Chronic obstructive pulmonary disease categorization using cardiovascular risk and inflammatory biomarkers in the Canadian population

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Abstract

Introduction: Cardiovascular disease (CVD) accounts for highest mortality in the patients with chronic obstructive pulmonary disease (COPD) and cardiovascular (CV) risk increases as airflow obstruction worsens. Therefore, CV risk should be assessed in the general Canadian population with mostly mild and moderate COPD.

Objectives: To determine 1) the prevalence and incidence of CVD, and 2) the prevalence of high CV risk groups using risk scores and inflammatory biomarkers, in those with COPD compared to no COPD.

Methods: The Canadian Cohort of Obstructive Lung Disease (CanCOLD) study prospectively followed 1561 participants divided into age and sex matched study groups: healthy (never smoked), at-risk (current/former smokers), mild and moderate+ COPD. Incidence and prevalence of CVD were self reported from questionnaires (ischemic heart disease (IHD), cerebrovascular disease (CBD) and heart failure (HF)), and CV risk was assessed using the Framingham risk score (FRS) and pooled cohort equations (PCE), and by measuring high levels of fibrinogen $\geq$3.5g/L, and c-reactive protein (CRP) $\geq$2mg/L.

Results: The prevalence of cardiac CVD (IHD or HF) was increased in COPD GOLD 2+ compared to no COPD with an odds ratio (OR) of 1.83 [1.25 - 2.68 95%CI] after adjusting for age, sex, smoking, BMI, hypertension and diabetes but prevalence of CBD was not. Subgroup analyses showed increased comorbid IHD OR of 1.57 [1.05-2.35] 95%CI and HF OR of 4.05 [1.71-9.60] 95%CI in GOLD 2+ COPD compared to no COPD. The incidence of CVD was increased in GOLD 2+ COPD with OR of 2.19 [1.07 - 4.50 95%CI] compared to no COPD after adjusting for age and sex but not for incident CBD. The prevalence and incidence of CVD was not

increased in mild COPD even after accounting for the same risk factors. There was no significant difference between CV risk observed between groups in men or women using either risk scores. In men, moderate+ COPD was associated with high levels of CRP compared to others, but not in women. High fibrinogen in men was evident in moderate+ COPD compared to all others except former/current smokers. Among women, high fibrinogen was seen in moderate+ COPD compared all others except mild COPD.

Conclusion: The presence of comorbid CVD or development of new CVD such as IHD and HF is increased in moderate+ COPD but not in milder disease compared to individual in the general population. Proportion of individuals considered high risk using CV risk scores were similar among groups even though proportion of men and women with high levels of biomarkers were greatest in moderate + COPD.

Abrege

Introduction: Les maladies cardiovasculaires (MCV) représentent la mortalité la plus élevée chez les patients présentant une maladie pulmonaire obstructive chronique (MPOC) et le risque cardiovasculaire (CV) augmente à mesure que l'obstruction des voies respiratoires s'aggrave. Par conséquent, le risque cardiovasculaire devrait être évalué dans la population canadienne en général atteinte principalement de MPOC légère et modérée.

Objectifs: Déterminer 1) la prévalence et l'incidence des maladies cardiovasculaires, et 2) la prévalence des groupes à haut risque CV à l'aide de scores de risque et de biomarqueurs inflammatoires, chez les personnes atteintes de MPOC par rapport à l'absence de MPOC.

Méthodes: L'étude CanCOLD a suivi de manière prospective 1561 participants répartis en groupes d'étude appariés selon l'âge et le sexe: en bonne santé (jamais fumé), à risque (fumeurs actuels / anciens), légers et modérés + MPOC. L'incidence et la prévalence des MCV ont été autodéclarées à partir de questionnaires (cardiopathie ischémique (IHD), maladie cérébrovasculaire (CBD) et insuffisance cardiaque (IC)), et le risque CV a été évalué à l'aide du score de risque de Framingham et de l'équation des cohortes regroupées, et en mesurant des niveaux élevés de fibrinogène $\geq$ 3,5 g/L et de protéine c-reactive (CRP) $\geq$ 2 mg / L.

Résultats: La prévalence des MCV cardiaques (IHD ou IC) était augmentée dans la MPOC GOLD 2+ par rapport à l'absence de MPOC avec un odds ratio (OR) de 1,83 [1,25 - 2,68 IC à 95%] après ajustement pour l'âge, le sexe, le tabagisme, l'IMC, l'hypertension et le diabète, mais la prévalence du CBD ne l'était pas. Les analyses de sous-groupes ont montré une augmentation de l'IC comorbide OR de 1,57 [1,05-2,35] IC à 95% et l'IC HF de 4,05 [1,71-9,60] IC à 95% dans GOLD 2+ MPOC par rapport à l'absence de BPCO. L'incidence des maladies cardiovasculaires cardiaques

a été augmentée dans la MPOC GOLD 2+ avec un OR de 2,19 [1,07 - 4,50 IC à 95%] par rapport à l'absence de MPOC après ajustement pour l'âge et le sexe, mais pas pour le CBD. La prévalence et l'incidence des maladies cardiovasculaires n'étaient pas augmentées dans la MPOC légère même après avoir tenu compte des mêmes facteurs de risque. Il n'y avait pas de différence significative entre le risque CV observé entre les groupes chez les hommes et les femmes en utilisant l'un ou l'autre des scores de risque. Chez les hommes, la modérée + était associée à des niveaux élevés de CRP par rapport aux autres, mais pas chez les femmes. Un taux élevé de fibrinogène chez les hommes était évident dans les cas de MPOC modérée + par rapport à tous les autres, à l'exception des anciens / actuels fumeurs. Chez les femmes, un taux élevé de fibrinogène a été observé dans les cas de MPOC modérée + par rapport à tous les autres, sauf pour la MPOC légère.

Conclusion: La présence de maladies cardiovasculaires concomitantes ou le développement de nouvelles maladies cardiovasculaires telles que l'IHD et l'IC est augmentée dans la MPOC modérée + mais pas dans la maladie légère par rapport à la population générale. La proportion d'individus considérés à haut risque en utilisant les scores de risque CV était similaire dans les groupes, même si la proportion d'hommes et de femmes présentant des niveaux élevés de biomarqueurs était la plus élevée dans les cas de MPOC modérée +.

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I would like to acknowledge McGill University and the Meakins-Christie Laboratories at the Research Institute of the McGill University Health Centre for the 2 awards I received during my graduate studies, that allowed me to progress and complete my Masters thesis in translational respiratory medicine. The Graduate Excellence Awards from the division of Experimental Medicine, Department of Medicine, McGill University (2017, 2018) and the Meakins-Christie Laboratories Collaborative Research Graduate Studentship Award, McGill University from 2017-2018. The Canadian Institute of Health Research provided funding as well as part of the project grant (#378405) "Chronic Obstructive Pulmonary Disease categorization using cardiovascular risk and levels of inflammatory mediators: a way toward more personalized care".

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academic committee and advisor for their guidance while I undertook my research. Lastly, I am extremely grateful to my family for their unconditional support in my endeavours.

Preface

The major goal of this Masters thesis was to evaluate cardiovascular risk among individuals in the early stages of chronic obstructive pulmonary disease (COPD) as compared to those at risk for COPD and healthy people with no respiratory disease from the general population. We used the presence of cardiovascular comorbidities and events, validated cardiovascular risk scores and levels of inflammatory biomarkers to achieve these goals. This thesis complies with the guidelines and requirements for a manuscript-based thesis at McGill University and has one manuscript that will be submitted for publication in 2020.

This thesis contains six chapters:

Chapter 1 is an introduction into the relationship between COPD and CVD, outlining the rationale for and objectives of this research project.

Chapter 2 provides background on COPD and cardiovascular disease (CVD), and the links between these two diseases.

Chapter 3 introduces the rationale, hypothesis and objectives of the thesis and manuscript.

Chapter 4 is the manuscript about categorizing cardiovascular risk in COPD.

Chapter 5 summarizes the research findings and provides a conclusion drawn from the thesis work.

Chapter 6 is the master list of references not included in the manuscript.

Contribution of Authors

Jean Bourbeau, my graduate thesis supervisor, was actively involved in the design, modification, and implementation of research activities for this thesis project and helped with feedback and support until completion of this thesis. Raquel Farias, a research associate and former member of Jean Bourbeau's research team, also helped in developing the study protocol and assisted with experiments exploring which biomarkers should be included in the final analyses. Pei Zhi Li aided in all the statistical analyses relevant to this thesis project. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi assisted with laboratory experiments designed to measure inflammatory and lipid biomarkers among certain study participants.

All chapters were written by Suurya Krishnan and reviewed for content and edited by Jean Bourbeau. All authors made substantial contributions to the development and implementation of this study.

Suurya Krishnan is first author on the manuscript in chapter 4. He was involved in designing, modifying, and implementing the study protocol and led initial experiments for the measurement and selection of inflammatory and lipid biomarkers to be used for the manuscript and thesis project. All statistical analyses were initially done by Suurya Krishnan and all final statistical analyses were done in collaboration with Pei Zhi Li at the Respiratory Epidemiology Research Unit of the McGill University Health Centre. All experiments performed by Suurya Krishnan were done in the laboratories of Simmon Rousseau at the Meakins-Christie laboratories at McGill in collaboration with Jean Bourbeau.

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lupus cancer, ulcerative colitis, Chron's disease, psoriasis, rheumatoid arthritis, or any type of cancer.

Manuscript Supplemental table 3. Odds ratios and 95% confidence intervals for the prevalence and incidence of CVD in COPD and non-COPD participants with COPD clinical "traits" (chronic bronchitis, high symptom burden using CAT or MRC dyspnea scores) compared to others without those traits adjusted for age, sex, smoking, hypertension, diabetes and FEV_1.

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List of Symbols, Abbreviations or Nomenclature

COPD: chronic obstructive pulmonary disease

CV: cardiovascular

CVD: cardiovascular disease

IHD: ischemic heart disease

HF: heart failure

CBD: cerebrovascular disease

FEV_1: forced expiratory volume in one second

FVC: forced vital capacity

AECOPD: acute exacerbations of COPD

CAT: chronic obstructive pulmonary disease assessment test

MRC: British Medical Research Council dyspnea scale

GOLD: Global Initiative for Chronic Obstructive Lung Disease

Mild COPD: GOLD 1

Moderate COPD: GOLD 2

Severe COPD: GOLD 3

Very severe COPD: GOLD 4

CRP: C-reactive protein

FRS: Framingham risk score

PCE: Pooled cohort equations risk score

CHAPTER 1: INTRODUCTION

The overall goal of our research is to assess risk for cardiovascular disease (CVD), one of the main causes of mortality in mild and moderate Chronic Obstructive Pulmonary Disease (COPD) [1]. All COPD patients are at increased risk to develop CVD such as ischemic heart disease (IHD), cerebrovascular disease (CBD) [2] and heart failure (HF) [3], and CV risk further increases after pulmonary exacerbations [4] and as airflow limitation worsens (decline in FEV_1) [5]. The coexistence of COPD and CVD could be attributed to chronic low-grade systemic inflammation. Several inflammatory biomarkers such as c-reactive protein (CRP) and fibrinogen have been proposed as predictors of mortality and adverse outcomes in COPD and in CVD. Current clinical guidelines have integrated CV risk assessment with levels of CRP to improve risk predictions in populations where CV risk is uncertain using traditional risk factors [6]**.** Given the strong relationship between COPD and CVD, it is of high relevance to collect epidemiological data on the prevalence of CV risk in people with COPD and longitudinal data to determine the impact of COPD on CVD events in the general Canadian population. The main objective of our study was to assess and compare CV risk among individuals with mostly mild and moderate COPD, and people with no COPD, (smokers, former smokers, and never smokers), all screened from the general Canadian population. CV risk was first assessed by comparing the prevalence and the incidence of CVD events and next by comparing the proportion of high-risk individuals categorized using validated CV risk scores and high levels of inflammatory biomarkers (CRP, fibrinogen) among these different groups. Our secondary objective was to determine if certain clinical "traits" of COPD or being at risk for COPD increased the likelihood of comorbid CVD.

CHAPTER 2: BACKGROUND

2.1 Chronic Obstructive Pulmonary Disease

2.1.1 COPD definition and overview

COPD is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2020 guidelines as the presence of airflow limitation and persistent respiratory symptoms, such as shortness of breath, cough, or increased sputum production. COPD is a treatable and preventable condition, usually caused by significant exposure to noxious particles or gases which result in airway and/or alveolar abnormalities. Tobacco smoking is the main risk factor for COPD but there are other environmental exposures including air pollution and exposure to biomass fuel, and a range of host factors such as abnormal lung development, genetic abnormalities, and accelerated aging [7]. The chronic airflow limitation in COPD is caused by a mixture of small airways disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). Chronic inflammation leads to structural changes, narrowing of small airways, and destruction of lung parenchyma, which results in the loss of alveolar attachments to small airways and decreases lung elastic recoil, decreasing the ability of these airways to remain open during expiration. While previous definitions of COPD have emphasized the terms emphysema and chronic bronchitis, these are not included in the definition of COPD from GOLD reports.

2.1.2 COPD diagnosis and severity

COPD should be considered in anyone with dyspnea, chronic cough, sputum production, or recurrent lower respiratory tract infections with exposure to risk factors for COPD. Airflow

limitation is measured by spirometry as is still the most reproducible and widely available test of lung function. Spirometry is required to make the diagnosis of COPD with a post-bronchodilator (post-bd) measure of forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) below 0.70 confirming persistent airflow limitation and thus a diagnosis of COPD [7]. Classification of severity of airflow limitation is based on GOLD criteria which uses post-bd measures of FEV_1 and calculates percent predicted FEV_1 (ratio of FEV_1 observed to FEV_1 expected for someone of the same age, sex, height, and race with no respiratory disease) [7]. The spirometry cut-off values, and definitions of COPD severity are given in table 1.

Table 1. Classification of airflow limitation severity in COPD based on post-bronchodilator spirometry in patients with $FEV_1/FVC < 0.70$.

GOLD 1:	Mild	$FEV_1 \geq 80\%$ predicted.
GOLD 2:	Moderate	$50\% \leq FEV_1 < 80\%$ predicted.
GOLD 3:	Severe	$30\% \leq FEV_1 < 50\%$ predicted.
GOLD 4:	Very severe	$FEV_1 < 30\%$ of predicted.

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

2.1.3 COPD symptoms, burden, and clinical traits

Chronic dyspnea or shortness of breath is the most common symptom experienced in COPD. This can be described as an increased effort to breathe, air hunger, chest heaviness or gasping [8]. Chronic cough is another common symptom that may be productive and can start intermittently earlier in the disease and later become more frequent. Cough with sputum production is experienced by 30% of COPD patients and symptoms may vary from day to day and person to person [7]. Other symptoms of COPD include wheezing and chest tightness, with the latter more likely to occur after physical exertion. The presence of any symptom may occur together with or independent of airflow limitation in COPD. Assessment of symptoms and their burden can be done using a simple measure of breathlessness such as the British Medical Research Council (MRC) dyspnea scale which is illustrated in table 2 below.

Table 2. British Medical Research Council dyspnea scale [9]

MRC Grade 1	I only get breathless with strenuous exercise.
MRC Grade 2	I get short of breath while hurrying on the level or walking up a slight hill
MRC Grade 3	I walk slower than people of the same age on the level because of breathlessness, or I have to stop for breath when walking on my own pace on the level.
MRC Grade 4	I stop for breath after walking about 100 meters or after a few minutes on the level.
MRC Grade 5	I am too breathless to leave the house or I am breathless when dressing or undressing.

MRC: British Medical Research Council dyspnea grade

Symptom burden can also be assessed using the COPD assessment test (CAT), which is a disease specific measure of the symptomatic impact of COPD on health status. The CAT score uses 8 items and scores a range of 0 to 40. The items, description, and scoring of the CAT is detailed in figure 1. GOLD guidelines define a high symptom burden as a CAT score above 9 or a modified medical research council (mMRC) (scale 0-4) greater than 1. [7]. The mMRC dyspnea grades are a modified version of the MRC, with MRC scores above 2 considered to be a high symptom burden.

CAT ASSESSMENT [7, 10]

I never cough	⓪①②③④⑤	I cough all the time
I have no phlegm (mucus) in my chest at all	⓪①②③④⑤	My chest is full of phlegm (mucus)
My chest does not feel tight at all	⓪①②③④⑤	My chest fells very tight
When I walk up a hill or one flight of stairs I am not breathless	⓪①②③④⑤	My chest is completely full of phlegm (mucus)
I am not limited doing any activities at home	⓪①②③④⑤	I am very limited doing activities at home
I am confident leaving my home despite my lung condition	⓪①②③④⑤	I am not at all confident leaving my home because of my lung condition
I sleep soundly	⓪①②③④⑤	I don't sleep soundly because of my lung condition
I have lots of energy	⓪①②③④⑤	I have no every at all

Total Score: ◯

Figure 1. Description of the various questions and scores used in the CAT. CAT: Chronic obstructive pulmonary disease assessment test.

Another important clinical feature of COPD is the history of acute exacerbations. An acute exacerbation of COPD (AECOPD) is characterized by a sudden worsening of respiratory symptoms that can lead to hospitalization and represents an important clinical feature of COPD that predicts mortality. Acute exacerbations are often recurrent, concomitant with cardiac pathologies and reduce long-term survival. Studies have failed to find convincing evidence that frequent exacerbators are at greater risk for adverse CV outcomes compared to those without exacerbations. However, patients with severe exacerbations (those requiring a hospital admission) are at a greater risk for a cardiac event with a relative risk (RR) of 4.03 [2.65 - 6.11 95%CI] in the 6 months following discharge from the hospital compared to before the exacerbation [4]. Furthermore, AECOPD requiring hospitalizations are often associated with pneumonia. Among patients hospitalized for acute COPD exacerbations in Canada, CT scans showed that 32.5% had consolidation and an additional 24.8% had "ground glass" abnormalities compatible with associated pneumonia [11]. There is evidence of the adverse long-term implications of pneumonia, mostly driven by cardiac diseases such as acute myocardial infarctions [12]. AECOPD may be similar to acute pneumonia in this regard as differentiating between the two is exceedingly difficult without a CT scan. AECOPD also tends to occur more frequently and with more severity as COPD progresses and airflow limitation worsens.

Chronic bronchitis is the presence of a chronic cough with sputum for at least 3 months in a year, for two consecutive years. Recent studies have found an association between mucus hypersecretion and a faster decline in FEV_1 [13], and chronic bronchitis is associated with an increased likelihood to develop COPD in young adult smokers. Older GOLD guidelines (from 2001) defined GOLD stage 0 or "at risk" for COPD as having normal spirometry with a chronic cough and sputum [14]. When combined with COPD, chronic bronchitis is associated with worse respiratory symptoms,

higher frequency and severity of AECOPD [15], and a higher risk of respiratory-related mortality [16].

2.1.4 COPD and comorbidities

COPD is a complex and multidimensional disease that often coexists with other chronic illnesses fulfilling the definition of comorbidities, irrespective of the mechanisms underlying the association. A recent meta-analysis found that COPD patients had significantly higher comorbidities compared to individuals with no COPD, including cardiovascular and cerebrovascular diseases, endocrine and metabolic disorders, psychiatric and neurological disorders, gastrointestinal diseases, musculoskeletal disorders, non-COPD respiratory conditions, and cancer [2]. Several of these comorbidities could be simply due to common demographic characteristics (male, ageing population) and/or risk factors (smoking). However, regardless of if these comorbidities are related or independent of each other, GOLD guidelines recommend identifying and treating all comorbidities as part of COPD management because comorbidities can have a serious impact on the prognosis of COPD patients [1, 17, 18].

2.2 COPD and cardiovascular disease

2.2.1 COPD and cardiovascular disease comorbidities

Cardiovascular (CV) comorbidities such as ischemic heart disease (IHD), heart failure (HF) and stroke often coexist in COPD patients, and this association was observed in population based studies with less advanced COPD as well [19-21]. CV comorbidities are among the most prevalent with COPD [2] and it has been shown to be related to the severity of airflow obstruction [22-24]. COPD

patients indeed are at increased risk for all comorbid diseases compared to those without COPD, but the most important ones are cardiac diseases OR of 1.90 [1.59-2.28 95%CI] and cerebrovascular diseases OR of 1.84 [1.47-2.31 95%CI] [2]. There is further evidence suggesting that the association between COPD and CVD persists even after adjusting for shared risk factors, such as age, sex and previous heart disease [25]. Comorbid CVD highly impacts on both COPD and CVD outcomes as these patients have a worse prognosis compared to those with COPD alone [26, 27]. While the leading cause of death has been shown to be respiratory failure in more severe COPD [28], a major cause of death in people with mild and moderate COPD is CVD [1, 5]. In a large multisite randomized clinical trial, the Study to Understand Mortality and Morbidity (SUMMIT), which enrolled over 16000 patients with moderate COPD and increased CV risk, CVD was found to be the leading cause of mortality, accounting for 43% of deaths compared to 13% from pulmonary causes [29]. In the Lung Health Study, a cohort of smokers mainly with asymptomatic airway obstruction, CVD was the second leading cause of death accounting for 22% of all deaths and a decrease of 10% in FEV_1, indicating worsening airflow obstruction, was associated with a 30% increase in CVD risk [30]. Lower levels of FEV_1 have also been associated with heart disease and stroke [6], a rapider rate of decline in FEV_1 further increases CVD risk [31]. FEV_1 is an independent predictor of mortality and incident CVD even in people with mild airflow impairment and a clinically normal FEV_1 range [32] . In a cohort of young adults aged 18 to 30 followed for 29 years, reduced lung function was independently associated with an increased risk of CVD for people entering middle age [33].

2.2.2 Risk assessment for cardiovascular events

The global risk for CV events can be obtained by calculating the Framingham risk score (FRS) or the pooled cohort equations (PCE), both validated risk scores assessing risk of a future CVD event [6, 34]. Guidelines for the primary prevention of CVD from the Canadian Cardiovascular Society (CCS) use the FRS and the American College of Cardiology/American Heart Association (ACC/AHA) clinical practice guidelines use the PCE to categorize CV risk. Furthermore, both guidelines recommend different intervention strategies depending on the level of CV risk [6, 35]. The current GOLD COPD guidelines recommend that ischaemic heart disease be considered in all COPD patients depending on their risk factor profile and assessed with a global CV risk calculator, while the CCS guidelines recommend global CV risk assessment in all COPD patients [6]. However, neither risk calculator (PCE or FRS) includes any markers specific to airflow limitation or COPD. This is in contrast with other chronic diseases such as diabetes mellitus and chronic kidney disease which are indications for statin therapy by the CCS and considered CV risk enhancing factors for clinical patient risk discussion according to the ACC/AHA. Indeed, COPD patients have a higher cardiovascular risk and higher mortality from cardiovascular events compared to people with no COPD, which further increase with COPD severity [36]. Despite this widespread knowledge, CV risk is not routinely assessed in COPD patients and no epidemiological studies in Canada have looked at the prevalence of different CV risk categories in COPD with different disease severities in the general population. The integration of cardiovascular risk assessment into current COPD guidelines could lead to more targeted therapies and improve outcomes in COPD. For instance, COPD patients with high CV risk could be treated with cardioprotective agents, lifestyle changes, and have closer monitoring to quickly diagnose and treat

exacerbations. When added to lung function data, Framingham risk score improves predictions for CVD events and total mortality [36].

2.2.3 General mechanisms linking COPD to cardiovascular comorbidities

2.2.3.1 Chronic systemic inflammation

The coexistence of CVD and COPD has often been attributed to shared risk factors, such as advanced age, sex, and cigarette smoking [37], however there is also evidence suggesting that the association between COPD and CVD persists even after adjusting for these shared risk factors [18, 25, 38]. There have been many proposed mechanisms that link these two diseases, such as lung hyperinflation, exacerbations, hypoxemia, arterial stiffness, and systemic inflammation, which may work synergistically with one another [39, 40]. Chronic low-grade systemic inflammation is an important feature of COPD that could further explain the association with increased CV risk, as it has been proposed to lead development of atherosclerosis and subsequent coronary artery disease [41]. The airway epithelium is the first line of defense against pathogens and pollutants and therefore induces an inflammatory response by macrophages in response to chronic stimuli such as cigarette smoke and acute damage by bacterial or viral infections. The airway epithelial cells release the pro-inflammatory cytokines interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) that can translocate from the lung into systemic circulation [42, 43]. These inflammatory mediators can stimulate bone marrow to release monocytes, granulocytes, and platelets, which can all contribute to the progression of atherosclerotic plaques. IL-6 and IL-1β induce the production of C-reactive protein (CRP), fibrinogen, and factor VIII by the liver. CRP is an acute phase reactant and important in

the clearance of pathogens while fibrinogen and factor VIII are involved in the clotting cascade [44, 45]. The release of these inflammatory mediators is thought to induce a pro-thrombotic environment and therefore increase the risk in COPD patients to develop coronary artery disease, arrhythmias, and heart failure [46, 47]. To counteract these effects, surfactant protein D (SP-D) is released from airway epithelial cells and attenuates inflammation by decreasing the expression of IL-6 [48, 49]. Chronic inflammation in the lung may further increase the circulation of cytokines and inflammatory mediators as COPD progresses, and has the potential to advance the development of atherosclerosis [50]. Smoking alone can cause systemic inflammation by increasing leukocyte circulation, however the extent of systemic inflammation is greater in patients with COPD [51]. While it is still unclear if these inflammatory mediators found in systemic circulation were "leaked" from the lung or due to another process in COPD, this chronic inflammation increases susceptibility of COPD patients to myocardial infarctions and ischemic stoke.

2.2.3.2 Acute inflammation, exacerbations, and pneumonia

The proposed theory suggests chronic inflammation from COPD aids in atherosclerotic plaque formation and progression through various inflammatory mediators. Acute inflammation and injury of the lung form respiratory tract infections, pneumonia or AECOPD can then induce the rupture of these plaques. COPD patients have higher levels of inflammation compared to general population and during AECOD, serum levels of IL-6 further increases and raises levels of plasma fibrinogen [52]. Acute lung inflammation and increased circulation of cytokines such as granulocyte-macrophage colony-stimulating factor may further increase circulating leukocytes which in turn increases the susceptibility of the plaque to rupture [53]. Acute lung injury also

induces systemic oxidative stress in addition to systemic inflammation which can increase permeability of the endothelium of blood vessels and cause acute endothelial dysfunction [54]. This combination of inflammation and oxidative stress can also destabilize atherosclerotic plaques making them more vulnerable to rupture by increasing the number of inflammatory cells, increasing lipid content, and thinning the fibrous cap of the plaque [55]. COPD patients have evidence of systemic inflammation and with acute lung injury and inflammation from AECOPD, are at higher risk to develop acute coronary and cerebrovascular disease (CBD) events. There is also epidemiological evidence that COPD patients are at increased risk for CVD for up to a year after a moderate or severe AECOPD, however CV risk is greatest in the first month following the exacerbation [56, 57]. In a secondary cohort analysis of the SUMMIT clinical trial, COPD patients who suffered a severe AECOPD requiring hospitalization were at much higher risk for CVD in the following month, with a hazard ratio (HR) of 9.9 [6.6-14.9 95%CI] compared to those with no exacerbation [57]. Pneumonic infiltrates have also been regularly observed during AECOPD in an outpatient COPD population and when present are associated with higher levels of systemic inflammation [58]. Pneumonia in hospitalized patients increases short-term risk for CVD similar to AECOPD, however pneumonia has also been implicated in precipitating and increasing long term 10-year CVD risk [59] , which has not been observed following AECOPD. Development of acute myocardial infarctions in patients hospitalized with pneumonia is common and should especially be considered in patients with respiratory failure [12]. Community acquired pneumonia has also been linked to increased systemic inflammation from circulating cytokines that can stay elevated for a week after diagnosis. Since it is hard to differentiate pneumonia from AECOPD in clinical practice without the use of radiography [11], the increased long term CVD risk resulting from pneumonia could also occur in COPD patients after severe AECOPD.

2.2.4 Biomarkers of Inflammation in COPD and cardiovascular disease

2.2.4.1 Inflammatory biomarkers in COPD

A recent meta-analysis found that COPD was associated with higher serum levels of CRP, leukocytes, IL-6, IL-8, and fibrinogen, but no association was found with TNF-α [60]. Our knowledge of these inflammatory mechanisms in COPD has led to the characterization of blood biomarkers as predictors of clinical outcomes. Most of the studies evaluating biomarkers in COPD have evaluated exacerbations and mortality as main outcomes. However, inflammatory biomarkers can be useful in management of COPD because of their ability to predict both disease progression and adverse outcomes longitudinally. In addition to fibrinogen, CRP, and SP-D, two more biomarkers have been associated with poorer clinical outcomes in COPD. Clara cell secretory protein (CC16) is an anti-inflammatory protein secreted by respiratory epithelium and can serve as a biomarker of lung epithelial injury. The receptor for advanced glycation end-products (RAGE) also plays a role in chronic inflammatory diseases such as COPD. sRAGE, the soluble form of RAGE acts as a decoy and prevents the inflammatory response mediated by RAGE activation [61], and has been proposed as a biomarker of disease severity in inflammatory conditions. CRP, fibrinogen and SP-D have been previously characterized as predictors of COPD exacerbations [62, 63] but there is also evidence that their prediction ability is not superior to clinical information such as history of a previous exacerbation [64]. High levels of SP-D and low levels of sRAGE have been associated with the presence and progression of emphysema [65]. Lower levels of sRAGE and higher levels of CC16 have also been found to be associated with decline in lung function among COPD patients [66]. The addition of CRP, fibrinogen, IL-6, IL-8 and SP-D, CC16, to established clinical factors improves risk stratification for all-cause mortality in COPD patients

and offers better precision than clinical variables alone [67, 68]. A recent sub-study of the SUMMIT randomized clinical trial found that systemic levels of the fibrinogen, CRP, CC16, SP-D and sRage were not associated with FEV_1 decline, exacerbations, or hospitalizations in COPD patients [69]. Furthermore, only fibrinogen and CRP were associated with an increase in deaths in these COPD patients. However, a comprehensive analysis of 2 large multi-centre cohorts (ECLIPSE and COPD gene) showed that the combination of these biomarkers improves risk prediction of mortality, lung function decline and AECOPD hospitalizations, compared to clinical information or use of a single biomarker alone [70]. Plasma fibrinogen is the first biomarker drug development tool for COPD that has been approved for use by the FDA [71, 72]. However, fibrinogen does not offer precision at the individual patient level and fibrinogen levels do not correlate with lung function decline in COPD patients [73], and therefore may be more useful when combined with other biomarkers. Elevated levels of CRP, fibrinogen, and leukocytes together in COPD patients has been associated with a two to four times increase in development of comorbid diseases such as myocardial infarction, diabetes, heart failure, lung cancer and pneumonia [62, 74].

2.2.4.1 Inflammatory biomarkers in CVD

There are several inflammatory biomarkers that have been linked to CVD and a few provide prognostic information for future CVD. Growth-differentiation factor 15 (GDF-15) is a cytokine that is released from macrophages and provides good prognostic information for CV mortality. [75]. GDF-15 is a strong predictor of CV mortality and marginally improves risk prediction for CVD when added to traditional risk factors including other biomarkers such as CRP [76]. Since GDF-15 is also associated with various CVD, such as coronary artery disease, myocardial

infarctions, and HF, independent of other established biomarkers of risk, it could be extremely useful for CV risk assessment. Uric acid is the product of purine metabolism and elevated levels have ben theorized to play a role in CVD development. Even at clinically normal levels, uric acid is thought to increase the susceptibility of individuals by enhancing oxidative stress, increasing inflammation and promoting endothelial disfunction [77]. There is conflicting evidence to whether uric acid is an independent risk factor for CVD death and its benefit in guiding therapy [78-80]. However, recent evidence has indicated that uric acid is an independent risk factor for CVD mortality, particularly sudden cardiac death [81]. CRP is another inflammatory biomarker that is associated with development of CVD such as coronary heart disease and ischemic stroke, and CVD mortality [82]. In a population with no history of CVD, the addition of CRP to CV risk assessment marginally improved CV risk prediction and identified high risk individuals for closer monitoring and therapy, and could therefore theoretically reduce CVD mortality [83]. Based on this evidence and further research, CRP has been characterized as a biomarker of enhanced CV risk when added to traditional CV risk factors and validated CV risk scores for persons with unusual or uncertain risk as per American Heart Association/American College of Cardiology primary prevention for CVD guidelines [6]. Fibrinogen has also been characterized as a biomarker for CVD and is involved in platelet aggregation, endothelial injury, plasma viscosity, and formation of thrombus. A large meta-analysis study found that increased levels of fibrinogen were independently associated with the development of coronary heart disease and stroke, even in the presence of another validated biomarker such as CRP [84]. Similar to CRP, the assessment of fibrinogen together with traditional CV risk factors showed a significant improvement in the prediction of CVD events [83]. The primary prevention guidelines on CVD by the European Society of Cardiology allow fibrinogen measurement as a part of the risk assessment in patients

with an unclear or moderate cardiovascular risk profile, but is not recommended for asymptomatic individuals categorized with low-risk [85]. In addition to being elevated in COPD, CRP and fibrinogen serve as predictors for the development of myocardial infarction, stroke and HF [83, 86-89]. COPD patients are known to have higher levels of CRP and fibrinogen compared to those without COPD [51, 73], and higher baseline CRP and fibrinogen levels are associated with increased CVD mortality [84, 90]. The benefit of fibrinogen and CRP as inflammatory biomarkers may be in identifying COPD patients at risk for CVD and related mortality, who could be offered preventive care earlier.

CHAPTER 3: RATIONALE, HYPOTHESIS AND OBJECTIVES

3.1 Rationale

CVD and COPD are among the leading causes of death around the world accounting for around 37% of all deaths in 2016 by global health estimates. These diseases often coexist together as comorbidities. Ischemic heart disease is responsible for the most deaths at 9.4 million followed by stroke at 5.8 million and then COPD which caused over 3 million deaths globally [91]. Epidemiological studies and large clinical trials have helped us understand that there are higher rates of CVD morbidity and mortality in COPD patients as compared to other individuals not having COPD [92]. Due to the importance of CVD comorbidities in COPD, we should give more attention to CVD in individuals with COPD. We need to know from the general population, the importance of CVD in milder to moderate COPD and see how it compares to the non-COPD population.

Furthermore, COPD is often undiagnosed in the general population, which accounts for over 60% of COPD in Canada [93], and therefore may be a much more important risk factor for CVD than previously imagined. This signifies the importance of early diagnosis of COPD screened from the general Canadian population. This will give us important information on recognition and management of undiagnosed COPD or mild to moderate COPD, individuals that may be at risk for CVD but have been overlooked in other study strategies. We should also include information on COPD symptom burden and clinical "traits", so we can identify any additional features of COPD that are associated with CVD.

As there is strong association between COPD and CVD, it is of great significance to gather epidemiological data on the prevalence of CVD and CV risk in people with mild and moderate

COPD, and see how it compares to the general population. This is essential if we want to have a picture that better reflects the reality in our population. Furthermore, it will be necessary to collect longitudinal data to determine the impact of COPD on CVD outcomes such as IHD, heart failure and stroke, so we can make temporal associations between these comorbidities. Better knowledge on CV risk assessment together with serum biomarkers may lead to a more precise identification of COPD individuals at risk for adverse CV events. Finally, the integration of CV risk assessment into current COPD guidelines could help identify COPD patients with high CV risk, providing useful information that could facilitate a more individualised approach to patient management.

Our research will stem from the 'Canadian Cohort Obstructive Lung Disease' (CanCOLD), a multi-center longitudinal study to assess COPD risk factors and outcome relationships. CanCOLD is one of the rare studies worldwide and the first observational cohort in Canada specific to COPD having recruited its participants from the general population rather than more convenient recruitment in clinical settings. This study embedded in CanCOLD better mirrors prevalent and incident COPD populations at large and provides proper representation of typically underrepresented groups in COPD studies; early disease, female population, individuals who have never smoked and those with undiagnosed disease. All these factors make CanCOLD a valuable cohort to test our hypotheses and accomplish our objectives.

3.2 Hypothesis

Our central hypothesis is that COPD has a relationship with CVD related to sustained inflammation secondary to tissue injury and independent of shared risk factors from the general population.

3.3 Objectives

3.3.1 General objective

To assess and compare individuals with high CV risk in varying severities of COPD, primarily mild to moderate, as compared to those at risk for COPD (former and current smokers) and those who never smoked from the general population.

3.3.2 Primary objectives

The main objective of our study was to assess and compare in the Canadian population primarily among individuals with mild/moderate COPD and people with no COPD (current/former smokers and never smokers with normal spirometry):

1) a. the prevalence and **b.** the incidence of CVD

2) the proportion of high-risk individuals categorized **a.** using CV risk scores and **b.** using inflammatory biomarkers (CRP, fibrinogen)

3.3.3 Secondary objectives

Our secondary objective was to determine if certain clinical "traits" (chronic bronchitis, significant burden of dyspnea, or decreased quality of life index) in people with COPD increases the prevalence of comorbid CVD as compared to others with COPD, and if being "at risk" for COPD increases prevalent CVD compared to those with no COPD and not "at risk".

CHAPTER 4: MANUSCRIPT "Chronic Obstructive Pulmonary Disease categorization using cardiovascular risk and levels of inflammatory mediators in the Canadian population: CanCOLD study"

4.1 ABSTRACT

Introduction: Cardiovascular disease (CVD) accounts for highest mortality in the patients with chronic obstructive pulmonary disease (COPD) and cardiovascular (CV) risk increases as airflow obstruction worsens. Therefore, CV risk should be assessed in the general Canadian population with mostly mild and moderate COPD.

Objectives: To determine 1) the prevalence and incidence of CVD, and 2) the prevalence of high CV risk groups using risk scores and inflammatory biomarkers, in those with COPD compared to no COPD.

Methods: The CanCOLD study prospectively followed 1561 participants divided into age and sex matched study groups: healthy (never smoked), at-risk (current/former smokers), mild and moderate+ COPD. Incidence and prevalence of CVD were self reported from questionnaires (ischemic heart disease (IHD), cerebrovascular disease (CBD) and heart failure (HF)), and CV risk was assessed using the Framingham risk score (FRS) and pooled cohort equations (PCE), and by measuring high levels of fibrinogen ≥3.5g/L, and c-reactive protein (CRP) ≥2mg/L.

Results: The prevalence of cardiac CVD (IHD or HF) was increased in COPD GOLD 2+ compared to no COPD with an odds ratio (OR) of 1.83 [1.25 - 2.68 95%CI] after adjusting for age, sex, smoking, BMI, hypertension and diabetes but prevalence of CBD was not. Subgroup

analyses showed increased comorbid IHD OR of 1.57 [1.05-2.35] 95%CI and HF OR of 4.05 [1.71-9.60] 95%CI in GOLD 2+ COPD compared to no COPD. The incidence of cardiac CVD was increased in GOLD 2+ COPD with OR of 2.19 [1.07 - 4.50 95%CI] compared to no COPD after adjusting for age and sex but not for incident CBD. The prevalence and incidence of CVD was not increased in mild COPD even after accounting for the same risk factors. There was no significant difference between in CV risk observed between groups in men or women using either risk scores. In men, moderate+ COPD was associated with high levels of CRP compared to others, but not in women. High fibrinogen in men was evident in moderate+ COPD compared to all others except former/current smokers. Among women, high fibrinogen was seen in moderate+ COPD compared all others except mild COPD.

Conclusion: The presence of comorbid CVD or development of new CVD such as IHD and HF is increased in moderate+ COPD but not in mild disease compared to the general population. Proportion of individuals considered high risk using CV risk scores were similar among groups even though proportion of men and women with high levels of biomarkers were greatest in moderate + COPD.

4.2 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD) are among the leading causes of death worldwide and often coexist together as comorbidities [1]. Several large population studies have demonstrated that cardiovascular (CV) comorbidities are among the most prevalent with COPD and are related to the severity of airflow obstruction [2-4]. While previously attributed to common risk factors, COPD patients are at increased risk for myocardial infarctions (MI), angina and heart failure even after accounting for shared risk factors such as advanced age, sex, smoking and previous heart disease [2, 5, 6]. In large multicenter clinical trials that include smokers and COPD patients, CV risk increased as airflow limitation got worse and CVD was the second highest cause of mortality [7-9].

The mechanisms that underlie the association between COPD and CVD beyond concomitant risk factors are still unknown, however low-grade systemic inflammation is suggested to be a key feature linking these two diseases [10]. In response to chronic stimuli such as cigarette smoke and acute damage from bacterial or viral infections, airway epithelial cells release pro-inflammatory cytokines into the systemic circulation which attract immune cells to the lung and induce the production of C-reactive protein (CRP) and fibrinogen by the liver [11, 12]. This response is thought to negatively impact the CV system by promoting a prothrombotic state and thus increasing susceptibility of COPD patients to coronary artery disease and consequently complications such as heart failure [13, 14]. Increased levels of CRP and fibrinogen have been linked to the development of myocardial infarction and heart failure [15-19], and CRP is a biomarker predicts risk for CVD when added to traditional CV risk factors [20].

In a recent meta-analysis comparing patients with COPD to the general population [21], COPD patients had a nearly two to five times higher risk of all CVD prevalence than non-COPD patients.

More specifically, there were over two times increased risk of major cardiovascular diseases such as ischemic heart disease and heart failure, and over 30% higher odds of hypertension and diabetes in patients with COPD compared to no COPD. However, they did not find an association between COPD severity and ischemic heart disease which was previously suggested to exist [5, 21]. The main limitations of these studies were the absence of longitudinal observations, the lack of adequate control groups and none of the cohorts screened for COPD from the general population. Finally, some studies focused only on moderate to severe or hospitalized COPD patients, and a large proportion of the studies relied mainly on administrative data with lack of clinical data.

It is therefore highly relevant to gather data representative of the Canadian population on CV risk in the earlier stages of COPD by assessing CVD prevalence/incidence as well as proportions of higher CV risk groups categorized using validated risk scores and inflammatory biomarkers from established guidelines [20, 22]. Furthermore, because COPD is often undiagnosed in the general population (over 60% of COPD in Canada [23]), COPD could be more important as a risk factor for CVD than expected. The main objective of the present study was to determine in the Canadian population among individuals with mild/moderate COPD: 1) a- the prevalence and b- the incidence of CVD; 2) the proportion of high risk individuals categorized using a- CV risk scores and b- inflammatory biomarkers (CRP, fibrinogen), as compared to people not having COPD (smokers and never smokers). The secondary objective was to determine if certain clinical "traits" (chronic bronchitis, significant dyspnea, or decreased quality of life index) in people with COPD increases the prevalence of comorbid CVD as compared to others with COPD, and if being "at risk" for COPD increases prevalent CVD compared to those without COPD and not "at risk". We made use of the data collected by the population-based Canadian Cohort Obstructive Lung Disease (CanCOLD) to achieve our objectives.

4.3 METHODOLOGY

4.3.1 Study population and design

At the time of conducting this analysis, CanCOLD had prospectively followed 1561 participants recruited from 9 sites across Canada over an average median time of 6 years. Participants from the prevalence study COLD [24], a random sample of non-institutionalized adults above the age of 40 years divided equally between men and women, were invited to participate in CanCOLD. A random sample of eligible individuals for each site was identified using Statistics Canada census data and individuals were recruited by random phone digit dialing. The mean participation rates across all sites was 74% ranging from 63 to 87% [25].

CanCOLD includes a large representation of individuals with mild airflow obstruction and of individuals with undiagnosed COPD and women with airflow obstruction. Post-bronchodilator (post-BD) spirometry of forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were used to define COPD ($FEV_1/FVC < 0.7$), and COPD severity was based on GOLD classification [26]. The sampling strategy was designed for a balanced recruitment of participants in four subsets: COPD participants GOLD 2+ (post-BD $FEV_1/FVC < 0.70$, and $FEV_1 < 80\%$ of predicted); GOLD 1 (post-BD $FEV_1/FVC < 0.70$, and $FEV_1 \geq 80\%$ of predicted); and two subsets of age- and sex-matched non-COPD subjects (post-BD $FEV_1/FVC \geq 0.70$), participants who were at risk for COPD (smokers and former smokers) and those who never smoked and had no reported respiratory disease. Eligibility and study design for CanCOLD has been previously described [27].

4.3.2 Study measurements

All participants were longitudinally assessed for 4 visits; they had an initial visit (Visit 0) for the prevalence study COLD (Canadian Cohort Lung Disease) followed by 3 CanCOLD visits spaced

at least 18 months apart with median follow-up time being about 6 years. Assessments relevant to this study included lung function, socio-demographic and clinical status questionnaires that included information on CVD and respiratory symptoms, and other study measurements needed to calculate CV risk scores including blood collected from participants stored in a biobank.

Lung function. Post-BD spirometry and lung volumes were obtained using the standard technique, and disease presence and severity was classified according to GOLD classification [26]. FEV_1 percent predicted was used for GOLD classification and was calculated using age, sex, height, and race (NHANES III) [28].

Cardiovascular disease events. At all visits, participants were asked to complete a detailed set of questions specific to comorbidities since their last visit. We defined CVD events as a self-reported diagnosis of CVD which includes ischemic heart disease (IHD) (stable/unstable angina, coronary artery disease or MI), cerebrovascular disease (CBD) (stroke or transient ischemic attack) and heart failure (HF). Self-reported questionnaires assessing angina, MI, and stroke have been validated and provide results similar to data from medical records [29, 30], while self-reported HF sensitivity is much lower although it has good specificity [30, 31].

Cardiovascular risk scores. The Framingham risk score for global CVD (FRS) and the pooled cohort equations (PCE) are validated risk scores that categorize high CV risk using age, sex, smoking, diabetes, systolic blood pressure, total cholesterol, high-density lipoprotein (HDL), and blood pressure medication [20, 32]. Age, sex, smoking, and diabetes, and blood pressure medication were all self-reported. Systolic blood pressure was measured while sitting and lipids were measured from frozen blood stored at the CanCOLD biobank. Only participants with no history of CVD or arrythmia were evaluated for CV risk scores as per guidelines, and we defined very-high risk as $\geq$ 20%, and intermediate-high risk as 10 to <20% in FRS and 7.5 to <20% in

PCE. All participants belonging to either intermediate-high or very-high risk groups were categorized as high risk [20, 32].

Inflammatory biomarkers. Levels of high sensitivity (hs)CRP and fibrinogen were obtained from analyses done on frozen blood samples stored at the biobank. Analyses for hs-CRP used the particle enhanced immunoturbidimetric assay [33] and fibrinogen levels were measures using the clotting principle of Clauss [34]. High CV risk levels of inflammatory biomarkers were defined using established CVD guidelines for CRP as $\geq$ 2 mg/L [20] and since no fibrinogen threshold exists in CVD guidelines, we used the same threshold as high risk for COPD outcomes and increased mortality which is $\geq$ 3.5 g/L [35]. Participants with levels of CRP $\geq$ 10 mg/L were not included because these high levels of inflammation are more likely to be result of an underlying infection or chronic inflammatory disease.

COPD clinical "traits". All assessments and scores were elicited from detailed questionnaires. *Chronic bronchitis*. Defined as the presence of cough/phlegm almost every day for 3 months for at least 2 years. "*GOLD 0" (alternative definition of at risk for COPD)* was defined as the presence of chronic bronchitis with no COPD, i.e., having normal spirometry.

Dyspnea. The British Medical Research Council dyspnea score (MRC) is a unidimensional scale out of 5 calculating breathlessness with scores above 2 indicating moderate or more breathlessness.

COPD assessment test (CAT). The CAT score is multidimensional scale equally encompassing cough, phlegm, breathlessness, activity level, sleep, energy, chest tightness and confidence for a score out of 40, with 10 or higher indicating reduced quality of life from COPD symptoms.

Using MRC or CAT, a high symptom burden is defined as MRC scores of 3 or more and COPD assessment test (CAT) scores of 10 or more [26].

4.3.3 Statistical Analyses

The cross-sectional analyses for the primary objectives 1a, 2 and secondary objective used data from CanCOLD visit 1, and the longitudinal analysis for objective 1b used COLD (visit 0) as the baseline and followed participants till completion of CanCOLD visit 3. We conducted descriptive statistics for socio-demographic factors and health outcomes. For the cross-sectional analysis we used post-BD spirometry measures from CanCOLD visit 1 to divide the population into COPD and non-COPD study groups. We derived adjusted odds ratios (OR) with 95% confidence intervals (CI) for prevalent CVD in different COPD severities and compared them to the control group after adjusting for age, sex, smoking, diabetes, hypertension, and body mass index (BMI). The longitudinal analysis in objective 1b used measures from COLD visit 0 to divide into COPD and non-COPD study groups and OR 95%CI for new CVD events were derived adjusting for age and sex. All OR for the prevalence and incidence of CVD were considered statistically significant if $p<0.05$. In the cross-sectional analysis for objectives 2a and b, we first divided the population by sex and then into COPD and non-COPD study groups using data from CanCOLD visit 1. Study groups of each sex were compared for proportion of individuals considered "high" risk by each CV risk score and biomarker doing multiple comparisons using the Tukey method and a $P <0.05$ was considered as a significant difference. For the secondary objective we derived OR for prevalent CVD among those with COPD and specific clinical "traits" compared to participants with COPD but no such trait. We then derived OR for those "at risk" for COPD (using two definitions) compared to other non-COPD participants adjusting all the secondary analyses models for FEV_1 in addition to age, sex, current smoking, hypertension, and BMI. Sensitivity analyses were performed for objective 1 by excluding all COPD GOLD 3+ participants and by comparing all COPD groups to only at-risk participants (former and current smokers). For objective 2b the

sensitivity analyses excluded all participants with inflammatory conditions, which were lupus cancer, ulcerative colitis, Chron's disease, psoriasis, rheumatoid arthritis, and cancer. All statistical analyses were conducted using SAS 9.4 software.

4.4 RESULTS

A total of 1561 subjects were enrolled in CanCOLD with the timeline and how the study populations were selected detailed in Figure 1. Population characteristics by study groups are given in Table 1 (cross-sectional population) and Table 2 (longitudinal population). Participants were on average 65 to 68 years at CanCOLD visit 1 for the cross-sectional analyses and 62 to 64 years old at COLD (baseline for the longitudinal analyses). There were more males than females except in the healthy group (those who never smoked without a respiratory condition) and the entire population was mainly Caucasian with less than 3% having severe COPD (GOLD 3+). Among COPD participants, 72% of the cross-sectional study population and 82% of the longitudinal study population were undiagnosed. A higher proportion of COPD GOLD2+ participants reported exacerbation like events in one year compared to the other groups and comorbidities such as diabetes, hypertension and arrythmia were higher in the GOLD 2+ group compared to others.

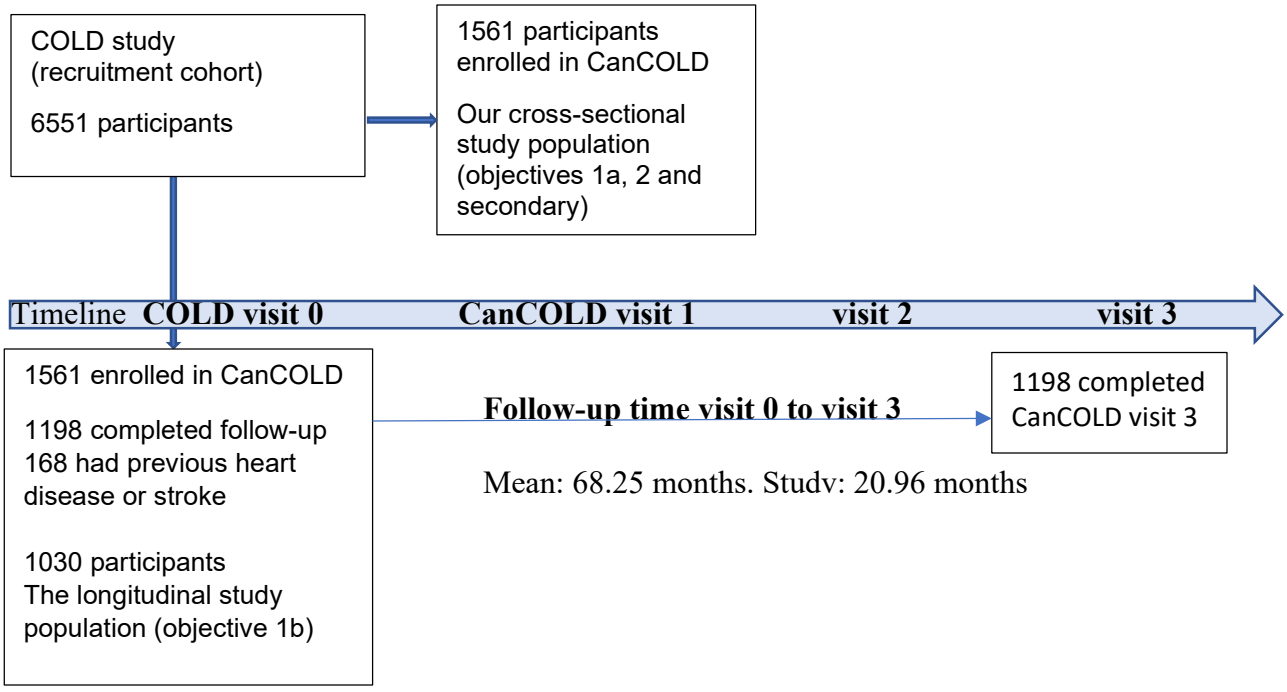

Fig 1. Flow diagram presenting the cross-sectional and longitudinal study populations with a reference timeline of study visits and how the populations were selected. COLD: Canadian cohort of obstructive lung disease prevalence study. CanCOLD: Canadian cohort of obstructive lung disease longitudinal study, Stdv: standard deviation.

Table 1. Demographics of the cross-sectional study population at CanCOLD visit 1 with differences among study groups.

Variables	Study Groups					
	Total N=1561	**Healthy N=347**	**At Risk N=475**	**COPD GOLD 1 N=408**	**COPD GOLD 2+ N=331**	
Demographics						
Age (years)	66.7 ± 9.8	66.4 ± 9.7	65.6 ± 9.4	68.1 ± 9.9	66.6 ± 10.3	*
Sex, male	876 (56.1)	157 (45.2%)	277 (58.3%)	263 (64.5%)	53.9%	*
Race, Caucasian	1486 (95.2)	325 (93.7%)	448 (94.3%)	397 (97.3%)	95.5%	
BMI	27.7 ± 5.3	27.4 ± 5.2	28.2 ± 5.3	27.0 ± 4.5	28.0 ± 6.1	*
Smoking						
Never smoked	546 (35.0)	347 (100%)	0	133 (32.6%)	66 (20.0%)	*
Former smokers	765 (49.0)	0	365 (76.8%)	220 (53.9%)	179 (54.2%)	*
Current smokers	250 (16.0)	0	110 (23.2%)	55 (13.5%)	86 (25.8%)	*
Pack-years	17.1 ± 22.9	-	20.1 ± 21.4	18.2 ± 23.0	29.5 ± 26.5	*
Exacerbations						
At least 1 in one-year	186 (13.5%)	27 (8.9%)	45 (10.7%)	39 (10.8%)	75 (22.7%)	
At least 1 in one-year with ER/hospital visit	14 (1.0%)	2 (0.7%)	2 (0.5%)	1 (0.3%)	9 (2.7%)	
Undiagnosed COPD	532 (72%)	-	-	341 (83.6%)	191 (57.7%)	*
Comorbidities						
Diabetes	161 (10.3%)	31 (8.9%)	57 (12.0%)	29 (7.1%)	44 (13.3%)	
Hypertension	419 (26.8%)	76 (21.9%)	120 (25.2%)	101 (24.8%)	122 (36.9%)	*
Arrythmia	161 (10.3%)	37 (10.7%)	42 (8.8%)	41 (10%)	31 (12.4%)	
Prevalent CVD						
CBD	81 (5.2%)	15 (4.3%)	24 (5.1%)	22 (5.4%)	30 (6%)	
IHD	160 (10.2%)	29 (8.4%)	48 (10.1%)	32 (7.8%)	51 (15.4%)	*
HF	27 (1.7%)	3 (0.9%)	6 (1.3%)	3 (0.7%)	15 (4.5%)	*
Any CVD	236 (15.1%)	43 (12.4%)	71 (14.9%)	50 (12.3%)	72 (21.8%)	*

COPD: chronic obstructive pulmonary disease GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease, CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, BMI: body mass index. n and % are given for all variables except for age, BMI and smoking pack-years where mean and standard deviation given. A statistically significant difference between groups in denoted by an * indicating a p value <0.05. CanCOLD: Canadian cohort of obstructive lung disease longitudinal study.

Table 2. Demographics of the longitudinal study population at baseline (COLD visit 0) and follow-up CVD events with differences among study groups.

	Study Groups					
Variables	**Total N=1030**	**Healthy N=223**	**At Risk N=269**	**COPD GOLD 1 N=328**	**COPD GOLD 2+ N=210**	
Demographics						
Age (years)	63.0 ± 9.5	62.2 ± 9.4	62.6 ± 9.0	64.2 ± 10.0	62.4 ± 9.4	*
Sex, male	560 (54.4%)	110 (49.1%)	153 (56.9%)	191 (58.6%)	106 (50.2%)	*
BMI	27.3 ± 5.3	27.5 ± 6.0	27.7 ± 5.2	26.0 ± 4.1	28.4 ± 5.8	*
Smoking						
Never smoked	469 (45.5%)	224 (100%)	38 (14.1%)	137 (42.0%)	70 (33.2%)	*
Former smokers	405 (39.3%)	0	172 (63.9%)	143 (43.9%)	90 (42.7%)	*
Current smokers	156 (15.1%)	0	59 (21.9%)	46 (14.1%)	51 (24.2%)	*
Pack-years	14.6 ± 20.4	-	17.7 ± 17.6	16.6 ± 21.7	23.0 ± 24.5	*
Exacerbations						
At least one in the past year	186 (13.5%)	27 (8.9%)	45 (10.7%)	39 (10.8%)	75 (22.7%)	*
Undiagnosed COPD	434 (82.0%)	-	-	295 (89.9%)	147 (70.0%)	*
Comorbidities						
Diabetes	73 (7.1)	9 (4.0)	25 (9.3)	21 (6.4)	18 (8.5)	
Hypertension	285 (27.7)	62 (27.7)	79 (29.4)	76 (23.3)	68 (32.2)	*
Incident CVD						
CBD	28 (2.7%)	6 (2.7%)	9 (3.3%)	8 (2.4%)	5 (2.4%)	
IHD	40 (3.9%)	10 (4.5%)	6 (2.2%)	13 (4.0%)	11 (5.2%)	
HF	8 (0.8%)	1 (0.4%)	0	2 (0.6%)	5 (2.4%)	
Any CVD	72 (7.0%)	15 (6.7%)	15 (5.6%)	22 (6.7%)	20 (9.5%)	

COPD: chronic obstructive pulmonary disease GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease, CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, BMI: body mass index. n and % are given for all variables except for age, BMI and smoking pack-years where mean and standard deviation given. A statistically significant difference between groups in denoted by an * indicating a p value <0.05. COLD: Canadian cohort of obstructive lung disease prevalence study.

Prevalence of CVD in COPD from a general population

The differences in study groups for the prevalence of CVD (Table 1) shows that over 15% of the population reported a history of CVD. The differences between those with COPD and no COPD after adjusting for age sex, smoking, diabetes, hypertension, and BMI are illustrated in Figure 2a. There was a statistically significant increase in the prevalence of combined CVD in GOLD 2+ OR of 1.55 [1.09-2.19] 95%CI but not in GOLD 1 OR of 0.71 [0.49-1.05] 95%CI compared to people without COPD. In subgroup analyses GOLD2+ participants had significantly higher OR for comorbid IHD, OR of 1.57 [1.05-2.35] 95%CI and HF, OR of 4.05 [1.71-9.60] 95%CI compared to the non-COPD group, but there was no statistically significant difference for prevalent CBD, OR of 1.08 [0.61-1.91] 95%CI. The COPD GOLD 1 group showed no statistically significant difference for prevalent IHD, HF, or CBD with ORs 95%CI of 0.65 [0.43-1.08], 0.38 [0.10-1.50], and 0.95 [0.55-1.67], respectively. Compared to participants with no COPD, there was no statistically significant difference for prevalence of a cardiac related CVD (IHD or HF) in COPD GOLD 1, OR of 0.67 [0.43-1.06] 95% but there was an increase in COPD GOLD 2+, OR of 1.83 [1.25-2.68] 95%CI.

Incidence of CVD in COPD from a general population

The incidence of CVD and demographics of the longitudinal study population with differences between COPD and non-COPD groups are presented in Table 2. A total of 1030 participants with no history of stroke or heart disease at baseline completed visit 3 for a median follow up time of 70 months, and 7% of the population reporting an incident CVD. Figure 2b shows the OR for the incidence of CVD in COPD participants compared to those without COPD after adjusting for age and sex. Incidence of combined CVD was not significantly increased in COPD GOLD 2+, OR of 1.64 [0.90-2.96]95% or GOLD 1 OR of 0.99 [0.55-1.76]95%CI compared to the non-COPD group.

Subgroup analyses showed no significant difference for IHD, OR 1.67 [0.76-3.69]95%CI or CBD, OR 0.76 [0.27-2.14]95%CI separately in COPD GOLD 2+ compared to people with no COPD. The COPD GOLD 1 group showed no statistically significant difference for prevalent IHD or CBD compared to those with no COPD with ORs 95%CI of 0.73 [0.76-3.69] and 1.02 [0.48-2.19], respectively. We did not have enough HF events to be included in subgroup analyses alone. Compared to participants with no COPD, there was no statistically significant increase for incidence of a cardiac related CVD (IHD or HF) in COPD GOLD 1, OR of 1.16 [0.56-2.39]95% but there was an increase in COPD GOLD 2+, OR of 2.19 [1.07-4.50] 95%CI.

High CV risk scores in COPD from a general population

The prevalence of high-risk groups using the two different CV risk scores are outlined in Figure 3. The proportion of high-risk individuals (intermediate-high or very-high risk) ranged in the study population from 79-91% using the FRS and 70-83% using the PCE in men among study groups. In women, 42-48%(FRS) and 42-51%(PCE) of women were considered high risk in the study groups. Participants categorized as very-high risk ranged from 39-55% by the FRS and 27-40% by the PCE in men and between 10-13% (FRS) and 16-22% (PCE) in women among different study groups. There was no statistically significant difference in the proportion of men or women classified high risk or very-high risk using either risk score among groups. More men were in the higher CV risk groups than women, and the proportions were higher using FRS in men and using PCE in women compared to the other risk score.

High levels of inflammatory biomarkers in COPD from a general population

High levels of inflammatory biomarkers CRP and fibrinogen in the study population and differences among study groups are illustrated in Figure 4. In men, high levels of CRP were most prevalent and significantly higher in the GOLD2+ group as compared to other groups. Proportion

of men with high fibrinogen levels were significantly higher in both COPD GOLD 2+ and the at-risk group compared to the healthy and COPD GOLD 1 groups. The number of women with high levels of biomarkers were higher than men in each study group. Women with COPD GOLD 2+ had the highest proportion of elevated CRP but there was no significant difference compared to women in other study groups. There was a significant difference in the proportion of high-risk women categorized using fibrinogen in the GOLD 2+ group at 27% compared to 13% in the healthy and at-risk groups. Women with COPD GOLD 1 also had a higher proportion with high fibrinogen levels at 22% compared to the non-COPD groups but there was no statistically significant difference.

COPD clinical traits, at risk for COPD, and CVD prevalence from a general population

Table 3 shows the CVD prevalence in COPD participants with specific clinical traits compared to those with COPD without these traits after adjusting for age, sex, smoking, BMI, hypertension and FEV_1. Prevalence of CVD was higher in COPD participants with a "high" symptom burden and high CAT score but not with chronic bronchitis or moderate plus dyspnea alone when compared to COPD participants without each symptom, respectively. There was increased prevalence for IHD of almost twice in COPD participants with high CAT scores compared to COPD participants with low CAT scores reaching statistically significance but not for CBD. COPD participants with high symptom burden (CAT≥10 or MRC dyspnea≥3) were also at increased risk for comorbid IHD of more than twice reaching statistical significance but not for comorbid CBD. Among those without COPD, the at-risk group (former/current smokers without COPD) were not at increased risk for CVD compared to those with no COPD who never smoked. Using the alternative definition of at-risk, GOLD 0 (chronic bronchitis with normal spirometry) participants were at increased risk only for IHD compared to other non-COPD participants without chronic bronchitis.

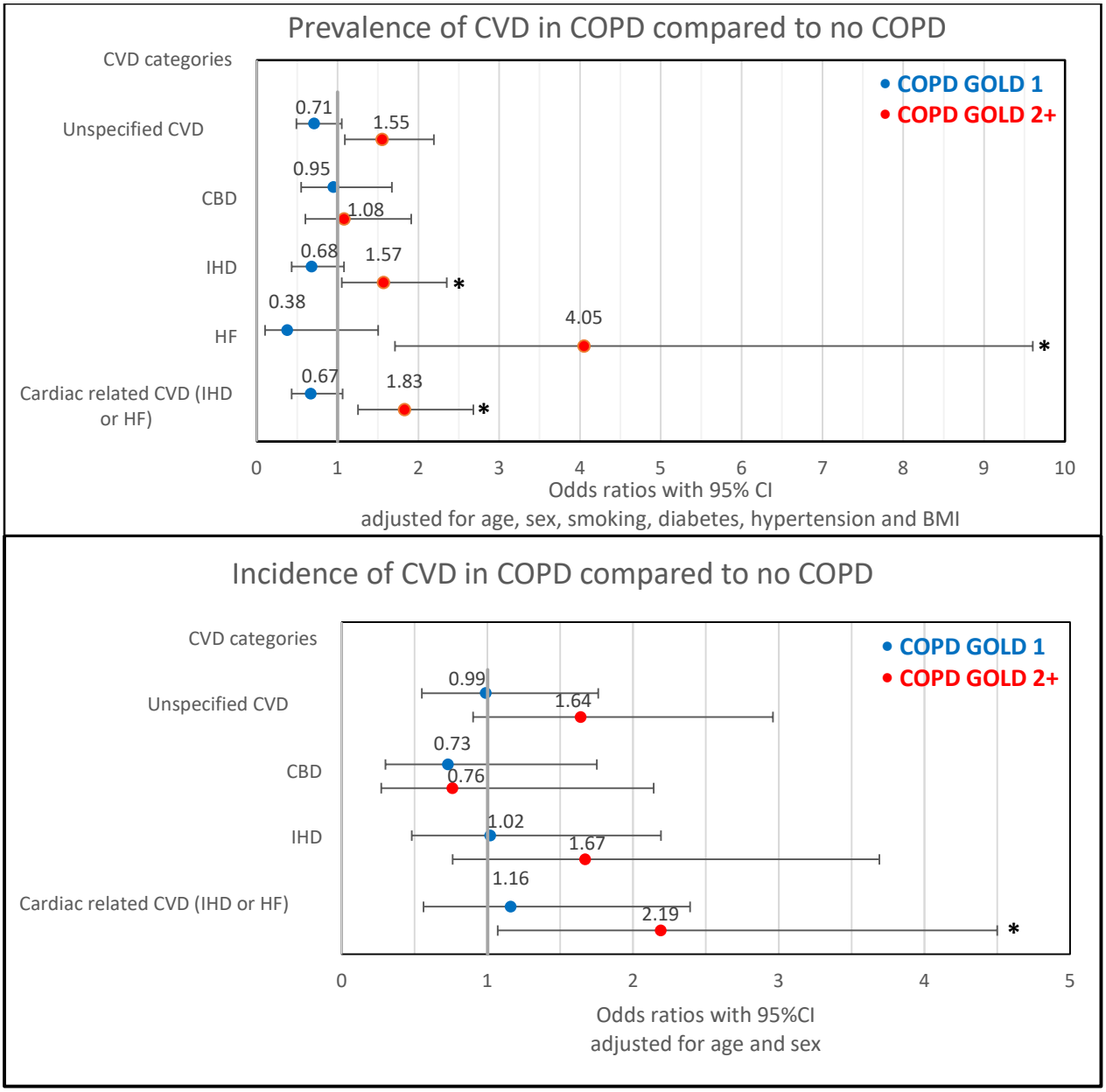

Fig 2a. Odds ratios and 95% confidence intervals for the prevalence of CVD in people with varying severities of COPD compared to those with no COPD adjusted for age, sex, smoking, diabetes, hypertension, and body mass index. **Fig 2b.** Odds ratios and 95% confidence intervals for the incidence of CVD in people with varying severities of COPD compared to people with no COPD adjusted for age and sex. COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, BMI: body mass index. Hypertension is a blood pressure $\geq$140/90 mm Hg (mercury) while sitting.

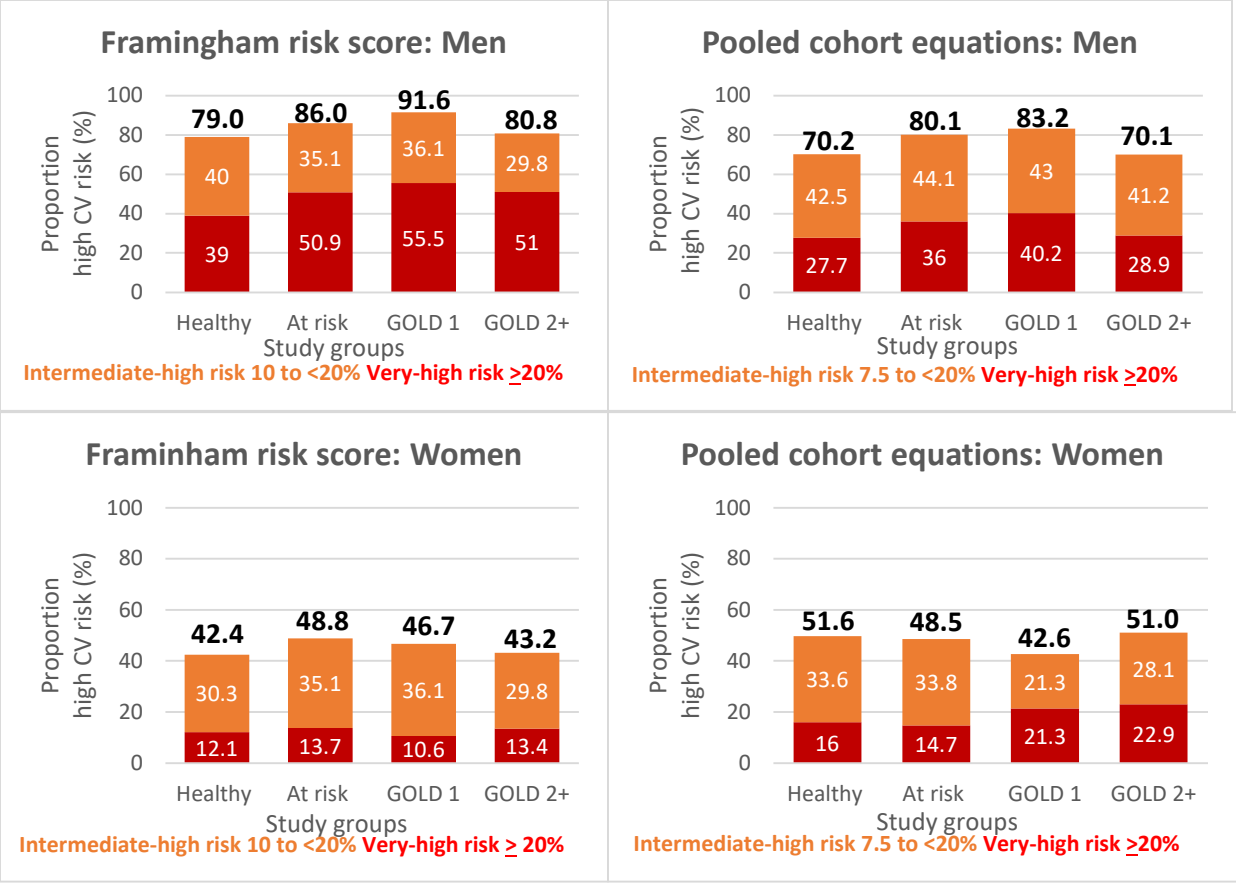

Fig 3. Proportions of individuals with high cardiovascular risk scores among different study groups in the study population. CV: cardiovascular, GOLD: global initiative for chronic obstructive lung disease stage.

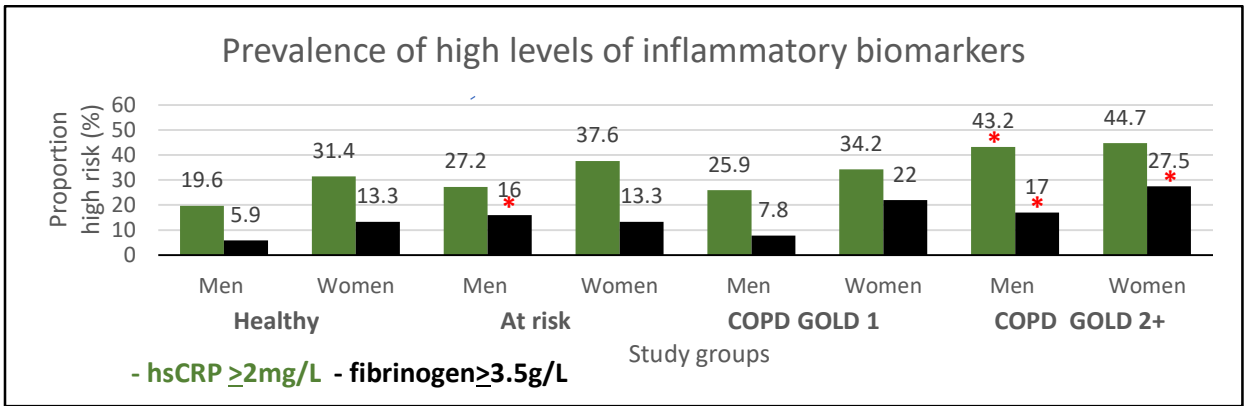

Fig 4. Comparing the prevalence of high levels of high sensitivity C-reactive protein and fibrinogen among different study groups in men and women. COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, hsCRP: high sensitivity C-reactive protein. An * indicates a statistically significant difference compared to other study groups of same sex.

Table 3. Odds ratios and 95% confidence intervals for the prevalence and incidence of CVD in COPD and non-COPD with COPD clinical "traits" (chronic bronchitis, high symptom burden using CAT or MRC dyspnea scores) compared to others without those traits adjusted for age, sex, smoking, hypertension, diabetes and FEV_1.

	Prevalence of CVD OR with 95%CI		
COPD clinical trait	Any CVD (CBD, IHD or HF)	CBD	IHD
COPD with chronic bronchitis compared to COPD with no chronic bronchitis	1.27 [0.76 - 2.13]	0.90 [0.38 - 2.10]	1.46 [0.81 - 2.65]
COPD with more dyspnea MRC≥3 compared to COPD with MRC <3	1.79 [0.88 - 3.61]	1.80 [0.59 - 5.47]	1.37 [0.60 - 3.12]
COPD with CAT ≥10 compared to COPD with CAT<10	1.80 [1.12 - 2.88]*	1.56 [0.75 - 3.27]	1.95 [1.12 - 3.40]*
COPD with high symptom burden (CAT≥10 or MRC≥3) compared to COPD with low burden.	2.04 [1.26 - 3.31]*	2.05 [0.97 - 4.33]	2.13 [1.20 - 3.79]*
Non-COPD clinical trait	Any CVD (CBD, IHD or HF)	CBD	IHD
At risk for COPD compared to Healthy with no COPD "GOLD 0" (no COPD and chronic bronchitis) compared to no COPD without chronic bronchitis	0.98 [0.61 - 1.58]	0.90 [0.41 - 1.95]	0.99 [0.57 - 1.72]
	1.69 [0.96 - 2.99]	1.25 [0.46 - 3.37]	1.95 [1.04 - 3.66]*

FEV_1: Forced expiratory volume in one second, COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease, CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, MRC: modified medical research council dyspnea, CAT: COPD assessment test, CI: confidence intervals. A * indicated a significant association with $p<0.05$. Hypertension is a blood pressure ≥140/90 mm Hg (mercury) while sitting.

Sensitivity analyses

Sensitivity analyses were performed by comparing prevalence/incidence in GOLD 2 COPD by excluding GOLD 3+ participants and found similar OR and corresponding 95%CI when compared to no COPD. All COPD groups were also compared to the "at-risk" groups (current and ex-smokers) and differences remained significant with ORs around the same or higher in some subgroups of CVD (see results in supplement table 1). Sensitivity analyses was performed comparing levels of biomarkers after excluding all inflammatory conditions such as lupus cancer, ulcerative colitis, Crohn's disease, psoriasis, rheumatoid arthritis, and cancer. Results show similar trends among groups (see results in supplement table 2).

4.5 DISCUSSION

In the Canadian population, this study demonstrated that moderate COPD (GOLD 2+) increases the risk of comorbid CVD compared to those without COPD after accounting for shared risk factors. People with COPD GOLD 2+ had 1.5 times increase likelihood of comorbid IHD and 4.0 times increased odds for comorbid HF, but no statistically significant difference for comorbid CBD when compared to the non-COPD population. Individuals with COPD GOLD2+ were more than twice as likely to develop an incident CVD (angina, coronary artery disease, MI, or heart failure) independent of age and sex in the longitudinal follow-up as compared to people without COPD. However, no difference could be shown in the prevalence or incidence of CVD for individuals with mild COPD (GOLD 1). Sensitivity analyses indicate that increases in the incidence and prevalence of cardiac related CVD in COPD compared to those without COPD was mainly due to moderate COPD (GOLD 2) in this population. Furthermore, the increased CV risk in COPD GOLD 2 was still present when compared to individuals at risk for COPD, only former and current

smokers with no COPD. The increased prevalence for all CVD, especially IHD, was also seen in people with some clinical traits, more specifically COPD with a higher CAT or dyspnea score. Individuals with COPD and chronic bronchitis did not have an increased prevalence of comorbid CVD compared to those with only COPD. Individuals at risk for COPD, specifically smokers or former smokers, were not at increased risk for IHD compared to those with no COPD who never smoked. However, being categorized as "GOLD 0" indicating chronic bronchitis with no COPD (also considered at risk for COPD) increased the likelihood of comorbid IHD compared to others without COPD. Therefore, being at risk for COPD with chronic bronchitis may also be an indication of being at-risk for comorbid CVD. Our findings suggest that some COPD clinical "traits" could be useful in defining CV risk, however we will need longitudinal research to assess their usefulness in predicting future CVD events. Chronic bronchitis symptoms may not be as benign as we have already thought considering previous evidence of its association with a higher risk of respiratory-related mortality [42].

The recent systematic review by Chen and colleagues showed that COPD patients were at increased risk of comorbid CVD with a twice as high risk for IHD and HF as compared to people without COPD [21]. In the general Canadian population with mostly mild and moderate COPD, we found that moderate+ COPD increases the risk of comorbid CVD compared to those without COPD, but mild COPD could not be shown to be an independent risk factor. This increased risk for IHD prevalence in GOLD2+ compared to people without COPD was slightly lower than what has been previously reported for COPD. However, many of the studies reporting these results are from clinical convenience sample where patients are more likely brought to the attention of physicians because of symptoms. Furthermore, we accounted for several shared risk factors such as advanced age, male sex, smoking, hypertension, diabetes, BMI, which have an increased

prevalence in COPD compared to the non-COPD population and still found a significant association between COPD GOLD 2+ and comorbid IHD. The systematic review did not find an association with IHD and COPD severity which was previously suggested to exist, while we found that COPD severity is associated with an increased risk of comorbid IHD in a mild and moderate COPD population. This study cannot comment on more severe COPD patients and how they relate to comorbid CVD, but the risk of comorbid CVD increases with COPD severity when randomly screening for COPD in the general Canadian population with mostly mild/moderate+ COPD.

We also found that people with moderate+ COPD were twice as likely to develop cardiac related CVD (IHD or HF) when followed longitudinally compared to those without COPD. While Chen and colleagues did not include any longitudinal studies in their review because of a lack of good comparator groups [21], other population based studies relying mainly on data from health administrative records have demonstrated a similar relationship between COPD and incident CVD [36-38]. This study's longitudinal analyses had age and sex matched control groups with current/former smokers and those who never smoked and found an increased risk of incident CVD in moderate COPD. While mild COPD did not significantly increase the likelihood of future CVD, further study needs to assess incident CVD with a longer follow-up.

While the prevalence and incidence of CVD was higher in COPD GOLD 2+ compared to the rest of the population, this was not reflected in the proportion of high CV risk scores, which were similar to people with mild COPD, those at risk for COPD (current/ex-smokers) and those with no COPD who never smoked. People with a history of CVD or arrythmia are at increased risk to develop a new CVD in the future compared to the general population and thus require closer monitoring by their physician, roughly one-fifth of the population. In people with no history of CVD or arrythmia, the FRS and PCE categorize high and very-high risk indicating which persons

require closer attention to prevent future CVD. Following the Canadian Cardiovascular Society guidelines in this population (mean age of 66.7 years) 86% of men and 42% of women were at high CV risk using the FRS. These people would require closer physician attention which may include statin therapy. In contrast, 78% of men and 48% of women were categorized high risk by the PCE following American College of Cardiology/American Heart Association clinical practice guidelines [20, 22]. This represents a remarkably high proportion of men and large proportion of women considered to have high CV risk and recommended for physician discussion and intervention. Those categorized very-high risk by either risk score are strongly recommended intervention that includes statin therapy by both the Canadian and American guidelines, which is 50% (FRS) and 35% (PCE) in men and 12%(FRS) and 18%(PCE) in women [20, 22]. Although there is variability in proportions of individuals classified high risk depending on the risk score and the thresholds for high risk, these proportions were not higher in COPD GOLD 2+ compared to the others as we would expect. It is particularly important to distinguish CV risk between those with and without COPD because moderate COPD is an independent risk factor for CVD, and has a larger proportion of individuals with traditional CV risk factors (diabetes, hypertension, smoking) compared to people with no COPD, which will further increase CV risk in this COPD population.

The American clinical practice guidelines mentioned earlier categorize CRP levels $\geq$ 2 mg/L to be a CV risk enhancing factor that can be added to CV risk scores in a population with intermediate-high risk or uncertain risk [20]. Around 44% of men and women with GOLD 2+ COPD had high levels of CRP, a known CV risk enhancing factor, which were the highest proportions among different groups in this population. COPD patients are known to have higher levels of CRP compared to those without COPD [39] and higher baseline CRP has been associated with

increased CVD mortality, with this association being stronger in men [40]. We found a significant difference in proportion of men with high CRP levels compared to others, but the difference was not significant in women. There was also a greater proportion of men and women with high fibrinogen levels in COPD GOLD 2+ compared to the rest of the study population. However, men at-risk for COPD and women with mild COPD (GOLD 1) also had similar proportions of individuals with high levels of fibrinogen as COPD GOLD 2+. Higher levels of fibrinogen are also associated with a lower survival rate of CVD consistently in both men and women [41]. Our results suggest that greater proportions of people with high risk levels of fibrinogen may be in the progression from "at risk for COPD" or mild COPD to the development of moderate plus COPD which we know is an independent CV risk factor. While we did not compare proportions of high levels of inflammatory biomarkers between men and women, we noticed in each study group the proportions were consistently higher in women.

4.5.1 Strengths and Limitations

The strengths of our study were in the ability to assess COPD and CVD in earlier subclinical stages and monitor their relationship and progression. The population-based cohort screened from the general Canadian population provides a large representation of undiagnosed COPD, mild COPD, and women with COPD, all groups that are underrepresented in many studies. CanCOLD is the first observational cohort specific to COPD having recruited its participants from the general population rather than more convenient recruitment in clinical settings which better mirrors prevalent COPD populations at large. CanCOLD also has post-BD spirometry measures and detailed information on symptoms that allows for a more accurate diagnosis of COPD and the severity using established diagnostic criteria and gold standard definitions for COPD clinical "traits". While many studies rely on health administrative data or COPD patients screened from

clinics or hospitals with more severe COPD, the current study allowed a better understanding of the relationship between CVD and mild/moderate COPD in the general population.

This study has certain limitations, the first being self-reporting on CVD outcomes. While there is good validity for IHD and CBD from self-reporting, it is much lower for HF. This may be the reason why the prevalence of HF was 1.7% and incidence was only 0.7% in this population, partly due to recall bias and partially because HF symptoms are similar to symptoms of COPD exacerbation and can be commonly mistaken for one another [43]. Even though we had a low number of HF events from self-reporting and similarities in symptoms between COPD exacerbations and HF, this most likely led to more underreporting in the COPD groups and therefore lower odd ratios than expected. However, HF has good specificity and we still found a substantial difference between GOLD 2+ and the non-COPD groups in the range of what was previously reported for COPD. We also had a shorter follow-up period compared to the 10-year minimum for most CV studies developing CV risk scores such as the PCE and FRS which both assess 10-year CVD risk. Another limitation of this study was the inability to assess the prediction capability of the different CV risk scores or inflammatory biomarkers for future CVD events which needs further research.

4.6 CONCLUSION

In the general Canadian population, individuals with moderate+ COPD (GOLD 2+) were shown to have an increased likelihood of having comorbid CVD (IHD or HF), and to develop a new CVD compared to those with no COPD. However, we could not demonstrate that having mild COPD (GOLD 1) was related or increased the risk of CVD. The increased CV risk in COPD as compared

to people with no COPD is driven by moderate COPD (GOLD 2) and remains high even when compared only to those at risk for COPD, i.e., current and former smokers with normal spirometry. A larger proportion of people with COPD GOLD2+ also had higher levels of inflammatory biomarkers (CRP and fibrinogen, which are CV risk enhancing factors) compared to people with no COPD or mild COPD, yet the proportions of individuals categorized as high risk using validated CV risk scores were similar in all study groups. Importantly, a high symptom burden of COPD (using either MRC dyspnea or CAT scores) as recommended in GOLD guidelines to guide COPD therapy also increases the likelihood of comorbid CVD when compared to others with COPD who do not have these clinical "traits". This important finding could have an impact in clinical practice, helping the physician to recognize mild to moderate COPD patients who may be at high risk of CVD. Also, of importance to note, having chronic bronchitis with normal spirometry "GOLD 0" also increases the likelihood of a comorbid IHD compared to those without chronic bronchitis.

Moderate COPD is an independent risk factor for CVD and while we were unable to definitively show an association between mild COPD and comorbid CVD, an association with the development of new CVD may exist but this will likely require a larger sample and/or a longer follow-up period. Inflammatory biomarkers such as CRP and fibrinogen are known predictors of CVD and further exploration is needed to assess their usefulness in predicting CVD for people with COPD and those at risk for COPD. Finally, validated CV risk scores such as the FRS and PCE do not categorize a higher proportion of the COPD population as high-risk compared to those without COPD, even as COPD severity increases and airflow limitation worsens. Canadian guidelines recommend screening all COPD patients using CV risk scores but do not assess the impact of COPD burden or airflow limitation on CV risk and the American guidelines do not include COPD as a risk enhancing factor [20, 22]. Further investigation is needed to assess if people with COPD will

benefit from CV risk assessment that includes COPD severity, inflammatory biomarkers, and COPD specific clinical "traits" in addition to traditional CV risk factors. An improved CV risk assessment tool might be needed for people with COPD as this could help physicians properly target high risk individuals for a more personalized care approach that highlights both CVD prevention and COPD progression strategies. The coexistence of COPD, cardiovascular disease, and major risk factors for cardiovascular disease highlights the crucial need for the development of strategies aimed at early detection for and to reduce CV risks associated with COPD.

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4.8 SUPPLEMENT

Supplemental table 1a. Odds ratios and 95% confidence intervals for the prevalence of CVD in people with moderate COPD (GOLD 2) compared to those with no COPD and those at risk for COPD (current/former smokers) adjusted for age, sex, smoking, diabetes, hypertension, and body mass index.

	Any CVD	CBD	IHD	HF	Cardiac CVD
GOLD 2 compared to non-COPD	1.64 [1.12 - 2.39]*	1.10 [0.57 - 2.13]	1.64 [1.07- 2.52]*	5.11 [1.83 - 14.25]*	1.83 [1.25 - 2.68]*
GOLD 2+ compared to "at risk" for COPD	1.54 [1.05 - 2.25]*	1.05 [0.56 - 1.97]	1.58 [1.02 - 2.45*	3.66 [1.38 - 9.67]*	1.78 [1.17 - 2.71]*
GOLD 2 compared to "at risk" for COPD	1.63 [1.08 - 2.46]	1.14 [0.55 - 2.37]	1.64 [1.03 - 2.63]*	4.46 [1.39 - 14.28]*	1.83 [1.22 - 2.78]*

COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, BMI: body mass index. Hypertension is a blood pressure $\geq$140/90 mm Hg (mercury) while sitting.

Supplemental table 1b. Odds ratios and 95% confidence intervals for the incidence of CVD in people with moderate COPD (GOLD 2) compared to people with no COPD adjusted for age and sex.

	Any CVD	CBD	IHD	Cardiac CVD
GOLD 2 compared to non-COPD	1.78 [0.97 - 3.26]	0.66 [0.21 - 2.05]	1.84 [0.83 - 4.10]	2.51 [1.22 - 5.15]*
GOLD 2+ compared to "at risk" for COPD	1.81 [0.90 - 3.63]	0.69 [0.23 - 2.11]	2.48 [0.90 - 6.84]	3.46 [1.32 - 9.12]
GOLD 2 compared to "at risk" for COPD	1.93 [0.95 - 3.91]	0.62 [0.19 - 2.06]	2.81 [1.02 - 7.77]*	3.89 [1.48 - 10.26]*

COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, CVD: cardiovascular disease CBD: cerebrovascular disease, IHD: ischemic heart disease, HF: heart failure, BMI: body mass index. Hypertension is a blood pressure ≥140/90 mm Hg (mercury) while sitting.

Supplement table 2: Proportion of participants with high CV risk in different study groups using inflammatory biomarkers (CRP and fibrinogen) after excluding participants with lupus cancer, ulcerative colitis, Chron's disease, psoriasis, rheumatoid arthritis, or any type of cancer.

	Study groups				
	Healthy	At risk	GOLD 1	GOLD 2+	P
Men with high CRP (≥ 2mg/L)	20.0%	25.4%	23.8%	40.0%	*
Women with high CRP (≥ 2mg/L)	30.0%	34.2%	38.4%	47.3%	
Men with high fibrinogen (≥3.5g/L)	7.2%	14.8%	8.0%	17.3%	*
Women high fibrinogen (≥3.5g/L)	15.1%	9.7%	17.8%	25.6%	*

GOLD: global initiative for chronic obstructive lung disease stage, CRP: c-reactive protein.

Supplemental table 3. Odds ratios and 95% confidence intervals for the prevalence and incidence of CVD in COPD and non-COPD participants with COPD clinical "traits" (chronic bronchitis, high symptom burden using CAT or MRC dyspnea scores) compared to others without those traits adjusted for age, sex, smoking, hypertension, diabetes and FEV_1.

	Non-COPD participants only		**COPD participants only**	
Model adjusted for age, sex, smoking, hypertension, diabetes.	OR	P	OR	P
Chronic bronchitis compared to no chronic bronchitis	1.57 [0.90 - 2.74]	0,108	1.46 [0.89 - 2.40]	0,132
MRC ≥3 compared to MRC<3	2.78 [1.19 - 6.51]	0.019*	2.51 [1.30 - 4.85]	0.006*
CAT score ≥10 compared to CAT score<10	2.13 [1.30 - 3.49]	0.003*	2.24 [1.43 - 3.49]	<0.001*
Model adjusted for age, sex, smoking, hypertension, diabetes and FEV_1.	OR	P	OR	P
Chronic bronchitis compared to no chronic bronchitis	1.61 [0.91 - 2.86]	0,103	1.27 [0.76 - 2.13]	0,359
MRC ≥3 compared to MRC<3	1.47 [0.51 - 4.19]	0,473	1.79 [0.88 - 3.61]	0,106
CAT score ≥10 compared to CAT score<10	1.79 [1.06 - 3.03]	0.031*	1.80 [1.12 - 2.88]	0.015*

OR: Odds ratios and 95% confidence intervals, FEV_1: forced expiratory volume in one second, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, MRC: modified medical research council dyspnea, CAT: COPD assessment test, CI: confidence intervals. A * indicated a significant association with $p<0.05$. Hypertension is a blood pressure ≥140/90 mm Hg (mercury) while sitting.

CHAPTER 5: SUMMARY AND CONCLUSION

The goal of this research was to assess CV risk in the general Canadian population and determine if there was a difference in risk among individuals with COPD compared to those with no COPD. Our findings suggest that individuals with moderate COPD are more likely to have comorbid CVD (IHD and HF) compared to their peers without COPD. We also found that individuals with no history of CVD and moderate COPD were more likely to develop a CVD compared to others without COPD. We did not find a significant association between mild COPD and CVD, and we did not find an association between CBD and mild or moderate+ COPD. This increased incidence and prevalence of CVD remained even when compared only to those at risk for COPD i.e., current/former smokers. CV risk assessed using validated risk scores found no difference in the proportion of high-risk individuals in mild or moderate COPD as compared to individuals at risk for COPD and those who never smoked. High levels of inflammatory biomarkers, indicative of higher CV risk, was also observed in a greater proportion of individuals with moderate+ COPD as compared to the remainder of the population. Certain clinical traits of COPD such as the CAT score, indicating a high symptom burden, was also associated with an increased risk of comorbid CVD. While we did not show that being at risk for COPD (current/former smokers) increased the likelihood of having a comorbid CVD, chronic bronchitis with normal lung function increased the odds of comorbid IHD compared to other with no COPD.

Our findings suggest that CV risk increases with COPD severity even among those with less severe disease. Treatable trait of high symptom burden and chronic bronchitis without COPD were also indicative of an increased risk of CVD. Furthermore, 70% of the COPD population with mostly mild and moderate disease, had COPD that was not diagnosed by a physician. Therefore, COPD may be an important risk factor for CVD even in the earlier stages of COPD that gets overlooked.

Our research highlights that risk for CVD increases with progression of subclinical COPD to moderate COPD. Considering the high proportion of individuals with undiagnosed COPD in the general Canadian population, this indicates that there may be important care gaps with respiratory and cardiac consequences.

People with moderate+ COPD from the general population have an increased prevalence and incidence for cardiac related CVD such as IHD and HF, and a larger proportion of the population with high levels of inflammatory biomarkers compared to those without COPD. However, the CV risk scores did not show a difference in the proportion of high CV risk individuals among those with moderate plus COPD compared to others, indicative of no increased perceived risk for a future CVD event in this COPD population compared to those without COPD. Furthermore, CV risk scores were much higher in men than women, with half of men eligible for recommended statin therapy and 85% requiring closer attention by the Canadian Cardiovascular society guidelines. Primary prevention of CVD as per these Canadian guidelines recommend screening all COPD patients using CV risk scores but does not assess the potentially additional impact of COPD burden or airflow limitation on CV risk. The American guidelines for primary prevention do not include COPD as a risk enhancing factor, even though they include other inflammatory diseases and even other comorbidities such as diabetes. Since there is a large proportion of the population who are considered to have high CV risk requiring some form of physician intervention, especially among men, it will be important to investigate how well these CV risk scores perform in predicting CVD events in a COPD population. The symptom burden of COPD is shared by CVD in relation to dyspnea, chest tightness and lower activity levels. However, other symptoms such as chronic bronchitis and airflow limitation are more specific to the lung yet still associated with CVD. This

warrants further investigation to assess if people with COPD will benefit from CV risk assessment that includes COPD clinical "traits" to traditional CV risk factors.

Although the incidence of cardiac CVD was increased in mild COPD (GOLD 1) compared to those without COPD, this did not reach statistical significance. This requires further investigation with a larger sample and/or longer follow-up period. Individuals at risk for COPD and those with mild COPD do have increased CV risk compared to people with no respiratory disease who never smoked, but this is mainly due to worsening of COPD. More attention should be given in clinical practice to monitor high risk persons to prevent both CVD and deterioration of lung function, which in turn increases CV risk. The largest proportions of people with high levels of inflammatory biomarkers are seen in COPD GOLD 2+ in both men and women, and persistently high levels of inflammatory biomarkers enhance CV risk. Since CRP and fibrinogen are known to be predictors of CVD, they may be useful in predicting CVD for people with COPD and those at risk for COPD. Validated CV risk scores such as the FRS and PCE need to be assessed in a COPD population with different disease severities, especially since there is heterogeneity among CV risk scores and thresholds of risk that are used. Understanding how well these CV risk scores perform in a COPD population will help to improve CV risk assessment in COPD. This will also allow us to determine if adding COPD severity and biomarkers of inflammation to these CV risk scores will improve CV risk prediction in the Canadian COPD population and others worldwide, and help target high risk individuals for a more personalized care approach.

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