant 25 ans et pourront être accédés par un tiers parti. Les échantillons biologiques seront gardés jusqu'à 5 ans après l'analyse. Si requis, les auditeurs et représentants de l'hôpital ou du comité d'éthique de l'Université McGill pourront avoir accès aux données de l'étude pour s'assurer de la conduite éthique de celle-ci.

10. PARTICIPATION VOLONTAIRE ET RETRAIT

Vous êtes complètement libre de participer ou non à cette étude de recherche. Si vous décidez de ne pas y participer, vos soins de santés présents et futurs à cette institution ne seront aucunement affectés par votre choix. Vous pouvez décider de vous retirer de cette étude n'importe quand. Votre participation à cette étude pourra être terminée par vous ou votre médecin de l'étude s'il le faut.

11. RÉSULTATS MÉDICAUX FORTUITS

Vous serez informé de résultats qui sont obtenus durant une évaluation ou analyse d'échantillons biologiques qui pourraient indiquer un problème de santé potentiel. Vous pourrez obtenir une copie de ces résultats pour votre médecin de famille afin de pouvoir en discuter plus en détails.

12. PERSONNES À CONTACTER

Vous avez le droit à n'importe quel moment de demander de l'information au médecin de l'étude sur votre condition. Vous pouvez aussi demander que cette information, que des résultats de l'étude ou que ce formulaire soient fournis à votre médecin.

Si vous avez des questions à propos de l'étude, on vous prie de contacter :

Investigateur principal: Dr. Jean Bourbeau: 514-934-1934 poste 32185
Technicienne de recherche: Katrina Metz 514-934-1934 poste 32489
Candidate au doctorat: Laura Labonté: 514-882-1074

Si vous avez des questions à propos de vos droits en tant que sujet de recherche, vous pouvez contacter l'Ombudsman de **L'Institut Thoracique de Montréal** (514) 934-1934 poste 35655

CONSENTEMENT ÉCLAIRÉ DU PATIENT

PAGE DE SIGNATURE

Exacerbation de MPOC: Comprendre le cours des changements et du rétablissement des issues extra-pulmonaires

Investigateur Principal: Dr. Jean Bourbeau

1. Je suis conscient que ceci est une étude de recherche.
2. J'ai lu toutes les pages de ce formulaire de consentement. Le personnel de l'étude m'a expliqué l'information et les procédures de l'étude. J'ai eu l'occasion de poser des questions et on m'a répondu d'une manière satisfaisante. On m'a donné le temps de considérer cette information attentivement et de décider de participer ou non à cette étude.
3. J'ai été informé que ma participation à cette étude est entièrement volontaire et que je peux refuser de participer, ou me retirer de l'étude n'importe quand, sans conséquences envers mes soins médicaux en cours à cette institution.
4. J'autorise l'accès à mes dossiers médicaux, pour les investigateurs de l'étude, pour les autorités réglementaires et pour le comité d'éthique de cette institution pour les buts de l'étude seulement. Cette autorisation est valide pour une période de 25 ans.
5. Je suis conscient qu'on me fournira une copie de ce consentement que je pourrai garder pour ma propre information, une fois signée. J'ai été informé qu'une copie de ce formulaire de consentement sera placée dans mon dossier médical pour que les fournisseurs de soins de santé à cette institution sachent que je participe à cette étude et sachent de quoi il s'agit.
6. J'ai été informé que je n'abandonne aucun de mes droits légaux en signant ce formulaire, ni ne libère les investigateurs, commanditaires, ni l'établissement où l'étude aura lieu de leurs responsabilités civiles et professionnelles.
7. Ma signature ci-dessous indique que j'accepte volontairement de participer à cette étude.

_____	_____	_____
Signature du sujet	Nom (en lettres moulées)	Date
_____	_____	_____
Signature de la personne Administrant le consentement	Nom (en lettres moulées)	Date

Je confirme avoir rencontré le sujet au moment de l'inscription à l'étude et d'avoir répondu à ses questions à propos de cette étude.

_____	_____	_____
Signature de l'investigateur	Nom (en lettres moulées)	Date

APPENDIX II
ENGLISH EXACERBATION QUESTIONNAIRE

CASE REPORT FORM
(CRF)

PHONE INTERVIEW

English Questionnaire

Please note the following:

1. The following phone interview is done every 3-months. The upcoming dates will always be calculated from the date were the first interview was done.

2. Update CanCOLD Database and indicate whether the subject completed the Phone interview or not (if not indicate reasons.)

3. Scan and e-mail all your new available phone interview forms in PDF to the National Coordination Office (NCO) on the 15th and the 30th of each month (for monitoring and data entry).

Version 13JUN2013

PHONE INTERVIEW

Subject: | | | | | | | **Date** (dd-mmm-yyyy): _____ **Interview:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 1 – GENERAL OVERVIEW
Mandatory page for all interviews

PLEASE HAVE ALL YOUR MEDICATIONS AND A CALENDAR, THIS WILL FACILITATE ANSWERING THE QUESTIONNAIRE

1. During the last 3 months (or since this questionnaire was last administered, on _____ _____ _____
read date to subject) have you smoked? dd mmm yyyy

No ○ Yes ○

2. In the last 3 months (or since this questionnaire was last administered) have you **experienced an episode with new or changes in any respiratory symptoms (**cough, phlegm, wheeze, breathlessness) that **became worse for at least 2 days**?

No ○ *(end of questionnaire)* Yes ○ ✎ Complete section on **Page 2 - Respiratory Symptoms**

If yes:

2.1 How many episodes* did you experience that became worse for **at least 2 days** that were separated by **at least 3 days**? _____

2.2 Have you had any increases, additions or changes in medication related to these respiratory symptoms?

No ○ Yes ○ ✎ Complete section on **Page 3 - Medication**

2.3 Have you felt as though your work has been affected by these respiratory symptoms?

I am retired ○ No ○ Yes ○ ✎ Complete section on **Page 4 - Work**

2.4. Have you had any health care visits related to these respiratory symptoms?

No ○ Yes ○ ✎ Complete section on **Page 5 - Health care visits** (including unplanned physician visits, emergency department use and hospitalization)

Notes to the coordinator:
- *If the answer to these 2.2, 2.3 and 2.4 is "No", then end the questionnaire.*
- *If subject experienced more than one episode of changes/new respiratory symptoms that lasted for at least 2 days and that are separated by at least 3 days, fill out a separate questionnaire for each episode.*

Completed by ID #:|_____|_____|_____|_____|

PHONE INTERVIEW

Subject: | | | | | | | **Date** (dd-mmm-yyyy): | | **Interview:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 2 - RESPIRATORY SYMPTOMS
Repeat this page for each episode of new or changes in respiratory symptoms occurring in the last 3 months (or since this questionnaire was last administered)

1. In the last 3 months (or since this questionnaire was last administered) when did you experience an episode with **new or changes in any respiratory symptoms** that became worse for at least 2 days:

_____ _____ _____
dd mmm yyyy

For approximately how many days did you experience these symptoms? _____
(minimum 2 days)

2. Did any of the following respiratory symptoms change :

Indicate with an X new or changes in the following respiratory symptoms

Major respiratory symptoms

○ Worsening dyspnea (worsening difficulty breathing)

○ Increased production of sputum (more mucus produced)

○ Increased sputum purulence (ex. change in colour from white to yellow, or yellow to green)

Minor respiratory and related symptoms

New onset of:

○ Cough

○ Wheezing

○ Sore throat

○ Fever or chills (A fever is a temperature above 38°C or 100°F)

○ Cold or flu-like symptoms *(coryzal symptoms)*

○ Chest tightness

○ Fatigue

○ Difficulty with expectoration

○ Night time awakening

[BLUE FORM]

PHONE INTERVIEW

Subject: | | | | | | | **Date** (dd-mmm-yyyy): | | **Interview:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 3 - MEDICATION

Repeat this page for <u>each episode</u> of new or changes in respiratory symptoms occurring in the last 3 months (or since this questionnaire was last administered)

Note to coordinator: *Identify the date that this episode started* _____ _____ _____
dd mmm yyyy

In the last 3 months (or since this questionnaire was last administered), because of your **respiratory symptoms**, did you:

1. Have to increase any of your regular medication?

Yes ○ No ○ *(go to question 2)*

1.1 If yes, did these include increases in:
Indicate with an X increases in the following respiratory medication

○ Bronchodilators

○ Inhaled corticosteroids

○ Both bronchodilators and inhaled corticosteroids

2. Begin taking any new medication related to these respiratory symptoms (new meaning not regular)?

Yes ○ No ○ *(go to question 3)*

2.1 If yes, did these include:
Indicate with an X the addition of the following respiratory medication

○ Short-acting bronchodilators (such as Ventolin, etc.)
What was the approximate date that you started to take short-acting bronchodilators? _____ _____ _____
dd mmm yyyy

○ Oral corticosteroids (such as Prednisone, etc)
What was the approximate date that you started to take oral corticosteroids? _____ _____ _____
dd mmm yyyy

○ Antibiotics
What was the approximate date that you started to take antibiotics? _____ _____ _____
dd mmm yyyy

○ Others (prescription)
What was the approximate date that you started to take any other medication? _____ _____ _____
dd mmm yyyy

3. Take any non-prescription medication (i.e. cough medicine, decongestant)?

Yes ○ No ○

4. Take any non-prescription natural medication?

Yes ○ No ○ [YELLOW FORM]

PHONE INTERVIEW

Subject: | | | | | | | **Date** (dd-mmm-yyyy): | | | **Interview:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 4 - WORK

Repeat this page for <u>each episode</u> of new or changes in respiratory symptoms occurring in the last 3 months (or since this questionnaire was last administered)

Note to coordinator: *Identify the date that this episode started* _____ _____ _____
dd mmm yyyy

1. In the last 3 months (or since this questionnaire was last administered), have you been working?

Yes ○ No ○ *(end of this section/page)*

1.1 If yes, did changes in your **respiratory** symptoms prevent you from going to work?

Yes ○ *(answer 1.1.1)* No ○ *(answer 1.1.2)*

1.1.1 If yes, how many days of work did you miss? _____

1.1.2 If no, was your job performance negatively affected by changes in your **respiratory** symptoms?

Yes ○ No ○

[PINK FORM]

PHONE INTERVIEW

Subject: | | | | | | | **Date** (dd-mmm-yyyy): | | **Interview:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 5 - MEDICAL VISITS

Repeat this page for <u>each episode</u> of new or changes in respiratory symptoms occurring in the last 3 months (or since this questionnaire was last administered)

Note to coordinator: *Identify the date that this episode started* _____ _____ _____
dd mmm yyyy

In the last 3 months (or since this questionnaire was last administered), because of changes in your **respiratory symptoms**, did you…

<u>Doctor visits</u>

1. Visit a doctor or did a doctor visit you (such as a doctor's office, clinic or at home)?

Yes ○ No ○ *(go to question 2)*

Doctor visit (event number)	**1**	**2**	**3**
1.1 If yes, month of visit			
1.2 If yes, was this visit unscheduled (meaning that you saw a doctor as an emergency)?	Yes ○ No ○	Yes ○ No ○	Yes ○ No ○

<u>Emergency visits</u>

2. Have to visit the Emergency Room?

Yes ○ No ○ *(go to question 3)*

ER visit (event number)	**1**	**2**	**3**
2.1 If yes, month of visit			
2.2 If yes, how long did you stay in the ER? (* *SAE log)*	○ ≥ 24 hrs *(*)* ○ < 24 hrs	○ ≥ 24 hrs *(*)* ○ < 24 hrs	○ ≥ 24 hrs *(*)* ○ < 24 hrs

*Indicate with an X if stay was greater or equal to 24 hours or less than 24 hours, 24 hours meaning one day time **AND** one night time.*
1. Approximate date admitted to the ER _____ *# of days* _____ *Reason* _____
2. Approximate date admitted to the ER _____ *# of days* _____ *Reason* _____
3. Approximate date admitted to the ER _____ *# of days* _____ *Reason* _____

<u>Hospital visits</u>

3. Have to be admitted to the hospital?

Yes ○ No ○ *(end)*

Hospitalizations (event number)	**1**	**2**	**3**
3.1 If yes, month of visit			
3.2 Were you admitted to the ICU during your hospitalization?	Yes ○ No ○	Yes ○ No ○	Yes ○ No ○
3.3 For approximately how many days* were you in the hospital? *(* SAE log)*			

1. Approximate date admitted _____ *# of days* _____ *Reason* _____
2. Approximate date admitted _____ *# of days* _____ *Reason* _____
3. Approximate date admitted _____ *# of days* _____ *Reason* _____

** Record in the SAE log each admission/ER visit lasting greater or equal to 24 hours (occurring after the subject has signed Consent Form and since last questionnaire was administered).*

[GREEN FORM]

FRENCH EXACERBATION QUESTIONNAIRE

CASE REPORT FORM
(CRF)

PHONE INTERVIEW

FRENCH Questionnaire

Please note the following:

1. The following phone interview is done every 3-months. The upcoming dates will always be calculated from the date when the first interview was done.

2. Update CanCOLD Database and indicate whether the subject completed the Phone interview or not (if not indicate reasons.)

3. Scan and e-mail all your new available phone interview forms in PDF to the National Coordination Office (NCO) on the 15th and the 30th of each month (for monitoring and data entry).

Version 13JUN2013

ENTREVUE TÉLÉPHONIQUE

Sujet: | | | | | | | **Date** (jj-mm-aaaa): | | **Entrevue:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 1 – APERÇU GÉNÉRAL
OBLIGATOIRE pour chaque entrevue

AYEZ TOUS VOS MÉDICAMENTS ET UN CALENDRIER AVEC VOUS LORSQUE VOUS RÉPONDEZ AU QUESTIONNAIRE

1. Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété, le ___ _____ _____ *lire la date au sujet*) avez-vous fumé?
jj mmm aaaa

Non ○ Oui ○

2. Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété) avez-vous éprouvé **un épisode de nouveaux symptômes respiratoires (toux,** purulence des sécrétions, sifflements, essoufflé) **ou de changements dans ceux-ci,** qui a persisté **au moins 2 jours**?

Non ○ *(fin du questionnaire)* Oui ○ ✎ Complétez la section **Symptômes respiratoires à la Page 2**

Si oui:

2.1 Combien* d'épisodes **d'au moins 2 jours** séparés par **au moins 3 jours** avez-vous éprouvés?: _____

2.2 Avez-vous eu des augmentations, additions ou changements de médicaments reliés à ces symptômes respiratoires?

Non ○ Oui ○ ✎ Complétez la section **Médicaments à la Page 4**

2.3 Avez-vous ressenti que votre travail a été affecté par ces symptômes respiratoires?

Je suis retraité ○ Non ○ Oui ○ ✎ Complétez la section **Travail à la Page 5**

2.4 Avez-vous reçu des soins de santé reliés à ces symptômes respiratoires?

Non ○ Oui ○ ✎ Complétez la section **Visites reliées aux soins de santé à la Page 6** (qui inclut les visites non-planifiées chez un médecin, à l'urgence ou une hospitalisation)

Notes pour le coordinateur :
- *Si la réponse aux 2.2, 2.3 et 2.4 est « Non » le sujet a fini de répondre au questionnaire.*
- *Si le sujet a éprouvé plus d'un épisode de nouveaux/changements de symptômes respiratoires qui a duré au moins 2 jours et qui sont séparés par au moins 3 jours, remplissez un nouveau questionnaire pour chacune de ces épisodes.*

ENTREVUE TÉLÉPHONIQUE

Sujet: | | | | | | **Date** (jj-mm-aaaa): | | **Entrevue:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Complété par ID #:|_____|_____|_____|_____|_____|

Page 2 – SYMPTÔMES RESPIRATOIRES

Remplissez cette page pour chacun des épisodes de nouveaux/changements de symptômes respiratoires qui a eu lieu dans les 3 derniers mois (ou depuis que le questionnaire a été complété).

1. Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété) quand avez-vous éprouvé **de nouveaux symptômes respiratoires ou des changements dans ceux-ci** pendant au moins 2 jours:

____ _____ _____
jj mmm aaaa

Pendant environ combien de jours avez-vous éprouvé ces symptômes? _____
(minimum 2 jours)

2. Avez-vous éprouvé les symptômes suivant :

Indiquez avec un X les nouveaux symptômes ou les changements de symptômes respiratoires suivants

Symptômes respiratoires majeurs

○ Aggravation de la dyspnée (aggravation de la difficulté de respirer)

○ Augmentation de la production des sécrétions (plus de mucus produit)

○ Augmentation de la purulence des sécrétions (ex. changement de couleur de blanc à jaune, ou de jaune à vert)

Symptômes respiratoires mineurs et symptômes liés

Nouveaux symptômes présentés:

○ Toux

○ Sifflements

○ Mal de gorge

○ Fièvre ou frissons (Une fièvre est une température au delà de 38°C ou 100°F)

○ Symptômes de grippe ou de rhume

○ Serrement de la poitrine

○ Fatigue

○ Difficulté d'expectorer

○ Éveils durant la nuit

ENTREVUE TÉLÉPHONIQUE

Sujet: | | | | | | | **Date** (jj-mm-aaaa): | | | **Entrevue:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

[PAPIER BLEU]

Page 3 - MÉDICAMENTS

Remplissez cette page pour chacun des épisodes de nouveaux/changements de symptômes respiratoires qui a eu lieu dans les 3 derniers mois (ou depuis que le questionnaire a été complété).

Note pour le coordinateur: *Identifiez la date approximative du début ce cet épisode* ____ _____ _____
jj mmm aaaa

Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété), à cause de vos **symptômes respiratoires**, avez-vous:

1. Augmenté vos médicaments réguliers?

Oui ○ Non ○ *(si non: répondez à la question 2)*

1.1 Si oui, est ce que ceci incluait des augmentations de:
Indiquez avec un X les augmentations dans les médicaments respiratoires suivant

○ Bronchodilatateurs

○ Corticostéroïdes inhalés

○ Bronchodilatateurs et corticostéroïdes inhalés

2. Commencé à prendre de nouveaux médicaments reliés à ces symptômes respiratoires (nouveaux voulant dire pas réguliers)?

Oui ○ Non ○ *(si non: répondez à la question 3)*

2.1 Si oui, est ce qu'ils incluaient:
Indiquez avec un X s'il y a eu un ajout des médicaments respiratoires suivants

○ Bronchodilatateurs à courte durée d'action (comme du Ventolin, etc)
Qu'elle était la date approx. à laquelle vous avez commencé à prendre ces bronchodilatateurs? ____ ____ ____
jj mmm aaaa

○ Corticostéroïdes oraux (comme de la Prednisone, etc)
Qu'elle était la date approx. à laquelle vous avez commencé à prendre ces corticostéroïdes? ____ ____ ____
jj mmm aaaa

○ Antibiotiques
Qu'elle est la date approx. à laquelle vous avez commencé à prendre des antibiotiques? ____ ____ ____
jj mmm aaaa

○ Autres (prescription)
Qu'elle est la date approx. à laquelle vous avez commencé à prendre ces autres médicaments? ____ ____ ____
jj mmm aaaa

3. Pris des médicaments qui ne requièrent pas de prescription (i.e. médicaments contre la toux, décongestionnant)?

Oui ○ Non ○

4. Pris des médicaments qui ne requièrent pas de prescription et qui sont naturels?

Oui ○ Non ○

[PAPIER JAUNE]

ENTREVUE TÉLÉPHONIQUE

Sujet: | | | | | | | **Date** (jj-mm-aaaa): | | **Entrevue:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 4 - TRAVAIL

Remplissez cette page pour chacun des épisodes de nouveaux/changements de symptômes respiratoires qui a eu lieu dans les 3 derniers mois (ou depuis que le questionnaire a été complété).

Note pour le coordinateur: *Identifiez la date approximative du début ce cet épisode* ____ _____ _____
jj mmm aaaa

1. Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété), avez-vous travaillé?

Oui ○ Non ○ *(fin de cette section/page)*

1.1 Si oui, est ce que des changements dans vos **symptômes respiratoires** vous ont empêché d'aller au travail?

Oui ○ *(répondez à la question 1.1.1)* Non ○ *(répondez à la question 1.1.2)*

1.1.1 Si oui, combien de jours de travail avez-vous manqués? _____

1.1.2 Si non, est ce que votre performance au travail a été négativement affectée par vos **symptômes respiratoires**?

Oui ○ Non ○

[PAPIER ROSE]

ENTREVUE TÉLÉPHONIQUE

Sujet: | | | | | | | **Date** (jj-mm-aaaa): | | | **Entrevue:** | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 |

Page 5 – VISITES RELIÉES AUX SOINS DE SANTÉ

Remplissez cette page pour <u>chacun</u> des épisodes de nouveaux/changements de symptômes respiratoires qui a eu lieu dans les 3 derniers mois (ou depuis que le questionnaire a été complété)

Note pour le coordinateur: *Identifiez la date approximative du début ce cet épisode* ____ _____ _____
jj mmm aaaa

Durant les 3 derniers mois (ou depuis la dernière fois que ce questionnaire a été complété), à cause de vos **symptômes respiratoires**, avez vous…

<u>Visites de médecin</u>

1. Visité un médecin ou est ce qu'un médecin vous a visité?

Oui ○ Non ○ *(répondez à la question 2)*

Visites de médecin (numéro de la visite)	**1**	**2**	**3**
1.1 Si oui, quel est le mois de la visite?			
1.2 Si oui, est-ce que cette visite était non-prévue (c'est à dire que vous avez vu ce médecin à cause d'une urgence)?	Oui ○ Non ○	Oui ○ Non ○	Oui ○ Non ○

<u>Visites de la salle d'urgence</u>

2. Visité une salle d'urgence?

Oui ○ Non ○ *(répondez à la question 3)*

Visites de la salle d'urgence (numéro de la visite)	**1**	**2**	**3**
2.1 Si oui, quel est le mois de la visite?			
2.2 Si oui, combien de temps êtes-vous resté dans la salle d'urgence? (** log des SAE)*	○ ≥ 24 hrs *(*)* ○ < 24 hrs	○ ≥ 24 hrs *(*)* ○ < 24 hrs	○ ≥ 24 hrs *(*)* ○ < 24 hrs

*Indiquez avec un X si le sujet est resté au moins 24 heures ou moins que 24 heures à l'urgence (24 heures voulant dire un jour **ET** une nuit).*
1. Date approx. de l'admission à l'urgence _____ *# de jours*_____ *Raison* _____
2 Date approx. de l'admission à l'urgence _____ *# de jours*_____ *Raison* _____
3. Date approx. de l'admission à l'urgence _____ *# de jours*_____ *Raison* _____

<u>Visites d'un hôpital</u>

3. Été admis à l'hôpital?

Oui ○ Non ○ *(fin)*

Visites d'un hôpital (numéro de la visite)	**1**	**2**	**3**
3.1 Si oui, quel est le mois de la visite?			
3.2 Si oui, étiez-vous admis aux soins intensifs lors de votre hospitalisation?	Oui ○ Non ○	Oui ○ Non ○	Oui ○ Non ○
3.3 Si oui, combien de temps êtes-vous resté* à l'hôpital? (** log des SAE)*			

1. Date approx. de l'admission _____ *# de jours*_____ *Raison* _____
2 Date approx. de l'admission _____ *# de jours*_____ *Raison* _____
3. Date approx. de l'admission _____ *# de jours*_____ *Raison* _____

** Inscrivez dans le log des SAE chaque visite à l'urgence/hospitalisation qui a duré au moins 24 heures (qui est arrivée après que le sujet a signé le consentement et la dernière fois que ce questionnaire a été complété).*

[PAPIER VERT]

APPENDIX III

OPEN ACCESS Freely available online

PLOS ONE

Alterations in the Expression of the NF-κB Family Member RelB as a Novel Marker of Cardiovascular Outcomes during Acute Exacerbations of Chronic Obstructive Pulmonary Disease

Laura Labonté[1,2], Patrick Coulombe[2], Michela Zago[1,3], Jean Bourbeau[1,2], Carolyn J. Baglole[1,3]*

1 Department of Medicine, McGill University, Montreal, Quebec, Canada, **2** Respiratory Epidemiology and Clinical Research Unit (RECRU), Montreal Chest Institute, McGill University Health Centre, Montreal, Quebec, Canada, **3** Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada

Abstract

Background: Chronic obstructive pulmonary disease (COPD) exacerbations are acute events of worsened respiratory symptoms and enhanced inflammation partly mediated by NF-κB activation. RelB, an NF-κB family member, suppresses cigarette smoke-induced inflammation but its expression in COPD is unknown. Moreover, there is no information on its association with clinical features of COPD. The objectives of this study were to assess RelB expression relative to markers of inflammation as well as its association with cardiovascular and pulmonary features of COPD patients at stable-state and exacerbation.

Methods: Data from 48 COPD patients were analyzed. Blood samples were collected from stable-state and exacerbating patients. After RNA isolation, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to assess RelB, Cox-2, IL-8 and IL-1β mRNA expression and their associations with measured clinical variables.

Results: Of the 48 COPD subjects, 18 were in stable-state and 30 were in exacerbation. RelB mRNA expression was lower than that of Cox-2, IL-8, and IL-1β in all cases (all $p<0.001$, except for IL-8 at exacerbation ($p=0.22$)). Cox-2, IL-8 and IL-1β were significantly associated with clinical features of patients in both stable-state and at exacerbation. There was no association with RelB expression and any clinical features in COPD subjects at stable-state. RelB mRNA levels were significantly associated with cardiovascular events such as systolic blood pressure during exacerbation.

Conclusions: RelB mRNA expression is lower than that of the other inflammatory mediators. Expression of Cox-2, IL-8 and IL-1β were related to clinical features in both stable-state and at exacerbation. However, RelB expression was associated with clinical features of patients only during exacerbation, suggesting that RelB may represent a novel marker of health outcomes, in particular cardiovascular, during exacerbation in COPD.

Citation: Labonté L, Coulombe P, Zago M, Bourbeau J, Baglole CJ (2014) Alterations in the Expression of the NF-κB Family Member RelB as a Novel Marker of Cardiovascular Outcomes during Acute Exacerbations of Chronic Obstructive Pulmonary Disease. PLoS ONE 9(11): e112965. doi:10.1371/journal.pone.0112965

Editor: Sanjay B. Maggirwar, University of Rochester Medical Center, United States of America

Received August 25, 2014; **Accepted** October 16, 2014; **Published** November 19, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This study was funded as an investigator-initiated grant (no. 5427) by GlaxoSmithKline Canada Ltd (http://www.gsk.ca/english/index.html). LL was funded by a Canadian Institute of Health Research Doctoral Award (http://www.cihr-irsc.gc.ca). Infrastructure for this project was supported by the Canada Foundation for Innovation (CFI) -Leaders Opportunities Fund (http://www.mcgill.ca/medresearch/research/funding/cfi) (CJB). CJB was supported by a salary award from the Fonds de Recherche du Quebec-Sante (FRQ-S) (http://www.frqs.gouv.qc.ca/en/index.shtml). GlaxoSmithKline Canada Ltd, had a role in study design but no role in data collection and analysis, decision to publish, or preparation of the manuscript. All other funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: JB has the following competing interests: grants from the Canadian Institute of Health Research, CanCOLD Operating grant Collaborative program Rx & D (Almirall, Astra Zeneca, Boehringer Ingelheim, GSK, Merck, Novartis, Takeda) and the Canadian Respiratory Research Network (CRRN), grants from Fonds de Recherche du Quebec-Sante (FRQS) RHN COPD, research and educational grants from Almirall, Astra Zeneca, Boehringer Ingelheim, GSK, Merck, Novartis for investigator initiated projects. LL, PC, MZ and CJB have declared that no competing interests exists. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. CJB has served as Academic Editor for this journal and is still currently in that role. This does not alter the authors' adherence to PLOS ONE Editorial policies and criteria.

* Email: Carolyn.baglole@McGill.ca

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, not fully reversible airflow limitation [1] and chronic inflammation [2]. The course of COPD is perturbed by acute events of worsened respiratory symptoms known as exacerbations [3–7]. Exacerbations are associated with heightened pulmonary and systemic inflammatory responses [8], and lead to significant morbidity and mortality, especially due to cardiovascular events, as well as decreased health-related quality of life and an accelerated decline in lung function [3,4,9–11].

COPD inflammation in stable-state and at exacerbation is partly mediated by nuclear factor-κB (NF-κB) activation [12-14]. NF-κB is a ubiquitous transcription factor family composed of five proteins [15] activated by cigarette smoke (CS) that collectively are involved in the regulation of gene expression for pro-inflammatory cytokines, chemokines and adhesion molecules [12,16]. These include interleukin (IL)-1β, a key orchestrator of the immune response in COPD [14] that can activate NF-κB and is produced by airway epithelial cells in response to CS or acute injury [14,17]. Other mediators include IL-8, which is involved in inflammatory cell recruitment in COPD [18–21], especially with bacterial infection at exacerbation [20,21]; and cyclooxygenase-2 (COX-2), an inducible enzyme that catalyzes arachidonic acid transformation into thromboxane and prostaglandins [12]. COX-2 induction can also occur via IL-1β activation of the NF-κB pathway and is enhanced in response to noxious stimuli such as CS [12,22].

Although activation of NF-κB is typically regarded as pro-inflammatory, we recently identified RelB, another member of the NF-κB family, as a potent suppressor of CS-induced inflammation [23–27]. Weih *et al*. [28,29] showed that mice deficient in RelB have severely increased multi-organ inflammation, suggesting that RelB may have a role in suppressing inflammation. Our *in-vitro* and *in-vivo* data provide further evidence for an anti-inflammatory role for RelB. We have shown that loss of RelB expression due to smoke exposure promotes pro-inflammatory mediator production (including IL-8 and COX-2 expression), whereas RelB reconstitution reduces inflammation associated with CS [23,24]. Reciprocally, overexpression of pulmonary RelB in mice exposed to CS is associated with decreased lung neutrophil infiltration, pro-inflammatory cytokine and chemokine production and COX-2/prostaglandin production [25].

Despite increasing experimental evidence regarding the anti-inflammatory abilities of RelB against CS, neither expression of RelB nor its expression relative to other inflammatory mediators has been studied in the context of COPD at stable state or during exacerbation.

It is also unknown whether RelB is associated with any clinically-relevant outcomes in COPD, including acid-base balance and cardiovascular events. Recent experimental evidence in human lung epithelial cells has shown that increased nuclear RelB correlates with hypercapnia-induced protection against lung injury. This suggests that RelB is carbon dioxide-sensitive and may contribute to the benefits of hypercapnia in pulmonary inflammatory diseases [30]. Given that in COPD acid-base disturbances are common, particularly during exacerbations [31] and are associated with poor health outcomes, we hypothesize that RelB may be associated with clinical variables involved in acid-base maintenance during COPD and its exacerbations. It is also well-described that the period following COPD exacerbations has been associated with enhanced risk of acute cardiovascular events [32–34] possibly due to increased inflammation. Experimental evidence supports a protective role for RelB in the cardiovascular system, including our data where RelB controls pulmonary endothelial intercellular adhesion molecule-1 (ICAM-1) levels in response to CS [27]. RelB-deficient mice also have inflammatory cell infiltrates in the heart [35]. Thus our data and that of others support that RelB expression is an essential suppressor of pulmonary and cardiovascular inflammation. We therefore hypothesize that systemic RelB expression will be associated with cardiovascular outcomes in COPD, particularly at exacerbation when inflammation is typically increased.

The objectives of this study were: (1) to assess systemic RelB mRNA expression relative to other inflammatory markers relevant to both COPD pathogenesis and whose expression is regulated by RelB (*e.g*. Cox-2, IL-8, IL-1β) in stable-state and exacerbating COPD patients and (2) to assess associations between these two subject groups in relation to acid-base, cardiovascular and pulmonary patient variables. Our results reveal for the first time that RelB is expressed in stable-state and at exacerbation in COPD. Although circulating RelB levels did not correlate with any clinical parameters at stable-state, we show for the first time that RelB expression is associated with both acid-base and cardiovascular features of COPD patients at exacerbation, suggesting that RelB may represent a novel biomarker of cardiovascular outcomes during COPD exacerbations.

Materials and Methods

Study subjects

48 COPD patients were recruited at the Montreal Chest Institute between February-September 2013, including 18 in stable-state (no-exacerbation in the 4 weeks before assessment) and 30 who were hospitalized with a primary diagnosis of exacerbation. Exacerbations were defined based on symptom-change lasting at least two consecutive days requiring treatment with corticosteroid and/or antibiotics and hospitalization [36,37]. All subjects had to be ≥40 years old, previously diagnosed with COPD [1] (post-bronchodilator forced expired volume in one second (FEV_1) to forced vital capacity (FVC) ratio <0.70) and have clinical evidence of cardiovascular disease and/or established risk factors (clinical signs or imaging studies; coronary artery disease; peripheral vascular disease; previous stroke or myocardial infarction (MI); diabetes mellitus with target organ disease; or treatment for hypercholesterolemia, hypertension, diabetes mellitus or peripheral vascular disease). This study was conducted in accordance with the amended Declaration of Helsinki. The McGill University Faculty of Medicine Institutional Review Board (IRB) approved the protocol and written informed consent was obtained from all patients.

Clinical assessment and blood sample collection

Subjects underwent post-bronchodilator spirometry, blood collection (biomarker, lipid profile, complete cell blood count, and metabolic panel analyses), pulse, oxygen saturation, capillary gas, and hemodynamic measurements (carotid-femoral pulse wave velocity (cfPWV) and pulse wave analysis). Peripheral blood was collected using PAXgene blood RNA tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). For those in stable-state, assessments occurred during respiratory clinic visits and for exacerbating subjects, assessments were made during the first 72 hours of hospitalization. Samples were frozen at −80 until analysis.

Analysis of gene expression

RNA was isolated using PAXgene blood RNA kits (PreAnalytiX GmbH), and quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware). Reverse transcription of total RNA was carried out using iScriptII Reverse Transcription Supermix (Bio-Rad Laboratories, Mississauga, Canada). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed by adding 1 μL cDNA and 0.5 mM primers (Table 1) to SsoFast EvaGreen (Bio-Rad). PCR amplification was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad). Melt curves were analyzed to confirm that non-specific products were absent. The fluorescence detection threshold was set above the non-template control background within the linear phases of PCR amplifications and the cycle threshold (Ct) of each reaction was detected. Gene expression was analyzed using

Table 1. qRT-PCR Primer sequences.

Primer	Forward sequence	Reverse sequence
hCox-2	TCACAGGCTTCCATTGACCAG	CCGAGGCTTTTCTACCAGA
hIL-8	GATGTCAGTGCATAAAGACATACTCCAAAC	GCTCTCTTCCATCAGAAAGCTTTACAATAA
hIL-1β	AAAAGCTTGGTGATGTCTGGTCCATATGAA	CTTATCATCTTTCAACACGCAGGACAGGTA
hRelB	TGTGGTGAGGATCTGCTTCCA G	GGCCCGCTTTCCTTGTTAATT C
β-actin	CTACAATGAGCTGCGTGTG	TGGGGTGTTGAAGGTCTC

doi:10.1371/journal.pone.0112965.t001

the ΔΔCt method and results are presented as fold-differences normalized to housekeeping gene (β-actin).

Statistical analysis

Analyses were performed using SAS version 9.3 software (SAS Institute. Inc., Cary, North Carolina). For mean comparisons, two-tailed T-tests (normal distribution) or Wilcoxon signed-rank tests (non-normal distribution) were used; Chi-squared tests were used for dichotomous variables. A p-value of 0.05 or less was deemed statistically significant. Associations between clinical variables and mediators were assessed using Pearson correlation coefficients. Multiple regression models were used to determine whether changes in mediator expression could predict changes in clinical variables (adjusted for age, sex, body mass index and smoking history).

Results

Patient characteristics

Both the stable-state and exacerbation subjects groups were statistically-similar in most of the clinical features examined except that the hospitalized group had a lower mean FEV_1 (% predicted and liters), included more current smokers, and had significantly greater blood pressure and elevated heart rate (Table 2).

Stable-state and exacerbation inflammatory mediator expression relative to RelB levels

Figure 1 shows the relative fold-difference in mRNA expression of RelB compared to Cox-2, IL-8 and IL-1β. When considering the Stable-state group only, RelB mRNA expression was 69.01 fold less than that of Cox-2, 4.42 fold less than that of IL-8 and 26.97 fold less than that of IL-1β ($p<0.001$ for all cases). When considering the subjects who were in exacerbation, RelB mRNA expression was 65.46 fold less than that of Cox-2 ($p<0.001$), 1.48 fold less than that of IL-8 ($p = 0.22$) and 32.16 fold less than that of IL-1β ($p<0.001$). Moreover in exacerbating patients, RelB mRNA expression was 1.43 fold lower than in stable-state patients ($p< 0.001$) (Figure S1).

Associations between inflammatory mediators and patient clinical features during Stable-state or Exacerbation

In the stable-state patients (Table 3), Cox-2 mRNA expression correlated negatively with FEV_1 (liters), FEV_1/FVC and the ratio of cholesterol to high-density lipoprotein (HDL). IL-8 mRNA

Table 2. Patient characteristics.

	Stable-state (n = 18)	Exacerbation (n = 30)	P-value
Mean age (years)	71.0	71.1	0.86
No. Male (%)	7 (39)	14 (47)	0.11
Mean FEV_1 % predicted	43.5	34.5	0.046*
Mean FEV_1 L	1.1	0.79	0.0082*
Mean FEV_1/FVC	0.44	0.48	0.30
Smoking status, n(%)			
Ex-smoker	17 (94)	23 (77)	0.083
Current smoker	1 (6)	7 (23)	0.014*
LTOT (%)	22	20	0.62
Mean pack-year smoking history	68.77	57.84	0.42
Mean body mass index	27.9	25.1	0.15
Mean no. reported exacerbations in past 12 months	2	3	0.57
Mean systolic BP	111	125	0.013*
Mean diastolic BP	54	66	0.0049*
Mean heart rate	78	92	0.0030*
Pulse oximetry (%)	94	94	0.57

*Denotes statistical significance. No: number, LTOT: long-term oxygen therapy, BP: blood pressure.

doi:10.1371/journal.pone.0112965.t002

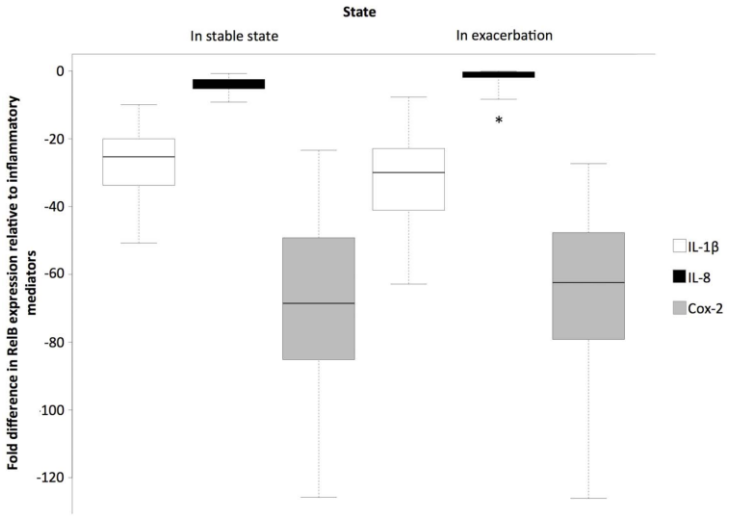


Figure 1. Fold difference in RelB mRNA expression relative to IL-1β, IL-8 and Cox-2 mRNA expression for patients in stable-state (n = 18) and those in exacerbation (n = 30). * Denotes non-statistically significant difference in expression (p = 0.22), for all other p<0.001.
doi:10.1371/journal.pone.0112965.g001

correlated negatively with systolic and diastolic blood pressure, and absolute basophil count, and positively with red blood cell (RBC) diameter width. IL-1β mRNA expression correlated positively with pack-year smoking history, white blood cell (WBC) count, absolute monocyte count and absolute neutrophil count. RelB mRNA expression was not correlated to any clinical variables in stable-state.

When considering mRNA expression in exacerbating subjects (Table 3), Cox-2 mRNA expression correlated negatively with systolic blood pressure, hematocrit (%) and anion gap, and positively with pack-year smoking history, calcium levels, RBC diameter width and absolute eosinophil count. IL-8 mRNA expression correlated negatively with systolic blood pressure, pH, anion gap and glucose level, and positively with the partial pressure of carbon dioxide (PCO_2), augmentation index (AIx) and absolute eosinophil count. IL-1β mRNA expression correlated negatively with cholesterol level, RBC count, hemoglobin level and hematocrit level. RelB mRNA expression correlated negatively with systolic blood pressure, anion gap, and glucose level.

Predictors of change in patient clinical features

When only considering the predictive relationships between inflammatory mediator mRNA expression and clinical features of stable-state patients (Figure 2a; Table 4), Cox-2 could predict negative changes in diastolic blood pressure, PCO_2, cfPWV, mean arterial pressure, AIx, cholesterol level, and low density lipoprotein (LDL). IL-8 expression could predict negative changes in systolic blood pressure and absolute basophil count, and positive changes in RBC diameter width. IL-1β expression could predict positive changes in pH, WBC count, and absolute neutrophil count. The expression of RelB could not significantly predict changes for any variables in stable-state.

For exacerbating patients (Figure 2b; Table 5), Cox-2 mRNA expression could predict negative changes in anion gap and positive changes in PCO_2, blood calcium level, RBC width, absolute eosinophil count, absolute basophil count and bicarbonate level. IL-8 mRNA expression could predict negative changes in systolic blood pressure, pH, and anion gap, and positive changes in PCO_2, augmentation pressure, AIx and absolute eosinophil count. IL-1β expression could predict negative changes in FEV_1/FVC, RBC count, hemoglobin level, and hematocrit level, and positive changes in calcium level. RelB mRNA expression could predict negative changes in systolic blood pressure and anion gap.

Discussion

Finding biomarkers of patient-relevant outcomes is a primary goal of COPD research [38]. Acute inflammatory changes that occur during exacerbations make it important to differentiate between biomarkers that might be useful for assessing disease activity and/or outcomes in stable-state from those found during exacerbation [38]. One such biomarker that offers potential in COPD is RelB, an NF-κB family member that is constitutively expressed in human lymphocytes and dendritic cells [39], suppresses cytokine production in lung epithelial cells [40] and is vital for thymus development and T cell function [41,42]. Importantly, RelB suppresses CS-induced inflammation by interacting with the aryl hydrocarbon receptor (AhR) [23–27] to collectively control Cox-2 expression in lung fibroblasts [24]. Previous studies of the importance of RelB on CS and lung diseases have been restricted to experimental *in-vitro* and *in-vivo* models. Although these studies conclusively support an anti-inflammatory role for RelB against CS, there is no data on RelB expression in COPD or associations with relevant clinical outcomes. We report RelB expression for the first time in COPD patients and provide novel evidence that RelB may be associated with clinically-relevant features of COPD patients during exacerbations. Consequently, our study is an important step to provide insights into RelB expression and its potential role in COPD. It

Table 3. Associations between inflammatory mediators and clinical features of patients in stable-state and at exacerbation.

	Inflammatory mediators							
	Cox-2 expression levels		IL-1β expression levels		RelB expression levels		IL-8 expression levels	
	Correlation coefficients		Correlation coefficients		Correlation coefficients		Correlation coefficients	
Clinical features	Stable-state n = 18	Exacerbation n = 30	Stable-state n = 18	Exacerbation n = 30	Stable-state n = 18	Exacerbation n = 30	Stable-state n = 18	Exacerbation n = 30
Pack-year smoking history	0.36	0.39*	0.49*	0.13	0.39	−0.020	0.050	0.18
Systolic BP	−0.18	−0.42*	0.13	−0.32	−0.090	−0.41*	−0.52*	−0.52*
Diastolic BP	−0.37	−0.29	−0.060	−0.36	−0.26	−0.17	−0.51*	−0.10
FEV_1 (L)	−0.51*	−0.060	0.11	0.070	−0.19	−0.080	−0.25	−0.20
FEV_1/FVC	−0.56*	−0.11	−0.33	−0.10	−0.37	−0.12	−0.11	−0.17
pH	0.33	0.11	0.42	0.22	0.16	−0.21	0.58	−0.46*
PCO_2 (mmHg)	−0.21	0.26	−0.34	−0.090	-0.10	0.41	−0.13	0.49*
Ca++ (mmol/L)	−0.070	0.47*	0.15	0.40	−0.57	0.35	0.080	0.19
Cholesterol/HDL	−0.51*	−0.060	−0.30	−0.15	0.080	0	−0.090	0.070
Cholesterol (mmol/L)	−0.25	−0.11	−0.34	−0.41*	−0.050	−0.36	−0.25	0.050
Aix (%)	−0.10	−0.15	−0.29	−0.13	−0.11	0.22	−0.17	0.46*
Hct (%)	0.26	−0.47*	0.48	−0.38	−0.030	−0.13	−0.10	−0.30
Hct (L/L)	−0.21	−0.36	0.30	−0.52*	−0.24	−0.11	−0.44	−0.18
WBC[a]	0.24	0.010	0.76**	0.33	0.32	−0.040	0.40	−0.12
RBC (10^{12}/L)	−0.15	−0.33	0.31	−0.47*	−0.11	−0.14	−0.37	−0.18
RBC diameter width (cV)	0.030	0.52*	0.19	0.29	0.26	0.22	0.67*	0.050
Hemoglobin (g/L)	−0.14	−0.34	0.30	−0.46*	−0.21	−0.16	−0.45	−0.17
Abs. MNC[a]	0.070	−0.05	0.63*	0.080	0.31	0.090	0.28	−0.060
Abs. neutrophil[a]	0.32	0	0.74**	0.35	0.29	−0.10	0.46	−0.15
Abs. basophil[a]	−0.020	0.25	0.21	0.20	−0.060	0.32	−0.61*	0.080
Abs. eosinophil[a]	0.010	0.49*	0.010	0.050	0.20	0.30	0.20	0.55*
Anion gap (mmol/L)	−0.050	−0.47*	0.13	0.020	0.21	−0.55*	0.15	−0.55*
Glucose (mmol/L)	−0.33	−0.31	0.14	0.15	0.050	−0.41*	0.12	−0.39*

Pearson correlation coefficients were used to determine associations.

*Denotes statistical significance $p<0.05$, ** denotes statistical significance $p<0.001$.

[a]units are (10^9/L).

BP: blood pressure, Ca++: calcium, Hct: hematocrit, Abs.: absolute, MNC: monocyte.

doi:10.1371/journal.pone.0112965.t003

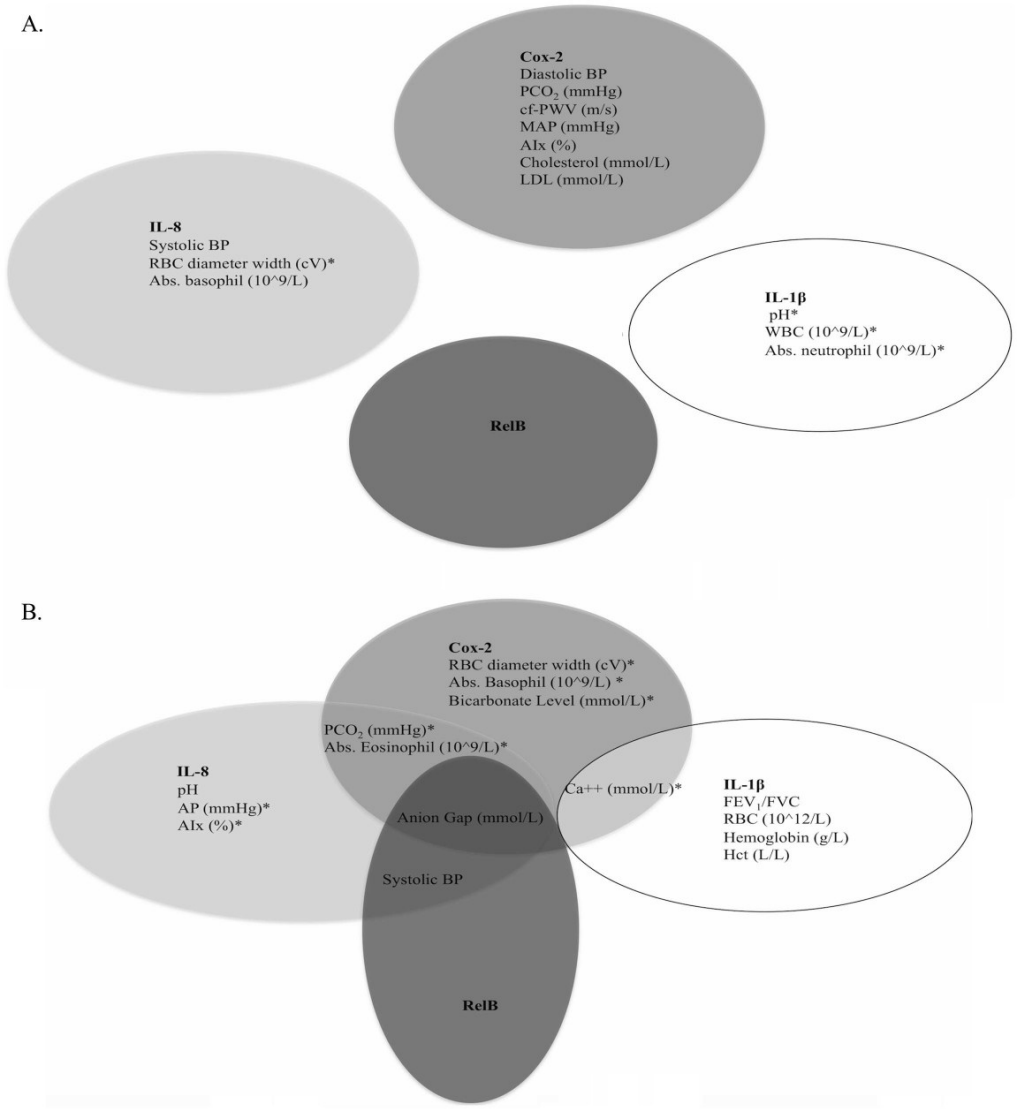


Figure 2. Ability of inflammatory mediators to predict changes in patient clinical features in a) stable-state (n = 18) and b) at exacerbation (n = 30). A linear regression model was used and adjusted for age, sex, body mass index and smoking pack-years. The inflammatory mediators were used as independent variables to predict changes in all assessed clinical features. * Denotes a positive association. BP: blood pressure, MAP: mean arterial pressure, Abs: absolute, AP: augmentation pressure.
doi:10.1371/journal.pone.0112965.g002

reports for the first time the associations between RelB and clinical parameters during COPD exacerbations.

One of our most intriguing findings is that although RelB mRNA expression was not associated with clinical outcomes in stable-state, at exacerbation RelB expression was negatively associated with several clinical parameters including systolic blood pressure. Our finding on RelB expression and systolic blood pressure is novel, as a relationship between RelB and blood pressure has not been reported to-date in humans. Experimentally RelB has been associated with balloon catheter injury in the rat carotid artery [43] and may be downregulated in response to treatment with DETA-NONOate- a nitric oxide donor [44].

Table 4. Predictors of clinical feature change in stable-state.

	Stable-state COPD (n = 18)							
	Cox-2 expression levels		IL-1beta expression levels		RelB expression levels		IL-8 expression levels	
Clinical features	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value	ß (95% CI)	p-value
Systolic BP	−637.13 (−1839.06–564.79)	0.27	48.89 (−2856.79–2954.56)	0.97	−66325.7 (−193597–60945.40)	0.28	−8410.48 (−16147.2–−673.76)	0.036*
Diastolic BP	−1173.04 (−2117.53–−228.56)	0.019*	−391.22 (−3170.54–2388.10)	0.76	−70756.5 (−191298–49784.85)	0.22	−7471.46 (−15178.5–235.53)	0.056
pH	2.85 (−0.64–6.33)	0.086	8.24 (1.35–15.13)	0.029*	182.37 (−357.62–722.36)	0.40	28.54 (−6.07–63.15)	0.084
PCO2 (mmHg)	−474.44 (−861.41–−87.47)	0.027*	−593.12 (−2343.89–1157.64)	0.40	−15073.0 (−98917.2–68771.15)	0.64	−1125.91 (−8586.88–6335.06)	0.70
cfPWV (m/s)	−634.31 (−1006.47–−262.14)	0.0030*	−807.59 (−1798.15–182.97)	0.10	−10424.8 (−62823.5–41973.93)	0.67	−557.57 (−4426.40–3311.26)	0.76
MAP (mmHg)	−1445.68 (−2228.01–−663.36)	0.0020*	−616.78 (−3197.05–1963.49)	0.60	−61331.8 (−172971–50307.08)	0.25	−5757.05 (−13787.5–2273.42)	0.14
Aix %	−931.72 (−1859.35–−4.09)	0.049*	−546.46 (−2782.59–1689.67)	0.59	−37079.5 (−138077–63918.39)	0.43	−1724.41 (−9533.71–6084.89)	0.63
Cholesterol (mmol/L)	−62.21 (−119.63–−4.80)	0.036*	−65.24 (−282.03–151.55)	0.52	86.86 (−8457.91–8631.63)	0.98	−302.84 (−1043.82–438.15)	0.38
LDL (mmol/L)	−61.38 (−117.57–−5.19)	0.035*	−85.14 (−294.24–123.95)	0.39	1469.95 (−6853.37–9793.26)	0.70	−319.33 (−1042.00–403.34)	0.35
WBC (10^9/L)	85.65 (−54.29–225.59)	0.21	504.49 (186.88–822.10)	0.0050*	3843.09 (−12921.6–20607.79)	0.62	913.43 (−368.64–2195.49)	0.15
RBC diameter width (cV)	44.43 (−136.38–225.24)	0.60	270.73 (−259.38–800.83)	0.29	8895.13 (−10807.2–28597.48)	0.34	1690.40 (386.50–2994.31)	0.016*
Abs. neutrophil (10^9/L)	85.84 (−51.73–223.41)	0.20	470.02 (138.86–801.18)	0.010*	2751.54 (−13865.3–19368.40)	0.72	1021.97 (−200.52–2244.47)	0.093
Abs. basophil (10^9/L)	−1.23 (−4.08–1.62)	0.36	−2.48 (−11.38–6.43)	0.55	−111.46 (−435.83–212.92)	0.47	−27.49 (−48.50–−6.48)	0.015*

Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. Inflammatory markers were used as independent variables to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in biomarker expression.
*Denotes statistical significance.
MAP: mean arterial pressure.
doi:10.1371/journal.pone.0112965.t004

Table 5. Predictors of clinical feature change at exacerbation.

	Exacerbation (n = 30)							
	Cox-2 expression levels		IL-1β expression levels		RelB expression levels		IL-8 expression levels	
Clinical features	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**	**ß (95% CI)**	**p-value**
Systolic BP	−677.58 (−1586.61–231.45)	0.14	−1390.54 (−3283.70–502.62)	0.14	−50749.6 (−92792.3–−8706.83)*	0.020*	−12649.1 (−24031.7–−1266.55)*	0.031*
pH	−0.13 (−2.15–1.90)	0.90	1.00 (−2.94–4.94)	0.60	−25.42 (−116.18–65.33)	0.56	−29.07 (−52.47–−5.67)*	0.018*
PCO_2 (mmHg)	360.06 (18.51–701.60)	0.040*	123.24 (−620.68–867.17)	0.73	12518.77 (−3831.36–28868.91)	0.13	5085.44 (564.18–9606.71)*	0.030*
Ca++ (mmol/L)	2.82 (0.46–5.19)	0.023*	5.41 (0.34–10.48)*	0.038*	85.87 (−33.15–204.90)	0.14	15.66 (−23.12–54.43)	0.40
FEV_1/FVC	−2.27 (−9.08–4.55)	0.50	−14.75 (−28.27–−1.23)*	0.034*	−157.61 (−492.65–177.42)	0.34	−46.09 (−140.18–48.00)	0.32
AP (mmHg)	−115.51 (−500.67–269.66)	0.54	−120.55 (−831.09–589.99)	0.73	12466.04 (−5030.98–29963.05)	0.15	5254.52 (1644.29–8864.76)*	0.0070*
Aix (%)	−348.85 (−1173.38–475.68)	0.39	−572.74 (−2098.13–952.65)	0.44	22125.77 (−16420.3–60671.88)	0.24	11956.20 (4295.85–19616.55)*	0.0040*
RBC (10^12/L)	−26.85 (−54.28–0.57)	0.060	−79.50 (−132.82–−26.18)*	0.0060*	−833.40 (−2267.21–600.42)	0.24	−233.26 (−634.42–167.91)	0.24
Hemoglobin (g/L)	−697.08 (−1584.64–190.48)	0.12	−2074.77 (−3864.48–−285.06)*	0.025*	−27604.0 (−72519.4–17311.45)	0.22	−5718.07 (−18463.6–7027.43)	0.36
Hct (L/L)	−2.18 (−4.87–0.51)	0.11	−7.33 (−12.46–−2.19)*	0.0070*	−63.61 (−202.16–74.94)	0.35	−17.94 (−56.53–20.65)	0.34
RBC diameter width (cV)	83.94 (12.69–155.20)	0.023*	42.68 (−122.55–207.91)	0.60	2309.70 (−1537.06–6156.46)	0.23	−247.14 (−1305.99–811.72)	0.63
Abs. eosinophil (10^9/L)	4.15 (0.79–7.51)	0.018*	−1.17 (−9.51–7.16)	0.77	134.32 (−45.59–314.24)	0.14	63.29 (18.69–107.89)	0.0080*
Abs. basophil (10^9/L)	1.89 (0.23–3.54)	0.027*	0.97 (−2.45–4.40)	0.56	77.14 (−8.13–162.41)	0.074	5.87 (−16.07–27.82)	0.58
Bicarbonate level (mmol/L)	214.54 (41.93–387.15)	0.017*	−28.89 (−431.63–373.85)	0.88	8733.02 (−227.46–17693.51)	0.056	1700.47 (−753.55–4154.48)	0.16
Anion gap (mmol/L)	−183.24 (−289.11–−77.38)	0.0020*	0.27 (−288.19–288.73)	1.00	−8316.41 (−13802.0–−2830.85)*	0.0050*	−2294.72 (−3798.95–−790.50)	0.0050*
Sodium (mmol/L)	111.10 (−89.66–311.86)	0.26	54.92 (−389.96–499.80)	0.80	5829.30 (−4079.80–15738.40)	0.24	−346.36 (−3193.78–2501.05)	0.80

Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. Inflammatory markers were used as independent variables to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in biomarker expression.
*Denotes statistical significance.
MAP: mean arterial pressure.
doi:10.1371/journal.pone.0112965.t005

Moreover, our recently published data show that RelB may suppress pulmonary ICAM-1 levels in response to CS [27]. When considered together with our current data, this supports a role for RelB in modulating endothelial function and blood pressure. This is potentially of importance, as it is well known that the period immediately following a COPD exacerbation is associated with increased risk of acute cardiovascular events [32–34]. In a separate analysis, we grouped stable-state and exacerbating subjects and found RelB expression to be negatively correlated to and able to predict negative changes in several outcomes including heart rate and PCO_2, while predicting positive changes in pulse pressure (Table S1). Thus, identifying novel biological targets that could be capable of maintaining cardiovascular stability in COPD is of significant clinical value.

In addition to cardiovascular events, RelB expression was also related to anion gap, a parameter commonly used to identify acid-base disorders and disturbances [45]. In COPD, acid-base disturbances occur frequently [31] and lead to poor patient outcomes. Although it was recently shown that RelB is carbon dioxide-sensitive and contributes to the benefits of hypercapnia in pulmonary inflammatory diseases [30], our study is the first to report on RelB expression in relation to anion gap. This renders it possible that RelB may play a role in acid-base regulation during COPD exacerbations. Together, these data lend further support for an association between RelB and cardiovascular function as well as the involvement of RelB in acid-base maintenance. Thus, given the relationship between RelB and CS-induced inflammation, it might be reasonable to speculate that alterations in RelB expression and/or activity during exacerbations in COPD contribute to cardiovascular manifestations.

RelB dampens the expression of numerous COPD-relevant inflammatory mediators. Therefore we also examined associations between these (*i.e.* Cox-2, IL-8 and IL-1β) and patient clinical features. As with RelB expression, several of these mediators exhibited strong associations with cardiovascular alterations. Cox-2 is induced by HDL [46] and LDL [47], and although an association between Cox-2 and blood pressure has been described [48,49], our study is first to suggest a relationship with cfPWV and AIx, two measures of arterial stiffening associated with cardiovascular risk. Moreover, the positive relationship between Cox-2 and RBC diameter width is novel and may be of clinical significance, as RBC diameter width-distribution is a powerful outcome predictor in chronic and/or acute left heart failure, and in COPD, can help identify right ventricle failure [50]. Associations between IL-8 and cardiovascular outcomes have been reported [51,52], although none specifically on AIx and augmentation pressure. In our study IL-8 was also linked to acid-base parameters (pH, anion gap and PCO_2) and fluctuations in these have been shown to alter pH and neutrophil IL-8 release [53]. Alterations in pH can also control IL-1β production by monocytes, not only supporting the relationship between IL-1β and pH [54] but also perpetuating and augmenting the inflammatory response in COPD.

Our study has strengths as well as limitations, the former including the fact that we recruited patients both in stable-state COPD and patients who were in acute exacerbation requiring hospitalization (*i.e.* severe exacerbation). Patients with severe COPD exacerbations are often difficult to recruit, as they are very sick and often refuse to participate in research studies. An additional strength of our study is that we also assessed patients within the first 72 hours of hospitalization, which allowed us to capture important physiological and clinical data that occur systemically and locally within the lung. A limitation of our study is that 25 of the 30 exacerbating patients took inhaled corticosteroids prior to blood collection. Corticosteroids can dampen Cox-2 and IL-8 expression via the NF-κB pathway by suppressing gene transcription [55,56]. Thus the relative expression levels we report may be an under-representation. Another perceived limitation is the reliance on mRNA levels to correlate with clinical parameters, as quantification of blood RelB protein expression remains to be determined. However, RelB protein expression mirrors that of mRNA levels during dendritic cell differentiation [57]. Thus, we expect a similar association between blood RelB mRNA and protein levels in COPD. It would also strengthen our observations presented herein to further investigate the relationship between RelB expression and pulmonary patient outcomes in COPD stages GOLD I–IV; these investigations are currently ongoing. Finally, we recognize that the subjects in our study are unpaired, thereby reducing the statistical power and rendering it possible that inter-subject variability may have impacted our measurements. Thus, investigation of RelB and associated clinical outcomes in paired subjects (stable-state and exacerbation from the same subjects) is warranted. To ultimately determine the suitability of RelB as a biomarker in COPD, a longitudinal relationship between expression and associated outcomes must be examined.

There has been growing evidence that RelB is a potent suppressor of CS-induced inflammation [24,25,27,40,58]. Thus despite the limitations of our study, the expression and function of RelB in COPD represents a burgeoning area of research, and our data on the associations of RelB expression in COPD are highly novel and clinically relevant. Moreover, the results of our study suggest for the first time that blood RelB expression may be a noteworthy marker of cardiovascular events during COPD exacerbations. Future longitudinal and mechanistic studies will undoubtedly shed light on the functional significance of RelB in COPD pathogenesis and its potential for therapeutic modulation.

Supporting Information

Figure S1 Mean fold difference (± standard error) in RelB mRNA expression at exacerbation (n = 30) relative to RelB mRNA expression in stable-state patients (n = 18). Fold decrease in RelB expression at exacerbation is 1.43 ($p<0.001$).
(DOCX)

Table S1 Associations between RelB expression levels and clinical features of both exacerbating and stable-state patients (n = 48) and its ability to predict changes in these features.
(DOCX)

Acknowledgments

We would like to thank all researchers, staff, in particular Katrina Metz for blood sample collection and Pei Z. Li for her help with statistical analyses, and to the COPD patients who participated in this study.

Author Contributions

Conceived and designed the experiments: LL JB CJB. Performed the experiments: LL PC MZ. Analyzed the data: LL CJB. Contributed reagents/materials/analysis tools: LL PC MZ JB CJB. Wrote the paper: LL JB CJB.

References

1. GOLD (2014) Global Strategy for the Diagnosis, Management and Prevention of COPD: Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2014. http://wwwgoldcopdorg/.
2. Tam A, Sin DD (2012) Pathobiologic mechanisms of chronic obstructive pulmonary disease. Med Clin North Am 96: 681–698.
3. Wedzicha JA, Seemungal TA, MacCallum PK, Paul EA, Donaldson GC, et al. (2000) Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. Thromb Haemost 84: 210–215.
4. Wedzicha JA, Donaldson GC (2003) Exacerbations of chronic obstructive pulmonary disease. Respir Care 48: 1204-1213; discussion 1213–1205.
5. Wedzicha JA, Seemungal TA (2007) COPD exacerbations: defining their cause and prevention. Lancet 370: 786–796.
6. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, et al. (2010) Susceptibility to exacerbation in chronic obstructive pulmonary disease. N Engl J Med 363: 1128–1138.
7. Aaron SD, Donaldson GC, Whitmore GA, Hurst JR, Ramsay T, et al. (2012) Time course and pattern of COPD exacerbation onset. Thorax 67: 238–243.
8. Wedzicha JA, Singh R, Mackay AJ (2014) Acute COPD exacerbations. Clin Chest Med 35: 157–163.
9. Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA (2000) Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 161: 1608–1613.
10. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, et al. (1998) Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 157: 1418–1422.
11. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA (2002) Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax 57: 847–852.
12. Barnes PJ, Karin M (1997) Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336: 1066–1071.
13. Barnes PJ (2006) Transcription factors in airway diseases. Lab Invest 86: 867–872.
14. Barnes PJ (2009) The Cytokine Network in Chronic Obstructive Pulmonary Disease . Am J Respir Cell Mol Biol 41: 631–638.
15. Grossmann M, Nakamura Y, Grumont R, Gerondakis S (1999) New insights into the roles of ReL/NF-kappa B transcription factors in immune function, hemopoiesis and human disease. Int J Biochem Cell Biol 31: 1209–1219.
16. Hayden MS, Ghosh S (2008) Shared principles in NF-kappaB signaling. Cell 132: 344–362.
17. Barnes PJ, Shapiro SD, Pauwels RA (2003) Chronic obstructive pulmonary disease: molecular and cellularmechanisms. European Respiratory Journal 22: 672–688.
18. van Eeden SF, Sin DD (2008) Chronic obstructive pulmonary disease: a chronic systemic inflammatory disease. Respiration 75: 224–238.
19. Sarir H, Henricks PA, van Houwelingen AH, Nijkamp FP, Folkerts G (2008) Cells, mediators and Toll-like receptors in COPD. Eur J Pharmacol 585: 346–353.
20. Celli BR, Barnes PJ (2007) Exacerbations of chronic obstructive pulmonary disease. Eur Respir J 29: 1224–1238.
21. Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, et al. (2002) Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax 57: 759–764.
22. Barnes PJ (2004) Mediators of chronic obstructive pulmonary disease. Pharmacol Rev 56: 515–548.
23. Thatcher TH, Maggirwar SB, Baglole CJ, Lakatos HF, Gasiewicz TA, et al. (2007) Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB. Am J Pathol 170: 855–864.
24. Baglole CJ, Maggirwar SB, Gasiewicz TA, Thatcher TH, Phipps RP, et al. (2008) 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 283: 28944–28957.
25. McMillan DH, Baglole CJ, Thatcher TH, Maggirwar S, Sime PJ, et al. (2011) Lung-targeted overexpression of the NF-kappaB member RelB inhibits cigarette smoke-induced inflammation. Am J Pathol 179: 125–133.
26. Zago M, Rico de Souza A, Hecht E, Rousseau S, Hamid Q, et al. (2014) The NF-kappaB family member RelB regulates microRNA miR-146a to suppress cigarette smoke-induced COX-2 protein expression in lung fibroblasts. Toxicol Lett 226: 107–116.
27. de Souza AR, Zago M, Eidelman DH, Hamid Q, Baglole CJ (2014) Aryl Hydrocarbon Receptor (AhR) Attenuation of Subchronic Cigarette Smoke-induced Pulmonary Neutrophilia is Associated with Retention of Nuclear RelB and Suppression of Intercellular Adhesion Molecule-1 (ICAM-1). Toxicol Sci.
28. Weih F, Warr G, Yang H, Bravo R (1997) Multifocal defects in immune responses in RelB-deficient mice. J Immunol 158: 5211–5218.
29. Weih F, Durham SK, Barton DS, Sha WC, Baltimore D, et al. (1996) Both multiorgan inflammation and myeloid hyperplasia in RelB-deficient mice are T cell dependent. J Immunol 157: 3974–3979.
30. Oliver KM, Lenihan CR, Bruning U, Cheong A, Laffey JG, et al. (2012) Hypercapnia induces cleavage and nuclear localization of RelB protein, giving insight into CO2 sensing and signaling. J Biol Chem 287: 14004–14011.
31. Bruno CM, Valenti M (2012) Acid-base disorders in patients with chronic obstructive pulmonary disease: a pathophysiological review. J Biomed Biotechnol 2012: 915150.
32. Patel AR, Kowlessar BS, Donaldson GC, Mackay AJ, Singh R, et al. (2013) Cardiovascular risk, myocardial injury, and exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 188: 1091–1099.
33. Donaldson GC, Hurst JR, Smith CJ, Hubbard RB, Wedzicha JA (2010) Increased risk of myocardial infarction and stroke following exacerbation of COPD. Chest 137: 1091–1097.
34. McAllister DA, Maclay JD, Mills NL, Leitch A, Reid P, et al. (2012) Diagnosis of myocardial infarction following hospitalisation for exacerbation of COPD. Eur Respir J 39: 1097–1103.
35. Lo D, Quill H, Burkly L, Scott B, Palmiter RD, et al. (1992) A recessive defect in lymphocyte or granulocyte function caused by an integrated transgene. Am J Pathol 141: 1237–1246.
36. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, et al. (1987) Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 106: 196–204.
37. Burge S, Wedzicha JA (2003) COPD exacerbations: definitions and classifications. Eur Respir J Suppl 41: 46s–53s.
38. Jones PW, Agusti AGN (2006) Outcomes and markers in the assessment of chronic obstructive pulmonary disease. Eur Respir J 27: 822–832.
39. Ammon C, Mondal K, Andreesen R, Krause SW (2000) Differential expression of the transcription factor NF-kappaB during human mononuclear phagocyte differentiation to macrophages and dendritic cells. Biochem Biophys Res Commun 268: 99–105.
40. Tully JE, Nolin JD, Guala AS, Hoffman SM, Roberson EC, et al. (2012) Cooperation between classical and alternative NF-kappaB pathways regulates proinflammatory responses in epithelial cells. Am J Respir Cell Mol Biol 47: 497–508.
41. Millet P, McCall C, Yoza B (2013) RelB: an outlier in leukocyte biology. J Leukoc Biol 94: 941–951.
42. Carrasco D, Ryseck RP, Bravo R (1993) Expression of relB transcripts during lymphoid organ development: specific expression in dendritic antigen-presenting cells. Development 118: 1221–1231.
43. Lindner V (1998) The NF-kappaB and IkappaB system in injured arteries. Pathobiology 66: 311–320.
44. Braam B, de Roos R, Dijk A, Boer P, Post JA, et al. (2004) Nitric oxide donor induces temporal and dose-dependent reduction of gene expression in human endothelial cells. Am J Physiol Heart Circ Physiol 287: H1977–1986.
45. Kraut JA, Madias NE (2007) Serum anion gap: its uses and limitations in clinical medicine. Clin J Am Soc Nephrol 2: 162–174.
46. Xiong SL, Liu X, Yi GH (2014) High-density lipoprotein induces cyclooxygenase-2 expression and prostaglandin I-2 release in endothelial cells through sphingosine kinase-2. Mol Cell Biochem 389: 197–207.
47. Taketa K, Matsumura T, Yano M, Ishii N, Senokuchi T, et al. (2008) Oxidized low density lipoprotein activates peroxisome proliferator-activated receptor-alpha (PPARalpha) and PPARgamma through MAPK-dependent COX-2 expression in macrophages. J Biol Chem 283: 9852–9862.
48. Hocherl K, Endemann D, Kammerl MC, Grobecker HF, Kurtz A (2002) Cyclooxygenase-2 inhibition increases blood pressure in rats. Br J Pharmacol 136: 1117–1126.
49. Cathcart MC, Tamosiuniene R, Chen G, Neilan TG, Bradford A, et al. (2008) Cyclooxygenase-2-linked attenuation of hypoxia-induced pulmonary hypertension and intravascular thrombosis. J Pharmacol Exp Ther 326: 51–58.
50. Sincer I, Zorlu A, Yilmaz MB, Dogan OT, Ege MR, et al. (2012) Relationship between red cell distribution width and right ventricular dysfunction in patients with chronic obstructive pulmonary disease. Heart Lung 41: 238–243.
51. Nymo SH, Hulthe J, Ueland T, McMurray J, Wikstrand J, et al. (2014) Inflammatory cytokines in chronic heart failure: interleukin-8 is associated with adverse outcome. Results from CORONA. Eur J Heart Fail 16: 68–75.
52. Testelmans D, Tamisier R, Barone-Rochette G, Baguet JP, Roux-Lombard P, et al. (2013) Profile of circulating cytokines: impact of OSA, obesity and acute cardiovascular events. Cytokine 62: 210–216.
53. Coakley RJ, Taggart C, Greene C, McElvaney NG, O'Neill SJ (2002) Ambient pCO2 modulates intracellular pH, intracellular oxidant generation, and interleukin-8 secretion in human neutrophils. J Leukoc Biol 71: 603–610.
54. Jancic CC, Cabrini M, Gabelloni ML, Rodriguez Rodrigues C, Salamone G, et al. (2012) Low extracellular pH stimulates the production of IL-1beta by human monocytes. Cytokine 57: 258–268.
55. Sethi S (2005) Pathogenesis and treatment of acute exacerbations of chronic obstructive pulmonary disease. Semin Respir Crit Care Med 26: 192–203.

56. Barnes PJ (2009) Role of HDAC2 in the pathophysiology of COPD. Annu Rev Physiol 71: 451–464.
57. Cejas PJ, Carlson LM, Kolonias D, Zhang J, Lindner I, et al. (2005) Regulation of RelB expression during the initiation of dendritic cell differentiation. Mol Cell Biol 25: 7900–7916.
58. Tully JE, Hoffman SM, Lahue KG, Nolin JD, Anathy V, et al. (2013) Epithelial NF-kappaB orchestrates house dust mite-induced airway inflammation, hyperresponsiveness, and fibrotic remodeling. J Immunol 191: 5811–5821.

Table S1. Associations between RelB expression levels and clinical features of both exacerbating and stable-state patients (n = 48) and its ability to predict changes in these features.

	Associations		Ability to predict change in clinical features	
Clinical features	**Correlation coefficient**	**P-value**	**ß (95% CI)**	**P-value**
Systolic BP	-0.42	0.0030*	-51205.3 (-89737.3 - -12673.4)	0.010*
Diastolic BP	-0.33	0.022*	-30750.3 (-62720.6 - 1219.91)	0.059
Pulse (BPM)	-0.38	0.0080*	-43450.1 (-78570.1 - -8330.11)	0.017*
O2 saturation (%)	0.070	0.62	2282.89 (-4423.89 - 8989.67)	0.50
pH	-0.18	0.33	-23.34 (-121.59 - 74.91)	0.63
PO2 (mmHg)	-0.12	0.50	-3703.96 (-31894.9 - 24486.97)	0.79
PCO2 (mmHg)	0.34	0.050*	11295.79 (-3690.84 - 26282.43)	0.13
Na+ (mmol/L)	0.29	0.11	6364.29 (-5672.46 - 18401.04)	0.29
K+ (mmol/L)	-0.29	0.11	-2956.32 (-6340.64 - 428.00)	0.084
Ca++ (mmol/L)	0.15	0.42	22.72 (-115.24 - 160.68)	0.74
Hct (%)	-0.14	0.44	-10363.1 (-26522.9 - 5796.77)	0.20
Approximate number of exacerbations reported during the previous year	-0.1	0.51	-1009.36 (-4995.93 - 2977.21)	0.62
FEV1 L	0.11	0.46	-54.56 (-863.62 - 754.49)	0.89
FEV1 % predicted	-0.07	0.63	-18874.4 (-49897.7 - 12148.90)	0.23
FEV1/FVC	-0.22	0.15	-300.82 (-569.17 - -32.48)	0.029*
mean cfPWV (m/s)	0.15	0.34	2012.17 (-9002.75 - 13027.09)	0.71
mean MAP (mmHg)	-0.2	0.21	-16046.9 (-50007.2 - 17913.50)	0.34
mean PP (mmHg)	0.31	0.051	29042.30 (3063.65 - 55020.94)	0.030*
mean AP (mmHg)	0.24	0.14	13136.28 (-971.24 - 27243.80)	0.067
mean Aix (%)	0.15	0.34	18482.26 (-10646.3 - 47610.86)	0.21
mean crPWV 1 (m/s)	0.12	0.44	841.56 (-2867.24 - 4550.37)	0.65
Cholesterol (mmol/L)	-0.15	0.32	-511.91 (-3010.68 - 1986.86)	0.68
Triglycerides (mmol/L)	0.040	0.82	272.50 (-1368.49 - 1913.49)	0.74
HDL (mmol/L)	-0.14	0.38	-156.49 (-1425.70 - 1112.71)	0.80
LDL (mmol/L)	-0.12	0.46	-478.54 (-2746.65 - 1789.57)	0.67
Cholesterol/HDL	0.030	0.82	-54.97 (-2426.23 - 2316.29)	0.96
WBC (10^9/L)	-0.12	0.42	-2813.16 (-12962.0 - 7335.69)	0.58
RBC (10^12/L)	-0.06	0.70	-737.39 (-2077.71 - 602.93)	0.27
Hemoglobin (g/L)	-0.18	0.23	-31628.3 (-71677.7 - 8421.00)	0.12
Hct (L/L)	-0.14	0.35	-80.22 (-198.90 - 38.46)	0.18
Mean cell vol. (fL)	-0.14	0.36	-3049.93 (-17685.5 - 11585.62)	0.68
Mean cell hemoglobin (pg/cell)	-0.18	0.23	-2086.62 (-7842.80 - 3669.56)	0.47
Mean cell hemoglobin Conc. (g/L)	-0.2	0.17	-11957.5 (-33895.0 - 9980.04)	0.28
RBC diameter Width (cV)	0.29	0.054	2650.85 (-1663.19 - 6964.90)	0.22
Platelet (10^9/L)	-0.18	0.24	-73614.0 (-271159 - 123931.5)	0.46
Platelet Hct	-0.13	0.40	-37.11 (-189.86 - 115.64)	0.63
Mean platelet vol. (fL)	0.16	0.30	563.72 (-1165.91 - 2293.35)	0.51
Platelet Dist. Width (cV)	0.030	0.86	342.31 (-838.73 - 1523.34)	0.56

Abs. Lymphocyte (10^9/L)	0.33	0.025*	1463.58 (-10.50 - 2937.66)	0.052
Abs. MNC (10^9/L)	0.20	0.18	239.08 (-572.63 - 1050.80)	0.56
Abs. Neutrophil (10^9/L)	-0.2	0.17	-5032.65 (-15249.6 - 5184.34)	0.33
Abs. Eosinophil (10^9/L)	0.44	0.0020*	273.19 (39.32 - 507.06)	0.023*
Abs. Basophil (10^9/L)	0.24	0.11	80.54 (-8.99 - 170.08)	0.077
Bicarbonate Level (mmol/L)	0.36	0.016*	7183.79 (344.14 - 14023.45)	0.040*
Anion Gap (mmol/L)	-0.46	0.0010*	-7400.53 (-12612.0 - -2189.07)	0.0070*
Creatinine (umol/L)	-0.08	0.62	-21089.4 (-76711.5 - 34532.62)	0.45
Glucose Random (mmol/L)	-0.45	0.0020*	-11766.3 (-20056.2 - -3476.45)	0.0070*
Chloride (mmol/L)	0.20	0.20	6280.30 (-3149.26 - 15709.86)	0.19
Potassium (mmol/L)	-0.09	0.58	-131.31 (-1059.12 - 796.51)	0.78
Sodium (mmol/L)	0.27	0.069	6063.56 (-1496.57 - 13623.70)	0.11

Pearson correlation coefficients were used to determine associations. Linear regression models were used to estimate predictors of change in clinical features and were adjusted for age, sex, body mass index, and smoking pack-years. RelB was used as an independent variable to predict changes in all clinical features of patients. ß represents the change in a clinical feature associated with a one-unit change in RelB expression. *Denotes statistical significance $p<0.05$. BPM: beats per minute, O2: oxygen, PO2: partial pressure of oxygen, PCO2: partial pressure of carbon dioxide, Na+: sodium, K+: potassium, Ca++: calcium, Hct: hematocrit, FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, BP: blood pressure, cfPWV: carotid-femoral pulse wave velocity, MAP: mean arterial pressure, PP: pulse pressure, AP: augmentation pressure, Aix: augmentation index, crPWV: carotid-radial pulse wave velocity, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WBC: white blood cell, RBC: red blood cell, vol.: volume, Abs.: absolute, MNC: monocyte.

Figure S1. Mean fold difference (± standard error) in RelB mRNA expression at exacerbation (n = 30) relative to RelB mRNA expression in stable-state patients (n = 18). Fold decrease in RelB expression at exacerbation is 1.43 ($p<0.001$).