Fractional Exhaled Nitric Oxide (FeNO) as Inflammatory Biomarker in Chronic Obstructive Pulmonary Disease (COPD) and Asthma-COPD Overlap (ACO)

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October 2017

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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ABSTRACT

Background and Objectives: Chronic obstructive pulmonary disease (COPD) and asthma are the most common inflammatory airway diseases. Some individuals share features of both asthma and COPD called asthma-COPD overlap (ACO) syndrome (ACOS). These individuals have worse symptoms and health status as well as lower pulmonary function than COPD-only. As well, the choice for pharmacotherapy is different in asthma and COPD patients. Therefore, it is crucial to differentiate ACO(S) patients from COPD-only. As a result, there is a remarkable need to have access to a biomarker that could be used in a clinical setting to be able to differentiate ACO(S) from COPD-only. Fractional exhaled nitric oxide (FeNO) is a promising biomarker identifying eosinophilic and T-helper cell 2 (Th2)-mediated airway inflammation in asthma. FeNO is recommended in the management of asthma. Its measurement is easy, sensitive, reproducible, and non-invasive. However, there are limited primary papers on FeNO measurement in COPD, the exact role of FeNO in COPD and in differentiating COPD from ACO(S) is still unclear and needs to be defined. We aimed to systematically search the literature to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize and aggregate findings from different studies. We considered specific questions/objectives to guide our review. Furthermore, we conducted a study embedded in the Canadian Cohort Obstructive Lung Disease (CanCOLD) to evaluate the role of FeNO and determine if there is a cut-off value that can differentiate ACO(S) from COPD-only in a population sampling based cohort that can better mirror the population of COPD patients at large.

Methods: Firstly, we conducted a systematic scoping review to determine key concepts, and to explore gaps within a developing field of research. The search strategy was conducted on several major databases, including Medline, EMBASE, CINAHL, Cochrane Library, Web of Science, BIOSIS. Secondly, we carried out a study embedded in CanCOLD with new measurement including FeNO level. The COPD participants were divided into ACO and COPD-only (non-ACO). ACO was defined according to 3 clinical definitions (def): i) >12% and >200 ml of increment in the FEV1 post-bronchodilator, iii) physician diagnosis of asthma; iii) atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire). The different levels of FeNO and its utility were assessed between ACO and COPD-only. The optimal cut-off values and the receiver operating characteristic (ROC) curves were obtained to evaluate the clinical utility of FeNO in diagnosing ACO(S).

Results: From the scoping review, 38 studies were selected, 24 were on modifying factors in FeNO measurement in COPD patients, 18 were on FeNO in COPD and compared to healthy subjects, 22 on FeNO and disease severity or progression, 7 on FeNO and ACO(S), 12 on FeNO and biomarkers, and 8 on FeNO and treatment response. From the original study embedded in CanCOLD, a total of 169 subjects were enrolled, of those 95 were COPD with ACO, N=46, and COPD-only, N=49. The mean FeNO level was higher but not statistically significant between ACO and COPD-only. The significant optimal cut-off values to differentiate ACO from COPD-only was for Def 1 FeNO ≥36 ppb with the sensitivity of 39%, specificity of 88% and AUC of 0.63, p=0.046, and Def 3 FeNO ≥23.5 ppb with the sensitivity of 80% and specificity of 50%, and area under the curve (AUC) of 0.65, p=0.047.

Conclusion: From the scoping review when measuring FeNO, the evidence is still lacking preventing us from recommending the general use of FeNO in clinical practice for COPD patients. Although FeNO level is higher in ACO(S) patients than COPD-only, it is still unclear if there is a FeNO cut-off that can be used to make the diagnosis of ACO(S) and/or to guide therapy with inhaled corticosteroids (ICS)/glucocorticoids (GCS) in COPD patients. After studying FeNO in a population-based sample of COPD, we were not able to show that FeNO levels could be used as a biomarker for differentiating ACO from COPD-only, and it is still too soon to be able to make a recommendation of using it in clinical practice. Future research should be done using a validated definition of ACO, i.e., having reference to the type of airway inflammation instead of relying on a clinical definition only, and to determine if FeNO could be part of a cascade of a therapeutic decisional algorithm and/or as an alternative to sputum induction for guiding COPD therapy.

RÉSUMÉ

Contexte et objectifs: La maladie pulmonaire obstructive chronique (MPOC) et l'asthme sont les maladies inflammatoires des voies respiratoires les plus courantes. Certaines personnes partagent des caractéristiques à la fois de l'asthme et de la MPOC, appelées syndrome de chevauchement de la MPOC (ACO)(S). Ces personnes ont des symptômes et une qualité de vie moins bons ainsi que des fonctions pulmonaires inférieurs à ceux de la MPOC seule. De plus, le traitement pharmacologique est différent. Par conséquent, il est crucial de pouvoir différencier les patients avec ACO (S) de ceux atteint de MPOC seule. En conséquence, il existe un besoin en clinique de pouvoir utiliser un test avec mesure d'un biomarqueur pour différencier les patients avec ACO(S) de ceux avec MPOC seule. L'oxyde nitrique exhalé fractionnel (FeNO) est un biomarqueur prometteur qui permet d'identifier l'inflammation des voies respiratoires médiée par les éosinophiles et les lymphocytes T auxiliaires 2 (Th2) dans l'asthme. Il est recommandé d'utiliser FeNO dans la prise en charge de l'asthme. Sa mesure est facile, sensible, reproductible et non invasive. Nous avons peu d'études sur la mesure du FeNO dans la MPOC, le rôle exact du FeNO chez les patients avec MPOC et la différenciation avec les patients ACO (S). Notre but était de faire une recherche systématiquement de la littérature dans le domaine d'intérêt, c'est-à-dire FeNO dans la MPOC, ainsi que de synthétiser et agréger les résultats des différentes études. Nous avons examiné des questions/objectifs spécifiques pour guider notre examen. De plus, nous avons mené une étude prospective en utilisant la population et la base de données CanCOLD (Canadian Cohort Obstructive Lung Disease) pour évaluer le rôle du FeNO dans cette population spécifique de MPOC et déterminer s'il existe une valeur seuil pour différencier les individus avec ACO (S) de ceux avec MPOC.

Méthodes: Dans un premier temps, une étude systématique « Scoping review » a été effectuée pour déterminer les concepts clés et explorer les lacunes dans le domaine de recherche en développement sur le FeNO et la MPOC. La stratégie de recherche a été menée sur plusieurs bases de données importantes, notamment Medline, EMBASE, CINAHL, la Bibliothèque Cochrane, Web of Science, BIOSIS. Dans un deuxième temps, l'étude incorporée dans CanCOLD avec mesures de FeNO ont été effectuées, en particulier chez les patients MPOC. Ces patients avec MPOC ont été divisés en ACO et MPOC seule (non-ACO). ACO a été cliniquement défini ACO en utilisant les 3 définitions (def) suivantes: i) >12% et >200 ml VEMs post bronchodilatateur; ii) diagnostic formulé par un médecin; iii) atopie avec un diagnostic formulé par un médecin (par questionnaire). Les différents niveaux de FeNO et leurs utilités pour différencier l'ACO (S) de la MPOC ont été évalués entre patients avec ACO et

ceux avec MPOC seule. Les courbes caractéristiques de fonctionnement du récepteur (ROC curve) ont été obtenues pour illustrer l'utilité clinique du FeNO dans le diagnostic de l'ACO(S). Les valeurs limites optimales ont également été déterminées.

Résultats: Les résultats de l'étude systématique « Scoping review » ont inclus 38 études, 24 articles sur les facteurs modificateurs de la mesure du FeNO chez les patients avec MPOC, 18 du FeNO chez les patients avec MPOC ou ceux avec MPOC comparés aux sujets sains, 22 du FeNO et de la sévérité ou de la progression de la maladie, 7 du FeNO et des patients avec ACO(S), 12 du FeNO et d'autres biomarqueurs, et 8 du FeNO et de la réponse au traitement. Les résultats de l'étude sur la population de CanCOLD, un total de 169 sujets, 95 sujets ont été divisés en un des 3 diagnostiques cliniques ACO (N=46) et MPOC seule (N= 49). Le niveau moyen de FeNO était augmenté chez les sujets avec ACO en comparaison au sujet avec MPOC seule mais la différence n'était pas statistiquement significative. Les valeurs de seuil optimales significatives pour différencier ACO de MPOC seule étaient pour la def1 FeNO $\geq$ 36 ppb avec une sensibilité de 39%, une spécificité de 88% et une AUC de 0,63, p = 0,046, et la def3 FeNO $\geq$23,5 ppb avec une sensibilité de 80% et une spécificité de 50%, et une aire sous la courbe (AUC) de 0,65, p = 0,047.

Conclusion: De l'étude systématique « Scoping review », il a été démontré que plusieurs facteurs peuvent affecter la mesure du FeNO. Les preuves de l'utilisation générale de FeNO dans la pratique clinique chez les patients atteints de MPOC font toujours défaut. L'étude sur la population de CanCOLD a démontré qu'il n'est pas possible d'utiliser la mesure du FeNO et/ou de confirmer un seuil de FeNO pouvant être utilisé pouvant différencier le diagnostic d'ACO(S) chez les individus avec MPOC et de guider le traitement avec corticostéroïdes inhalés/systémique (ICS)/glucocorticoïde (GCS). Il sera important de planifier dans le futur des études utilisant une définition de l'ACO avec référence au type d'inflammation bronchique au lieu seulement d'une définition clinique, et de déterminer si la mesure du FeNO pourriat faire partie d'une cascade d'un algorithme décisionnel thérapeutique et/ou une alternative à l'induction d'expectoration pour guider la thérapie des patients avec MPOC.

ACKNOWLEDGEMENTS

Throughout my graduate studies, I received two Graduate Excellence Awards in Experimental Medicine from the Division of Experimental Medicine, Department of Medicine, McGill University, and a scholarship for the CIHR - Quebec Respiratory Health Training Program (CIHR-QRHTP) from the Canadian Institutes of Health Research and the Health Respiratory Network of the FRQS and Laval University for the year 2016.

I would like to express my heartfelt gratitude to my supervisor, Dr. Jean Bourbeau, and thank him very much. Dr. Bourbeau embodies the merits of a remarkable and valuable mentor. The door to Dr. Bourbeau's office was always open whenever I ran into a trouble spot or had a question about my research projects or even regarding my presentations. He consistently allowed research projects to be my own work but steered me in the right direction whenever he thought I needed it. He continually tried to solve complicated and hard aspects of research and scholarships and guided me on the best way. He had an incredible eager and enthusiasm for teaching, helping and guiding not only me and his own other trainees but also the research staffs as well as other trainees in the research institute or even out of the institute, which was inspirational. I would like to thank him for encouraging me throughout my graduate studies and for allowing me not solely to grow as a research scientist, but also as an individual. I learned a lot from him not only details of medical research methods and how to be an outstanding and good research scientist, but also methods of life, problem-solving and how to be an outstanding and useful person in the social community and my life. This thesis would not be possible without his support, help, and guidance. I would like to appreciate all of his efforts again.

I would also like to sincerely thank my thesis committee members for serving as invaluable mentors who provided insightful and productive comments and suggestions on any aspect of my projects. I appreciate their patience and efforts in guiding me every step of the way.

I thank the members at the Respiratory Epidemiology and Clinical Research Unit for their constant encouragement and support. It was a privilege to work with and learn from this truly collaborative, supportive and diverse research group. I thank Palmina Mancino, research coordinator of CanCOLD, for her great help and support in gathering research subjects and finding missing data as well as helping in performing any measurement in the project that I needed. I thank Miriam Barrecheguren MD, respirologist and research fellow, for her help in solving problems that I confronted during my projects as well as reviewing and mentioning useful comments regarding my scoping review. I thank Pei Zhi Li, data analyst at the RECRU,

for her great help in analyzing my data. I am eternally grateful to Louise Auclair, administrative assistant, for handling management tasks as well as her support in urgent situations.

I would like to thank Aerocrine for its support and providing the measurement equipment, Niox Mino, for my project. Also, I would like to thank all the current and past sponsors of CanCOLD, including Canadian Respiratory Research Network (CRRN), Astra Zeneca Canada Ltd, Boehringer Ingelheim Canada Ltd, GlaxoSmithKline Canada Ltd; Novartis, CIHR (CIHR/ Rx& D Collaborative Research Program Operating Grants- 93326), the RI MUHC and the Respiratory Health Network of the FRSQ.

I would like to express my special thanks to my wife, Nadia Naderi, who has wholeheartedly support and encourage me not only during all of my life but also during my graduate study, research projects and writing my thesis.

Finally, I must express my very profound special thanks to my parents for providing me with endless love, support, and encouragement throughout my years of study as well as all of my life.

PREFACE AND CONTRIBUTION OF AUTHORS

One of the major goals of the projects constituting this Master thesis was to investigate exact role of FeNO in COPD patients and find gaps in this research topic. This thesis complies with the Graduate and Postdoctoral Studies' guidelines and general requirements of a manuscript-based (article-based) Master's theses at McGill University. This thesis consists of three manuscripts that address important research topics related to COPD/ACO patients. This thesis contains eight chapters: chapter 1 is a brief introduction and introduces the thesis background, rationale and objectives; chapter 2 provides a comprehensive literature review on the chronic obstructive lung disease (COPD), asthma-COPD overlap (ACO), inflammatory biomarker, including fractional exhaled nitric oxide (FeNO) in asthma and COPD. Chapters 3 to 5 include the manuscripts, which constitute my thesis. Chapter 3 is regarding full methodology of systematic scoping review called scoping review protocol; chapter 4 is a scoping review (the first scoping study on this topic) and presents results of this comprehensive review; chapter 5 constitutes the results of thesis objectives on FeNO role in different COPD population (Canadian Cohort Obstructive Lung Disease (CanCOLD)). Chapter six summarises the overall findings and final conclusions; chapter seven provides complete reference list, chapter eight contains supplementary materials from scoping review and its protocol in the form of appendix.

Chapters one and two were written and completed by Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi. They were revised according to my supervisor`s, Jean Bourbeau, review, revisions and critical comments.

Chapters three and four are formatted as manuscripts for submission to a peer-reviewed journal according to the respective journal's specifications. The manuscript in chapter three has been re-submitted after revision to the BMJ Open journal on October 17, 2017, and is under revision for a final decision. Figures, tables are provided at the end of each manuscript, except protocol manuscript (embedded in the manuscript according to the BMJ Open journal's specifications). My thesis supervisor, Jean Bourbeau, efficiently and usefully contributed to all stages of the research from the conception and design of the work to an active discussion of results and thoughtful and meticulous manuscript revisions. Pei Zhi Li assisted in data acquisition and imparted valuable support on the statistical analyses.

For the scoping review protocol (Manuscript 1), all authors made substantive intellectual contributions to the development of this protocol. All authors were involved in developing the review questions and the review design. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi

and Jean Bourbeau were involved in writing this manuscript. Nafiseh Naderi, Miriam Barrecheguren, and Abolfazl Dehghan commented critically on several drafts of the manuscript. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi, Nafiseh Naderi, and Jean Bourbeau were involved in conceptualizing this scoping review protocol. All authors approved the final version of the protocol manuscript to be submitted and re-submitted to the journal.

For the scoping review (Manuscript 2), Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi was the lead reviewer and the first author, while Jean Bourbeau and Nafiseh Naderi contributed to the conception, planning, and design of the review. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi was the lead reader/reviewer and Nafiseh Naderi was the second reader/reviewer for the screening of citations, full-text review, study selection and data extraction. Miriam Barrecheguren and Abolfazl Dehghan commented critically on several drafts of the manuscripts. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi wrote, prepared and revised the drafts of the manuscript and incorporated editorial and methodological advice from Jean Bourbeau before approving the final draft to be submitted to a journal.

For the study in CanCOLD (Manuscript 3), which is still ongoing and in preparation, Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi is the lead author, while Jean Bourbeau contributed to the conception, planning, and design. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi gathered data. Data analyses was conducted with Pei Zhi Li. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi, Jean Bourbeau, and Pei Zhi Li have provided advice for the analyses and assisted in the interpretation of results. Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi wrote and prepared the first draft of the manuscript and Jean Bourbeau revised the draft critically and provided editorial advice. Othe co-authors may be added to this manuscript for the purpose of reviewing and commenting on the final version of the manuscript before submitting to a journal.

Chapters six, seven and eight were written, completed by Seyed-Mohammad-Yousof Mostafavi-Pour-Manshadi. They were revised according to my supervisor`s, Jean Bourbeau, review, revision and critical comments.

CHAPTER 1: INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common obstructive pulmonary disease in adult, which is characterized by airflow limitation that is not completely reversible. Asthma-COPD overlap (ACO) syndrome (ACOS) is a distinct clinical phenotype that represents a subset of COPD patients who share features of asthma. It is not known if ACO is the consequence of a unique pathogenic mechanism or the additive result of two distinct chronic airway diseases, i.e., COPD and asthma. Importantly for clinicians, ACO is marked by worse respiratory symptoms and rates of exacerbation and hospital admissions than patients with COPD-alone. Initiation of pharmacotherapy for the treatment of these two diseases is different, patients with COPD-alone should usually be started on long-acting bronchodilators mono or combined therapy and those recognized with ACO should have combined inhaled corticosteroids and long-acting bronchodilators. Therefore, differentiating patients with COPD-alone from those who show asthma-like symptoms is clinically relevant, especially for the need of ensuring close monitoring of ACO patients who have worse outcomes and in guiding treatment decision. There is lack of gold standard for the diagnosis of ACO and diagnostic criteria have often been established primarily based on consensus opinion. There has been a debate to consider biomarkers commonly used in the diagnosis and management of asthma. Fractional exhaled nitric oxide (FeNO) is one of the inflammatory biomarkers that have recently attracted the attention of clinicians as well as researchers. Until further studies can be performed, it is still unclear if FeNO could be used as a tool to the diagnosis of ACO, assisting in the differentiation with COPD-alone and guiding the clinician in personalizing therapy.

The present MSc thesis will contribute by adding evidence to the understanding and potential use of FeNO in COPD patients with ACO. The thesis includes: i) a scoping review regarding the FeNO in COPD/ACO(S) along with its protocol (chapter 3 and 4) and; ii) an observational longitudinal study (embedded in the Canadian Cohort Obstructive Lung Disease, i.e., CanCOLD) regarding the use of FeNO in differentiating COPD and ACO(S) patients and trying to introduce an optimal cut-off value for this purpose (chapter 5).

1.1 Study Rationale, Hypothesis, and Objectives

1.1.1 Study Rationale

Few studies have reported on the use of the FeNO level for monitoring ACO(S) patients undergoing inhaled corticosteroid (ICS) treatment (1). While there have been a number of preliminary studies on measuring FeNO in COPD, literature defining the role of FeNO and the practical cut-off value in patients with COPD-alone and ACO(S) are minimal (2). FeNO has been recommended to be used for the management of asthma. Its main use is for identifying eosinophilic airway inflammation which could assist the clinician in monitoring and evaluating corticosteroid responsiveness for patients with asthma (3). However, the exact role of FeNO in COPD, and more specifically for monitoring ACO and patients undergoing inhaled corticosteroid therapy is still unclear and needs to be defined (1, 4). In summary, the following are the rationale of our study:

- Literature with original studies defining the role of FeNO, its association and the practical cut-off value in patients with ACO(S) and established COPD is minimal;
- There are concerns regarding the applicability and utility of FeNO in clinical practice, especially in phenotyping COPD patients, COPD-alone versus ACO(S).

1.1.2 Central Hypothesis

FeNO as a surrogate marker of eosinophilic airway inflammation could be a useful non-invasive marker to identify in COPD patients those with concomitant asthma, i.e., ACO(S) and therefore, COPD patients that would more likely benefit from treatment with inhaled corticosteroid (ICS).

1.1.3 Objectives

1.1.3.1 General Objectives

1. To review the state of knowledge of FeNO in COPD patients (Manuscripts 1 and 2- Scoping review protocol and Scoping review manuscript).

2. To determine the usefulness of FeNO as a biomarker in differentiating patients with COPD and concomitant asthma, i.e., ACO(S) from those with COPD-alone (Manuscript 3)

1.1.3.2 Specific Objectives

1. Specific objectives **(Manuscript 2- Scoping review)** were to assess

i) factors that modify the level of FeNO, the role of FeNO and useful cut-off value in differentiating ACO(S) from COPD alone; ii) its relationship with other biomarkers commonly used (immunoglobulin E (IgE), blood/sputum eosinophils) and outcomes; and iii) its response to ICS therapy.

2. Specific objectives **(Manuscript 3- Observational longitudinal study)**

Using data collected since 2009 and adding new measurements (FeNO, IgE, blood eosinophils) from the Canadian Cohort Obstructive Lung Disease (CanCOLD), a prospective, multi-center study including COPD individuals based on spirometric GOLD 1+, at risk and healthy control subjects, were:

a. To assess differences of FeNO levels in subjects with COPD according to GOLD 1-4, those at risk and healthy controls;

b. To determine if there are differences in FeNO levels and cut-off value differentiating ACO(S) based on 3 commonly used clinical definitions (def) compared to COPD-alone:

Def1) >12% and >200 ml of increment in the FEV1 post-bronchodilator;

Def2) Physician diagnosis of asthma (as reported in a self-reported questionnaire);

Def3) Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

c. To determine if FeNO level in COPD subjects is associated with disease severity (lung function, exacerbations and health status) and can predict those at risk of future lung function decline (diseases progression).

CHAPTER 2: LITERATURE REVIEW

2.1 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease, which is slow and progressive, characterized by permanent non-completely reversible airflow obstruction primarily in the bronchioles. As well, the COPD patients are characterized by decreased lung function, shortness of breath, reduced capacity, and poor quality of life (5, 6).

Chronic exposure to toxic substances and gases, especially tobacco smoke leads to abnormal inflammatory pulmonary and systemic response causing in susceptible individuals the onset of COPD (7, 8). However, cigarette smoking is considered as the leading cause of COPD, up to 25 percent of COPD patients are those who have never smoked (9). In fact, COPD emerges due to multiple factors, including genetic disorders (the only one known being alpha-1 antitrypsin (AAT) deficiency) and environmental factors (biomass fuel exposure and possibly air pollution) (10, 11). According to the GOLD 2017 (10), "COPD is a preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases." The chronic airflow limitation that is characteristic of COPD is caused by a mixture of small airways disease (e.g., obstructive bronchiolitis) and parenchymal destruction (emphysema), the relative contributions of which vary from person to person.

2.1.1 Epidemiology of COPD

COPD is a crucial challenge in public health and also is a major cause of chronic morbidity and mortality all over the world. COPD is currently the fourth leading cause of death in the world (12) but is predicted to be the 3rd leading cause of death by 2020 (10). The projection for 2020 demonstrates that COPD will be the fifth leading cause of years lost through handicap or early mortality (disability-adjusted life years) (13). In 2012, the death from COPD was 6% of all deaths globally, which means more than 3 million people died due to COPD (10). In coming decades, due to consecutive exposure to many risk factors as well as population aging, COPD burden is anticipated to growth (10, 14). According to the Global Burden of Disease (GBD) studies, COPD led to about 5% of global disability-adjusted life years (DALYs) (76.7 million), and 5% of total deaths (2.9 million) (12, 15, 16). COPD is responsible for high death rates,

early mortality, and significant cost to the healthcare system. While COPD was once more common in men than women, interestingly, it is now being reported more in women than in men under age 75 (17). According to the recent Burden of Lung Disease (BOLD) study using measured post-bronchodilator lung function data (18), the prevalence of COPD among randomly sampled Vancouver residents aged 40 or older was estimated 19%; the prevalence of moderate-to-severe COPD was 8% (19). Similar prevalence of COPD who had a history of smoking, 21 %, was reported by the results of lung function measurements for primary care patients aged 40 or older in Ontario; surprisingly, only one-third of these individuals knew that they had COPD (19). This reflects the current problem of underdiagnosed COPD.

2.1.2 Airway Inflammation and Pathogenic Mechanisms of COPD

Hyperplasia of mucus gland, chronic inflammation, and elevated goblet cells are airway abnormalities have been observed in COPD patients. As well, alveolar wall destruction in emphysema causes loss of tethering that leads to reduced elastic recoil, narrowing, and reduction in the number of small airways along with airway collapse (20). The appearance of CD8+ T-lymphocytes (even CD4+ but CD8+ is more than CD4+), neutrophils, and CD68+ monocytes/macrophages has been observed in the airways of COPD (21-23).

Some lung inflammations are observed in all cigarette smokers, but COPD develops in susceptible smokers due to enhanced or abnormal response to inhaling toxic substances leading to hypersecretion of mucus/sputum (chronic bronchitis), destruction of airway sacs (emphysema), and small airway inflammation and fibrosis (bronchiolitis) due to dysfunction of mechanism of normal repair and defence (21).

Generally speaking, the inflammatory and structural changes that occurred in COPD patients are associated with disease severity. These changes will be persistent even after smoking cessation. An imbalance between proteases and anti-proteases and an imbalance between oxidants and antioxidants (oxidative stress) in the lungs are also other components in the pathogenesis of COPD (21). Moreover, the severity of inflammation in COPD patients is related to the degree of airflow obstruction. During this inflammation process, many cytokines and mediators are released from inflammatory cells.

The inflammatory pattern seen in COPD patients is really different from the one observed in asthma patients (21). In COPD, there is an increase in levels of many inflammatory factors, such as Leukotriene B4, T cell chemoattractants produced by macrophages, neutrophils, and epithelial cells, chemotactic factors, including the CXC chemokines interleukin 8 and growth-related oncogene produced by macrophages and epithelial cells. These cells play a role as a cell attractions and absorb many cells from the circulation and increase pro-inflammatory responses (21).

Oxidative stress is initiated by cigarette smoke and inflammation leading to release a combination of proteases and inactivates several antiproteases from inflammatory cells. The proteases produced by neutrophils (including the serine proteases elastase, cathepsin G, and protease 3) and macrophages (cysteine proteases and cathepsins E, A, L, and S), and various matrix metalloproteases (MMP-8, MMP-9, and MMP-12) are considered to be the most important inflammatory cells. Alpha 1 antitrypsin, secretory leucoprotease inhibitor, and tissue inhibitors of metalloproteases are the main antiproteases in the pathogenesis of emphysema (21). In COPD, there is an increase in oxidative stress response. Cigarette smoke and reactive oxygen and nitrogen species are the sources of oxidants. Inactivation of antiproteases or stimulation of mucous production is due to oxidative stress. As well, oxidative stress can increase the activation of transcription factor (such as nuclear factor B) and therefore, gene expression of pro-inflammatory mediators leading to elevation of inflammation (21).

We may be able to differentiate smokers who do and do not develop COPD by using the presence or non-presence of CD8+ T lymphocytes respectively. Also, an association between T-cell numbers, the amount of alveolar destruction, and the severity of airflow limitation has been observed (21, 24). The mechanism of $CD8^+$ T lymphocytes accumulation in the airways of the lungs of COPD patients is still unclear. In COPD patients, T cells in peripheral airways enhance the expression of CXCR3, a receptor activated by interferon-inducible protein 10 (21). As well, elevated expression of interferon-inducible protein 10 is observed in bronchiolar epithelial cells (21). Moreover, there is an increase in the number of CD8+ cells in the patients with COPD who do not smoke (25). As well as increasing in the number of CD8+ cells in COPD patients, there is an elevation in the number of CD4+ cells in these patients, especially during the disease progression (26). This chronic immune stimulation may be because of colonization of the lower respiratory tract of COPD patients by bacterial and viral pathogens (27). It has been reported that there is an increase in the number of B lymphocytes and

bronchial-associated lymphoid tissue in small airways of COPD patients as they experience progression of their disease (27).

As mentioned before, the role of T cells in the pathogenesis of COPD is not clearly identified yet. There is a potentiality for $CD8^+$ cells to secrete tumor necrosis factor α, perforins, and granzymes, in addition to activating the Fas-Fas ligand apoptotic pathway. There is a relationship between $CD8^+$ cells and apoptosis of alveolar epithelial cells in COPD patients with emphysema (28).

It seems that neutrophils are other crucial inflammatory cells in the pathogenesis of COPD patients. They are able to release serum proteinases such as neutrophil elastase, cathepsin G, and proteinase 3, as well as matrix metalloproteinase 8 (MMP-8) and MMP-9. These proteases can lead to alveolar destruction and also cause strong stimulation of mucus secretion (29). It has been reported that there is an association between neutrophil numbers in bronchial biopsy specimens and induced sputum with the disease severity and the rate of lung function decline (30, 31). In COPD patients, attaching of neutrophils to endothelial cells causes the upregulation of adhesion molecule E-selectin in the airway epithelial cells (32). Then, under the control of chemotactic factors, including leukotriene B4, interleukin 8 (IL-8), and related CXC chemokines such as growth-related oncogene-α and epithelial cell−derived neutrophil attractant 78 neutrophils can migrate to the respiratory tract. In the airways of COPD patients, there is an increase in these chemotactic factors (21, 33, 34).

2.1.3 Clinical Diagnosis of COPD

One crucial aspect of recognizing COPD patients is considering COPD symptoms including dyspnea, chronic cough or sputum production, and/or history of exposure to risk factors for the disease. Another important aspect is a detailed medical history of a new patient who is known, or suspected, to have COPD. Diagnosis of COPD in the clinical setting requires spirometry (10, 18). The diagnosis is defined as the presence of a post-bronchodilator FEV1/FVC < 0.70 confirming persistent airflow limitation in COPD patients with appropriate symptoms and significant exposures to noxious substances. Spirometry is the most reproducible, non-invasive and readily available test for measuring airflow limitation. However, it cannot be considered as a reliable test alone due to its week specificity despite its good sensitivity (10, 35).

2.1.4 COPD Treatment

Smoking cessation is one of the most important and one of the only treatments to prevent COPD and disease progression. (36, 37)

Pharmacotherapy for COPD aims at decreasing symptom burden, reducing the frequency and severity of exacerbations, and improving exercise tolerance and health status. Inhaled bronchodilators are the primary medication with a known effect on FEV1and/or change other lung function parameters, and improvement of symptoms and quality of life (38, 39).

Antimuscarinic drugs are the primary pharmacotherapy for COPD. Short-acting antimuscarinics (SAMAs) include ipratropium and oxitropium and long-acting antimuscarinic antagonists (LAMAs), tiotropium, aclidinium, glycopyrronium bromide and umeclidinium (40, 41). There are also short-acting (SABA) and long-acting (LABA) beta2-agonists that are known to be effective. Combination therapy using LABA and LAMA is recognized to lead to better and higher degree of bronchodilation as well as lower side effects in comparison to increasing the dose of a single bronchodilator (42).

To date, exacerbations are the main reason to administer anti-inflammatory agents such as ICS in combination with long-acting bronchodilator therapy. Indeed, a combination of ICS and LABA in patients with moderate to very severe COPD and exacerbations is more effective than either component alone in improving lung function, health status and reducing exacerbations (43, 44). However, more recently, combination LABA and LAMA has shown to be non-inferior and potentially superior to ICS/LABA in reducing exacerbations (45). There is still a debate in COPD who should be prescribed combination ICS/LABA and combination LABA and LAMA. The risk benefits also need to be considered, the adverse effects of ICS being well recognized over those of the long-acting bronchodilators.

To help reduce potential adverse effects, inhaled delivery of medications is preferred over the oral route. In case the patients are able to get optimal effective delivery of the medications by using an inhaler, a spacer or nebulizer may be beneficial (46, 47). The choice of inhalation devices is also important in the decision of which inhaled medication should be prescribed for a given patient. This choice will be decided based on efficacy and potential for side effects but also based on access, cost, and patient preference.

2.2 Asthma-COPD Overlap (ACO) Syndrome (ACOS)

In real life, COPD patients can have overlapping clinical features of both COPD and asthma which makes it difficult to determine a clear diagnosis of either asthma or COPD (30). For instance, patients with asthma may present features of COPD such as history of smoking, poor response to ICS therapy due to the predominance of airway neutrophilia or evidence of a fixed airflow obstruction (30). In contrast, patients with COPD may demonstrate characteristics related to asthma, including significant reversibility, which is defined as an improvement in lung function test after administration of bronchodilator therapy, and also sputum/blood eosinophilia (30). Due to the exclusion of ACO(S) patients from clinical studies, available evidence regarding their diagnosis, treatment, and the prognosis is still a matter of debate. Recently, the Global initiative for chronic Obstructive Lung Disease (GOLD) and the Global Initiative for Asthma (GINA) have proposed criteria to assess and diagnosed COPD patients with features of asthma, patients having asthma-COPD overlap syndrome (ACOS) (48). However, recently, they have proposed to stay away from a syndrome and they have suggested the new terminology asthma-COPD overlap (ACO) (49). It is generally taken into account that patients with ACO(S) should be considered when they share clinical features of both asthma and COPD (30, 48).

In general, ACO(S) is clinically defined as patients presenting with symptoms of increased variability but incomplete airflow obstruction (50, 51). However, ACO(S) has a variety of operational definitions across studies (52). Unfortunately, there is no consensus on a unified definition or diagnostic criteria for ACO(S). Moreover, a variety of methods have been used to diagnose ACO(S), most commonly it includes but it is not limited to the utilization of self-report concomitant asthma and COPD diagnoses, retrospective identification from chart reviews and/or using the International Classification of Diseases (ICD-9) coding, and the presence of significant bronchodilator reversibility (30).

GINA and GOLD have come up with a definition (48). They proposed eleven clinical features that can be scored (but not weighted) and having these features support the diagnosis of asthma, COPD or ACO(S). These clinical features are as follows: 1) age at the onset of the disease, 2) pattern of symptoms, 3) lung function, 4) lung function between symptoms, 5) past history (previous doctor's diagnosis of asthma or COPD, history of tobacco smoke) and family history of asthma or allergy, 6) time course (seasonal symptoms, improvement after bronchodilator or ICS therapy, progressive worsening over time), and 7) chest radiography (hyperinflation). The

patients demonstrating three or more above COPD or asthma clinical features, in the absence of those for the alternative diagnosis, can be diagnosed as ACO(S). In other words, when a similar number of COPD and asthma features is available, ACO(S) should be diagnosed.

Other studies have suggested having objective measures such as a "large" acute response of FEV1 after bronchodilator therapy, increased sputum eosinophil count or serum IgE, in addition to a combined history of asthma and COPD (53, 54). Although there is heterogeneity and diagnostic criteria remain non-validated, there is an agreement among most guidelines regarding ACO(S). Accordingly, ACO(S) has three major components, including i) the demonstration of persistent airflow limitation in adults equal or more than 40 years old; ii) a significant history of smoking or biomass exposure; and iii) atopy or asthma history (54).

According to GOLD, persistent airflow limitation has been defined as post-bronchodilator FEV1/FVC <0.70 (6). Bronchodilator response is the most controversial aspect of ACO(S); according to the American Thoracic Society (ATS) criterion, a FEV1 or FVC improvement of >200 mL or 12% from baseline values is considered as a significant bronchodilator response (55). However, this cut-off is not able rationally to separate asthma from COPD. For the purpose of improvement in the diagnostic criteria, the use of 15% and 400 mL as the cut-off for patients with only one spirometric measurement or the traditional 12% and 200 mL cut-off for those with multiple measurements has been recommended by the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) (48). Similarly, the GOLD/GINA document (56) recommends the use of 15% and a 400 mL cut-off for ACO(S). However, in order to make a clear difference between asthma and COPD, none of these cut-off values have been exhibited to be successful. Furthermore, in general, bronchodilator response (BDR) in COPD patients is highly variable over time (57). Therefore, the utility of BDR for diagnosing ACO(S) is not clear.

2.2.1 Inflammatory and Pathogenic Mechanisms of ACO(S)

The mechanisms underlying ACO(S) remain controversial. There are two long-standing hypotheses concerning underlying ACO(S) mechanisms, while the "Dutch hypothesis" suggests that asthma and COPD are manifestations of the same basic disease process (58), the "British hypothesis" suggests that asthma and COPD are distinct diseases established by different mechanisms (59). However, some recent studies support both hypotheses (60).

In ACO(S) patients, the scale of the contribution of the underlying mechanisms of COPD and asthma may differ significantly between individuals due to different factors, including genetic predisposition, the initiating and environmental condition, and the alteration of the natural history of each patient (61). Interactions between host and environmental factors stimulate the pathogenic processes, which is similar in COPD, asthma, and ACO(S) patients (62). Studies suggest that asthma is an independent risk factor for COPD (51, 63-66). For instance, a longitudinal, prospective study of 6-7 years children, followed to age 50 years, indicated that risk of developing COPD was remarkably greater in children with asthma than children without asthma (64). Furthermore, another cohort study showed that developing COPD was 12.5 times greater in asthma patients than healthy subjects (65). Finally, the Behavioral Risk Factor Surveillance System Asthma Call-back Survey demonstrated that the prevalence of physician-diagnosed COPD was higher in active asthma patients than in patients with inactive asthma (63). Some asthma patients with long-standing exposure to cigarette smoke and/or biomass smoke or other environmental noxious gases may probably complicate with COPD that may lead to ACO(S) (51, 66-68).

There are several potential pathways that may lead to the manifestation of ACO(S). One of these pathways is defined as exposure of patients with early-onset asthma to cigarette smoke later in life that might lead to the development of fixed airflow limitation and COPD in many of these patients (69). Another potential pathway is described as patients with a history of lifetime smoking, diagnosed as COPD, and manifest late-onset features of asthma later in life (adult-onset eosinophilic asthma and aspirin-exacerbated respiratory disease) (69).

Although there is no clear evidence regarding underlying mechanisms of inflammation in ACO(S), some evidence has shown a strong role of eosinophils, similar to their role in asthma with a Th2-high profile (69). Similarly, mucosal eosinophils increase in acute exacerbations of mild COPD, a feature normally seen in asthma (70, 71). This similarity in inflammatory responses may be one pathophysiologic link to the clinical phenotype of asthma-COPD overlap (ACO) syndrome (ACOS) (71). According to different studies, significant sputum eosinophilia is a predictor of good response to ICS, both in patients with COPD and ACO(S) (72-74). On the other hand, the elevated sputum neutrophils are associated with a worse prognosis in patients with asthma (75); therefore, patients with ACO(S) have some evidence of the Th-1 pattern (characteristic of COPD) and some evidence of Th-2 pattern (characteristic of asthma) (69). Moreover, particularly bronchial infiltrate of CD8+ T cells and CD68+ macrophages, and epithelial remodeling being similar to COPD-like features can be manifested in smokers with

asthma (76). While asthma cytotoxic immune response is represented by granzyme A and B, in smoking asthmatic, perforin and 8-OHdG are additionally involved, being similar to the immune response of COPD (77). Mitochondrial dysfunction due to oxidative stress, which is presented in COPD, also plays a role in the pathogenesis of ACO(S) (78).

Finally, another factor which may be important in the pathogenesis of ACO(S) is genetic. The genetic variants associated with ACO(S) reported in the literature include single nucleotide polymorphisms in the genes CSMD1 and GPR65 (79).

Despite above findings, the exact pathogenic mechanism of ACO(S) is still unclear except the evidence that eosinophils and Th2 profile play an important role in its inflammatory and pathogenic mechanism.

2.2.2 Epidemiology of ACO(S)

2.2.2.1 Prevalence of ACO(S)

The exact prevalence of ACO(S) is unknown. Variable definitions make the accurate assessment of the prevalence of ACO(S) difficult (80). However, an estimated 13-55 % of all COPD patients fulfill potential criteria for ACO(S) (51, 81-85). Some reports suggest that at least 50 % of individuals over the age of 60 with obstructive airway disease (51) and approximately 25 % of adult patients with severe asthma fulfill ACO(S) criteria (86).

Using the Spanish National Consensus Conference criteria defined for identifying ACO(S) (54), Miravitlles et al. (87) observed a prevalence of ACO(S) of 5 % among 279 COPD patients. In the COPD Gene study, physician-diagnosed asthma was reported in 13 % of COPD patients (81). Using a post-bronchodilator FEV1/FVC < 0.7 and asthma diagnosis as ACO(S) criteria in epidemiological studies such as the PLATINO study performed in Latin America, a prevalence of 11.6 % was found (83). In the Spanish EPISCAN epidemiological study, the prevalence of 17.4 % of ACO(S) was reported among the COPD population (88). Other epidemiologic studies report an estimated prevalence of 20% (82, 89).

The prevalence of ACO(S) increases with age, in a similar pattern with the increased prevalence of COPD (63, 90, 91). In a two-stage multicenter study, the prevalence of ACO(S) in the general population was 1.6%, 2.1%, and 4.5% in the age groups of 20–44 years, 45–64 years, and 65–84 years, respectively (90). Furthermore, its prevalence in COPD patients was

reported between 12.1% and 55.2% (51, 91-94), and in patients with asthma, between 13.3% and 61% (95, 96). The prevalence of ACO(S) varies widely depending on the source and the criteria utilized to identify ACO(S). In general, it seems its prevalence ranges between 1.6% and 4.5% in the general adult population and between 15% and 25% in the adult population with chronic airflow obstruction (97).

2.2.2.2 Disease Characteristics and Outcomes

ACO(S) patients have worse lung function, more respiratory symptoms and frequent exacerbations, and a lower health-related quality of life than either COPD or asthma alone (98, 99). They use medical therapy/care as much as 2 to 6 times higher than asthma or COPD patients (100). In addition, they have more air trapping with inspiratory and expiratory identified in computed tomography scans (81, 90).

Clinically, ACO(S) patients are younger than those with COPD but older than those with asthma (101). Their asthma associated features include atopy, wheezing, elevated total IgE levels, allergic rhinitis, positive skin prick testing, and hay fever (74, 90, 100, 102). Furthermore, while COPD patients have neutrophilic airway inflammation, airway inflammation in ACO(S) patients is more eosinophilic (74, 103). Regarding lung function tests, ACO(S) patients demonstrate a higher carbon monoxide diffusing capacity (DLCO) and a more bronchodilator reversibility than COPD patients (74, 83, 103). Computerized tomography (CT) scan of the chest in ACO(S) shows more gas trapping, less emphysema, and greater bronchial wall thickening compared to COPD patients (81, 83). A history of asthma, either self-reported or physician-diagnosed, is strongly associated with ACO(S) (101).

2.2.2.3 ACO(S) vs. COPD (Prognostics and Outcomes)

Compared to patients with COPD-only (81), patients with ACO(S) are often taken into consideration to have different clinical manifestations, with more respiratory symptoms such as dyspnea and wheezing, worse impaired health-related quality of life (HRQL), more frequent COPD exacerbations (104), and more comorbidities (49). However, these differences between COPD and ACO(S) have not been globally reported and it is still debate concerning how ACO(S) might differ from COPD in terms of clinical characteristics and/or prognosis (105).

Differentiating ACO(S) from COPD is crucial due to the significant therapeutic difference between ACO(S) and COPD-only patients. Since ACO(S) patients demonstrate some eosinophilic inflammations (33), they respond better to corticosteroids compared to the more neutrophilic inflammations basically observed in COPD-only patients. Furthermore, the frequency of emergency room visits, intensive care unit admissions and hospital utilization are higher among ACO(S) patients compared to patients with COPD-only (106, 107). Moreover, overall healthcare cost for patients with ACO(S) is almost twice the cost for patients with asthma-only (108).

Regarding the radiological features, ACO(S) patients have similar features to COPD. However, recent studies showed that ACO(S) patients have less emphysema but greater post-bronchodilatation variations in air trapping, compared to COPD patients (109). Furthermore, interstitial changes detected in 23.3% of ACO(S) patients are associated with age and smoking history. In addition, a higher rate of fungal sensitization is observed in ACO(S) with interstitial changes (110). In addition, there is a higher percentage of the total cross-sectional area of pulmonary vessels less than 5 mm^2 in ACO(S) than COPD patients (111).

2.2.3 Treatment of ACO(S)

Although there is some evidence that ICS therapy should be the most remarkable part of therapy in most patients with ACO(S) due to their demonstration of degrees of eosinophilic airway inflammations, the evidence is not enough either to refuse or to prove this kind of therapy in ACO(S) patients (112).

Use of long-acting beta agonist (LABA)/ICS combination therapy is recommended by most studies in ACO(S) patients (97). In addition, an appropriate choice of therapy in patients with more severe symptoms, especially in the presence of frequent exacerbations is the use of “triple” therapy (long-acting muscarinic antagonist (LAMA), LABA, and ICS) (113).

Already in 2007, the Canadian guidelines stated that: “if the asthma component (in COPD) is prominent, earlier introduction of ICS may be justified” (114). Later, in 2010, the Japanese guidelines of COPD (115) indicated that ICS combined with long-acting beta-2 agonists (LABAs) would be the first choice of treatment regardless of the level of airflow obstruction. Very recently, the Spanish guidelines of COPD, the Czech Republic guidelines and the Finnish

guidelines recommend the indication of ICS in the ACO(S) patients in all stages of severity (68, 116, 117).

New treatments for the ACO(S) are evolving. One of them targets a reduction in eosinophilic concentrations such as the anti-IL5 benralizumab, which has shown the promising result in improving FEV1 in patients with the high eosinophilic count, and therefore a decrease in exacerbations (118).

2.3 Biomarkers in Chronic Airway Diseases

The National Institutes of Health defines biomarker as a ‘‘characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention’’ (119).

Respirologists usually use symptoms and pulmonary function tests to diagnose and manage airway diseases; however, this approach is not optimal. Symptoms are difficult to measure and often nonspecific, resulting in misclassification of diagnosis and prognosis (120). Another limitation is related to spirometry, measuring disease severity rather than activity, and associate only weakly with clinical outcomes, including symptom burden, exacerbations, and health status (121). Furthermore, sometimes there is a limitation to access spirometry in some settings as well as requiring a highly trained staff to perform it and interpret data appropriately. A surrogate marker could improve patient care by providing additional information to the clinician. In respiratory medicine, biomarkers have been introduced, which are related to airway diseases such as asthma and COPD. These biomarkers have been more promising in managing asthma than in COPD. They include sputum cell counts, blood eosinophil counts, immunoglobulin E (IgE) and exhaled gases (120, 122-124). On the other hand, for COPD, the identification of biomarkers has been very limited. At best, plasma biomarkers have demonstrated their potentiality use to anticipate the risk of COPD exacerbations and disease severity although their use beyond the clinical assessment of a COPD patient still remains very limited (120).

2.3.1 Biomarkers in Asthma

2.3.1.1 Sputum Biomarkers

One remarkable technique to study respiratory tract in patients with asthma is sputum analysis (125). It is often necessary to perform sputum induction because asthma patients have not productive cough. Due to eosinophilic nature of most asthma patients, it has been recommended applying eosinophil counts to support the diagnosis of this disease (120). Due to cases of non-eosinophilic asthma as well as COPD patients showing eosinophilic predominant; definition of four inflammatory phenotypes of asthma has been established by studies conducting on induced sputum. These phenotypes include eosinophilic, neutrophilic, mixed and paucigranulocytic pattern (126). Other biomarkers, which increase in sputum of asthma patients, include IL-5, IL-17A and IL-25 mRNA (127). Patients having these biomarkers in their sputum are in risk of uncontrolled asthma more than those who have not these biomarkers (127). IL-13 is another biomarker that can be found in the sputum of patients with asthma, which is useful to identify well-controlled asthma patients (128).

2.3.1.2 Blood Biomarkers

Due to asthma pathogenesis, the most widely studied markers consist of B-cell and T-helper 2 (Th2) derived molecules (129). As mentioned before, high blood eosinophil count is an indication of Th2 cell phenotype and may be helpful to anticipate corticosteroid therapy response (129). It has been shown that blood eosinophils have the highest accuracy value suggesting the blood eosinophils assessment may make asthma management easy as well as helping the search for guiding individualized treatment (130).

Periostin is a very promising molecule as a potential biomarker of Th-2 dependent eosinophilic activation (125). Although it has not been utilized in clinical practice nor accurately assessed in other airway diseases (125). It seems that the levels of periostin in blood are associated with airway eosinophilia, even more than blood eosinophil count and IgE levels (131). Furthermore, recently, immunoglobulin E (IgE) as a marker for B-cell activation has taken attention of researchers. Serum immunoglobulin E (IgE) levels are known to be associated with asthma (132).

2.3.2 Biomarkers in COPD

2.3.2.1 Sputum Biomarkers

Sputum is a surrogate of an important diagnostic tool for evaluating airway inflammation in COPD patients (125). It can be either spontaneous or induced by inhalation of hypertonic saline solution (133). The typical feature in the sputum of COPD patients is an increased number of neutrophils. While one study (134) revealed a significant association between neutrophil count and GOLD stage, the ECLIPSE study (135) did not report an association with pulmonary function. At all COPD stages, there are higher levels of neutrophils and macrophages, in later stages, lymphocytes are added to neutrophils and macrophages (136, 137) along with increased sputum concentrations of type 1 CD-8+ T-cells (138). Several pro-inflammatory cytokines such as TNF-a, IL-b, and IL-6 were shown to be elevated in the sputum of COPD patients (139-141).

Interestingly, some COPD patients have increased eosinophil counts. Higher levels of eosinophils seem to be associated with a better responsiveness to corticosteroids and bronchodilators (142). Chemokines play a crucial role in the recruitment of inflammatory cells, especially neutrophils, to the lungs of COPD patients. CCL2, CXCL8, CXCL1, CCL5, CXCL9, CXCL10 and CXCL11 levels markedly increase in sputum of patients with COPD (143, 144). Moreover, CXCL9, CXCL10, and CXCL11 are associated with the disease severity of COPD patients as well (145).

Higher levels of granulocyte macrophage colony stimulating factor (GM-CSF) have been demonstrated in induced sputum cells of COPD patients (146). As well, COPD patients have an increased vascular endothelial growth factor (VEGF) in their induced sputum and its levels are negatively associated with lung function (147).

Myeloperoxidase (MPO) is contained in neutrophils granules and in monocytes (125). Compared to normal controls, the sputum levels of MPO were higher during exacerbations and in stable disease (148). 8-isoprostane is an important prostaglandin isomer, which is associated to the physiopathology of oxidative damage. COPD patients have an elevated 8-isoprostane in their sputum, which is associated with smoking state and the decrease of FEV1 and FEV1/FVC (148).

Matrix metalloproteinases (MMPs) are involved in the destruction of extracellular matrix components (125). One study (149) suggests that MMP-8, which seems to be associated with lung function, can be able to differentiate smokers and who are at risk of developing COPD among chronic smokers. In addition, MMP-9 and MMP-12 increase in symptomatic smokers but none of them are definitely able to differentiate healthy from symptomatic smokers (149).

Leukotriene B4 (LTB4) is a pro-inflammatory leuckocyte, which is an arachidonic-derived molecule released by both neutrophils and macrophages (125). LTB4 can be considered as a reliable predictor of COPD exacerbations (150).

2.3.2.2 Blood Biomarkers

Fibrinogen, interleukin (IL)-6, IL-8, and C-reactive protein (CRP) are the biomarkers of systemic inflammation that have been most broadly studied (151). These biomarkers are able to differentiate patients with COPD from controls with acceptable sensitivity; however, their specificity is low (120). Due to low specificity of these biomarkers and for the purpose of identifying proteins that could be able to better reflex the airway environment, other molecules, including extracellular matrix such as metalloproteinases (MMPs) 8 and 9 and lung-derived markers such as surfactant protein-D (SP-D), Clara cell protein-16 (CC-16) and CCL-18 have also been investigated (151).

SP-D as a promising marker is important in pulmonary system immunity and surfactant homeostasis. COPD patients and smokers have shown higher blood median levels of SP-D (125). Even though SP-D levels are not associated with GOLD stages, peak blood levels seem to be related to the risk of exacerbations and are associated with the extension of emphysema documented by CT scan, and also its progression (152, 153).

Recently, fibrinogen is considered as a promising biomarker in COPD, which is an acute phase plasma protein. Although fibrinogen levels are significantly associated with the number of COPD exacerbations (153, 154), and rate of hospitalizations (155, 156), it is not able to predict lung function decline (154).

CRP is another remarkable plasma biomarker, which is an acute-phase protein and involved in COPD pathogenesis together with other inflammatory molecules such as matrix metalloproteinases (125). There is an association between CRP levels at baseline and lung function decline (125). In addition, increased CRP levels are inversely associated with forced expiratory volume in the first second (FEV1) (125). Similar findings with other biomarkers such as MMP-1, 7 and 9 are reported both in COPD associated with tobacco smoking and biomass smoke exposure (157, 158).

Recently, blood eosinophilia in COPD patients has attracted the attention of researchers as well as clinicians. It has been shown that COPD patients with exacerbation have higher blood eosinophilia than COPD without exacerbations (159). Also, COPD patients with blood eosinophilia experience a higher rate of exacerbation than those without eosinophilia (160). Furthermore, these patients respond better to the corticosteroid than COPD patients without blood eosinophilia (161).

2.3.3 Fractional Exhaled Nitric Oxide (FeNO) as a Biomarker

Many resident cells such as airway, airway epithelial cells, circulatory endothelial cells, and trafficking inflammatory cells within the respiratory tract produce Nitric oxide (NO) (162, 163). Endogenous NO may play a remarkable role in the physiological control of airway function and in the pathophysiology of airway diseases (164). NO is synthesized in response to IL4 and 13 via STAT-6 pathway in the epithelial cells of the bronchial walls (2). Enzyme NO synthase (NOS) converts the amino acid L-arginine to L-citrulline leading to the synthesis of endogenous NO, which includes three distinct isoforms (165). Two isoforms called neuronal (nNOS, type I) and endothelial (eNOS, type III) found in the airway epithelium where they secrete picomolar concentrations of NO, are constitutive and calcium-dependent (163, 166). The third one (iNOS, type II) is induced by several stimuli including endogenous mediators (chemokines and cytokines) and exogenous factors (bacterial toxins, viral infection, allergens, environmental pollutants, etc.) (167) and calcium-independent expressed in vivo in the bronchial epithelial cells in both healthy and asthmatic individuals and its activity elevates during certain inflammatory processes (163). As well, it is expressed in vitro following induction by endotoxins, cytokines, and lipopolysaccharides and its stimulation is blocked by glucocorticoids (168, 169). Nanomolar concentrations of NO, which is stable in the gaseous phase and can be evaluated, is produced by inducible NOS (170). Human pulmonary vasculature, the bronchial tree, and the parenchyma contain NOS isoforms as well (171). In addition, NOS is expressed in the many cell types, including arterial and venous endothelial cells, epithelial cells, mast cells, macrophages, neutrophils, eosinophils, non-adrenergic non-cholinergic nerves, smooth muscle, fibroblasts cells and platelets (171). Recently, fractional exhaled nitric oxide (FeNO) has been the interest of researchers as a potentially useful biomarker for the evaluation of airway inflammation both in undiagnosed patients with non-specific respiratory symptoms and in those with established airway disease (172).

Measurement of FeNO levels can be performed easily and in close to real time by utilizing chemiluminescence, electrochemical detection or laser spectroscopy devices (173). FeNO measurement can recognize patients who respond better to corticosteroid therapy as well as those with T-helper cell 2 (Th2)-mediated airway inflammation (174). As well, FeNO measurement is potential to recognize individuals who will get benefit from future anti-inflammatory treatments, particularly inhaled corticosteroid (ICS) (175-177). In addition, FeNO measurement may provide the ability to monitor and manage the treatment of patients who suffer from inflammatory airway diseases (178).

2.3.3.1 FeNO Measurement and Interpretation

The measurement of the fractional of exhaled nitric oxide (FeNO) is non-invasive, easy and reproducible and is well established in research (2, 179). The standardization of technique has recently been done by American Thoracic Society (3). The FeNO in human breath can be measured easily and rapidly by the NIOX MINO device (Aerocrine, NewYork, USA) in order to assess airway inflammation (3, 180). Exhaled NO is usually measured by chemiluminescence or electrochemical sensing (181). Due to no significant difference in FeNO levels between both devices, the use of handheld analyzers becomes increasing (182).

For the purpose of measurement method, first subjects exhale completely and then inhale through the machine. After inhalation to total lung capacity, patients must exhale immediately into the NO analyzer with the constant speed until a steady plateau is reached. According to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guideline 2005 (183), exhalation should be stable at a targeted flow rate of 50 mL/sec and last for 6-10 seconds. Accordingly, FeNO should be measured twice and a third measurement is required if there is a more than 10% difference between first two measurements (183). In addition, ATS/ERS guideline 2005 (183) also recommend considerations regarding different factors when FeNO is measured. The patient characteristics including age, sex, menstrual cycle, and pregnancy should be recorded at the time of measurement. It is recommended that NO analysis be performed before spirometry as well as other respiratory maneuvers because these maneuvers can lower FeNO. The nasal clip should not be utilized as it can influence closure of the soft palate leading to contamination with NO derived from the nasal cavity. It is recommended to record the time of last bronchodilator administration and some measures of airway caliber, such as FEV1. When it is possible subjects should refrain from eating and drinking for 1 hour before

exhaled NO measurement. Furthermore, it is recommended to perform serial NO measurements in the same period of the day and always record the time. Subjects should not smoke one hour before exhaled NO measurements, and short- and long-term active and passive smoking history should be recorded. If there is an upper and/or lower respiratory infection, FeNO measurements should be deferred until recovery or the infection should be recorded in the chart. The subjects should avoid strenuous exercise for 1 hour before the measurement. Moreover, according to the ATS/ERS guideline 2005 (183), all current medications and the time administered should be recorded.

Regarding the interpretation of FeNO values, according to the ATS guideline 2011 (3), which comes from asthma literature, after correct measurement, and with reference to factors which may be influencing the measurement, interpretation can be made as follows: "FeNO > 25ppb (>20ppb in children): eosinophilic inflammation and responsiveness to ICS (post-bronchodilator FEV1) are unlikely, >50ppb (> 35ppb in children): eosinophilic inflammation is likely; responsiveness to ICS (post-bronchodilator FEV1) is likely." "Values between 25ppb and 50ppb (20–35ppb in children) must be interpreted cautiously with reference to the clinical context. An increase of 20% and more than 25ppb (20ppb in children) may be significant but there are wide inter-individual differences." "A reduction of an elevated FeNO of more than 20% that often occurs 2–6 weeks after initiation of anti-inflammatory therapy supports that the treatment was successful in reduction of inflammation."

2.3.3.2 FeNO in Asthma

As a surrogate of eosinophilic airway inflammation, fractional exhaled nitric oxide (FeNO) is elevated in patients with asthma (184). The presence of eosinophils may be used to direct treatment as patients without eosinophilic inflammation are thought to be less responsive to ICS treatment (185). Furthermore, FeNO can be utilized as a marker to assess the severity of airway inflammation in patients with asthma (186). FeNO is crucial in the recruiting and activation of eosinophilic granulocytes (176, 187). Evaluation of airway inflammation by FeNO may be useful for asthma diagnosis, particularly when bronchial challenges and/or spirometric maneuvers cannot be correctly performed (177). A recent systematic review based on six different randomized control trials (RCTs) emphasize the importance of FeNO monitoring in asthma management especially in disease severity assessment which may be beneficial in controlling severe exacerbations (188).

High FeNO at 50 mL (>45 ppb) has been considered as a marker for steroid therapy response (189, 190) including improvement in spirometry tests such as FEV1 and/or FVC and airway hyperresponsiveness in most mild asthma patients (177). According to the ATS clinical practice guideline for exhaled nitric oxide in asthma, potential easier recognition of eosinophilic airway inflammation and predicting corticosteroid responsiveness are provided by adding FeNO monitoring (191). Moreover, ATS clinical practice guideline recommends using FeNO measurement in the management of asthma patients (191).

2.3.3.3 FeNO in COPD

FeNO levels in COPD are of conflict (167), but it seems that smoking status and disease severity are the most important factors affecting exhaled NO levels in these patients (192). Current smokers (193) and severe COPD patients (particularly in combination with cor pulmonale) (194) show lower levels of exhaled NO than ex-smokers and mild/moderate COPD. Elevated exhaled NO levels have been demonstrated in hospitalized patients during an exacerbation of COPD (195). Interestingly, months after discharge of steroid-treated patients, exhaled NO levels returned to control values showing different inflammatory process in COPD in comparison to the highly steroid-sensitive asthma patients (195). Moreover, exacerbated COPD patients have an increased FeNO levels compared to stable COPD (196)**.** Several studies (197-201) showed that increased FeNO in patients with COPD may be considered as a signal for elevated response to ICS measured with spirometry tests.

In COPD, due to lack of randomized, double-blind, control studies regarding the FeNO, the exact role of FeNO assessment in therapeutic intervention, especially in a clinical setting and for monitoring ACO and those undergoing inhaled corticosteroid therapy, is still unclear and needs to be defined (1, 4, 162). Moreover, literature defining the role of FeNO and the practical cut-off value in patients with ACO and established COPD is minimal (2). Moreover, there is no comprehensive review, neither a scoping nor a systematic review regarding role of FeNO measurement in COPD/ACO. For the sake of conducting a review to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize findings from different studies, as this topic of FeNO in COPD covers a wide range of research questions and because of its exploratory nature, a scoping review is more appropriate.

CHAPTER 3: MANUSCRIPT 1: INVESTIGATING FRACTIONAL EXHALED NITRIC OXIDE (FENO) IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AND ASTHMA-COPD OVERLAP (ACO): A SCOPING REVIEW PROTOCOL

The following manuscript is the protocol for conducting a scoping review regarding investigating the role of FeNO in COPD/ACO(S) patients. The full methodology of scoping review has been stated in this protocol. This protocol has been revised according to the journal's reviewers' comments and re-submitted to the BMJ Open journal on October 17, 2017 and is under the review for a final decision (Mostatavi-Pour-Manshadi, Naderi, Barrecheguren, Dehghan, Bourbeau, 2017). It has been formatted according to the journal's specification.

Title:

Investigating Fractional Exhaled Nitric Oxide (FeNO) in Chronic Obstructive Pulmonary Disease (COPD) and Asthma-COPD Overlap (ACO): A Scoping Review Protocol

Authors:

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Abstract

Introduction

During the last decade, many articles have been published, including reviews on fractional exhaled nitric oxide (FeNO) utility in clinical practice and for monitoring and identifying eosinophilic airway inflammation, especially in asthma, and evaluating corticosteroid responsiveness. However, the exact role of FeNO in patients with chronic obstructive pulmonary disease (COPD) and its ability to distinguish COPD patients and those having concomitant asthma, i.e., asthma-COPD overlap (ACO) is still unclear and needs to be defined. Due to the broad topics of FeNO in chronic airway disease, we undertook a scoping review. The present article describes the protocol of a scoping review of peer-reviewed published literature specific to FeNO in COPD/ACO over the last decade.

Methods and Analysis

We utilized Joanna Briggs Institute Reviewers'Manual scoping review methodology as well as Levac et al's and Arksey et al's framework as guides. We searched a variety of databases, including Medline, EMBASE, CINAHL, Cochrane Library, Web of Science, BIOSIS on June 29, 2016. Additional studies will be recognized by exploring the reference list of identified eligible studies. Screening of eligible studies will be independently performed by two reviewers and any disagreement will be solved by the third reviewer. We will analyze the gathered data from article bibliographies and abstracts.

Ethics and Dissemination

To investigate the body of published studies regarding the role of FeNO in COPD patients and its usefulness in the clinical setting, a scoping review can be utilized as a modern and pioneer model, which does not need ethics approval. By this review, new insights for conducting new research specific to FeNO in COPD/ACO population will emerge. The results of this study will be reported in the scientific meetings and conferences, which aim to provide information to the clinicians, primary care providers, and basic science researchers.

Strengths and Limitations of This Review

- To the best of our knowledge, this will be the first scoping review undertaken on FeNO and COPD patients; the intent of our scoping review will be to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize and aggregate findings from different studies.

- This scoping review will include all languages but it will be limited to the year 2005 onwards, as this was the year that the first ATS/ERS guideline regarding FeNO measurement was published.
- Due to the nature of this review, i.e., a scoping review, it will mainly enable to identify where research is lacking and a better determination of the feasibility of a systematic review.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common obstructive pulmonary disease, which is characterized by airflow limitation (202, 203). Asthma-COPD overlap (ACO) (49) syndrome (ACOS) (204) is a distinct clinical phenotype that represents a subset of COPD patients who share features of asthma (12). Initiation of pharmacotherapy for the treatment of these two diseases is different (107), patients with COPD alone should usually be started on bronchodilators mono or combined therapy and those recognized with ACO should have combined bronchodilators and inhaled corticosteroids (49, 205). Therefore, differentiating patients with COPD alone from those who show asthma-like symptoms is clinically relevant, especially for the need of ensuring close monitoring of ACO patients who have worse outcomes and also in guiding treatment decision.

There is a lack of gold standard for the diagnosis of ACO (51, 203), and diagnostic criteria have often been established primarily based on consensus opinion. Fractional exhaled nitric oxide (FeNO) is one of the inflammatory biomarkers that have recently attracted the attention of clinicians as well as researchers. FeNO can be measured noninvasively, fast, reproducibly, and in an easy way in close to real time (172, 206). It is suggested using FeNO for the management of asthma and also for monitoring airway inflammation, identifying eosinophilic and T-helper cell 2 (Th2)-mediated airway inflammation and evaluating corticosteroid responsiveness during asthma follow-up (3, 207). The exact role of FeNO in COPD, and more specifically for monitoring ACO and patients undergoing inhaled corticosteroid therapy is still unclear and needs to be defined (1, 4). Moreover, literature defining the role of FeNO and the practical cut-off value in patients with ACO and established COPD is minimal (2).

Our preliminary search showed no comprehensive review, neither a scoping nor a systematic review with a view of the role of FeNO measurement in patients with COPD and/or ACO.
As this topic of FeNO and COPD covers a wide range of potential questions and because of its exploratory nature, a scoping review will be conducted. The intent of our scoping review is to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize and aggregate findings from different studies. We are considering specific questions/objectives to guide our review but through our search of the literature, we may have opportunities to refine some of these questions. The objectives of this scoping review will be i) to investigate COPD patients' factors that can modify FeNO measurements including but not limited to age, cigarette smoking, sex, glucocorticoids (ICS/GCS), bronchodilators, and

exacerbations; ii) to evaluate the FeNO role and if a useful cut-off value can be used in differentiating COPD patients from healthy individuals; iii) to determine the relationship of FeNO with disease severity and/or progression (lung function, health status, and exacerbations); iv) to assess the role of FeNO and if a useful cut-off value can be used to differentiate patients with COPD-only from those with concomitant asthma (ACO); v) to determine the relationship of FeNO with inflammatory markers (Immunoglobulin E (IgE), blood/sputum eosinophils) and; vi) to assess the utility of FeNO measurement in treatment response of COPD/ACO patients, especially inhaled corticosteroid (ICS)/glucocorticoids (GCS) therapy with or without inhaled bronchodilators.

Methods

Different types of systematic approaches available for reviewing published literature have been taken into account and eventually, a scoping review of peer-reviewed published articles was selected as the most appropriate method. This scoping review will provide the readers and researchers with an overview of the topic, determining key concepts, and exploring gaps within a developing field of research (208). Compared to a systematic review, the research questions defined for a scoping review are broader than for a systematic review (208). A scoping review is appropriate for the topic of FeNO in COPD because the purpose of this study is to have a comprehensive review in an area that is relatively complex. However, there are limitations regarding scoping reviews. These limitations include missing some relevant studies (209) which is related to the database search, exclusion of gray literature (209), lack of critical quality appraisal of included studies, and therefore difficulty in addressing the gaps in the evidence base (209, 210), and limitation of depth of analysis (211, 212). It would be a huge challenge to assess quality among the wide range of study designs and a large volume of literature that will be included in the scoping review. The balance between breadth and depth of analysis is also a challenge (209). To minimize this, we are planning to aggregate findings from different studies under themes and synthesize the data under each of these themes.

This study will be conducted as per the methodology outlined in the Joanna Briggs Institute Reviewers'Manual (213) and reported as per the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement (214). It is also according to the Levac et al's (215) and Arksey et al's (208) framework for a scoping review.

Eligibility Criteria

To be eligible, studies of all languages, from 2005 onwards and including n>10 will be considered. Diagnosed COPD/ACO patients according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (216) and GOLD-Global Initiative for Asthma (GINA) (205), respectively will be included. Any intervention will be taken into consideration, except the ones that have no focus on FeNO measurement. Table 1 shows inclusion and exclusion criteria for selecting eligible studies, i.e. types of study, participants and outcomes.

Table 1. Inclusion and exclusion criteria for selecting eligible studies of the scoping review

Inclusion criteria	**Exclusion criteria**
Type of study	
Randomized clinical trials (RCT), cohorts, longitudinal studies, cross-sectional studies	Reviews, letters, reports, comments, opinions, editorials, case studies and case series conferences, and meeting abstracts as well as other non-peer-reviewed abstracts/articles, gray literature
Participants	
COPD and/or asthma-COPD overlap	Other pulmonary diseases such as asthma
Outcomes	
Clinical usefulness and reproducibility of FeNO alone or combined with other inflammatory biomarkers	Without any focus on FeNO

COPD: Chronic obstructive pulmonary disease; FeNO: Fractional exhaled nitric oxide

Information Source (Databases), Literature Search and Search Strategy

A structured comprehensive literature search was conducted in major databases including Medline (via OvidSP), EMBASE (via OvidSP), CINAHL (via EBSCO host), Cochrane Library (via Wiley Online), Web of Science, BIOSIS Previews, BIOSIS Previews Archives on June 26, 2016 and an updated search was performed on June 29, 2017. Additional studies will be recognized by exploring the reference list of identified eligible studies. The first inception of database searches was conducted without date limitation but it will be limited to the year 2005 onwards as this was the year when the ATS/ERS (American Thoracic Society/European Respiratory Society) guideline (183) concerning FeNO and its measurement was published. There is no limitation regarding language in this search strategy. We used a variety of

keywords/text words and database subject heading such as [COPD OR Chronic Obstructive Lung Disease OR Emphysema OR Chronic Bronchitis OR ACOS OR Asthma-COPD Overlap Syndrome OR Concomitant asthma] AND [FeNO OR Fractional Exhaled Nitric Oxide].

Study Selection

Two reviewers (SMY Mostafavi-Pour-Manshadi and Nafiseh Naderi) will independently review the title and abstract of retrieved articles from the database searches for the purpose of screening. Then, the full text of potential articles, which will be retrieved from first screening, will be investigated as the second screening. Discrepancies will be solved by reaching the consensus between two reviewers according to the criteria eligibility. If the two reviewers could not reach the consensus concerning the specific article(s) or both were suspicious about including/excluding the articles, these papers will be reviewed by the third reviewer (Jean Bourbeau) and the issue will be solved.

Data Extraction

Data collection/extraction will be done by using a designated data extraction form and gathered electronically. We will use PICOS (214, 217) approach for designing the form and extracting data as well, which will be developed from our research questions. The form will be reviewed and revised again by the reviewers after completing to reach the consensus among reviewers. Data extraction will be independently done by two reviewers (SMY Mostafavi-Pour-Manshadi and Nafiseh Naderi). The data will include study title, first author's name, publication year, the name of the journal, sample size, sample description, setting description, and outcomes. Concerning outcomes, the data will be as follows but not limited to FeNO values, eosinophil level/IgE in sputum and/or in blood, pulmonary function tests, computed tomography (CT) scan findings, and exacerbations (symptoms-based or evidence-based, i.e. requiring antibiotics or non-inhaled/systemic corticosteroids, emergency or hospital admission). The information from the studies will be summarized by producing descriptive summary tables. Table 3 shows the data extraction framework.

Table 3. Data extraction framework of the scoping review

Bibliometrics	**Comments**
• Author(s)	• First author, et al.
• Title	• Full title
• Year	• Year of Publication
• Country	• Country of conducted study
Data extraction	
• Aim of study	• Full aim, regardless of our research questions
• Design of study, if applicable	• Type of study
• Intervention, if applicable	
• Methods	
• Setting/Sample of study	• Outpatient or inpatient or as described by the author(s)/ population number (N) in analysis (including N in total/COPD if it is different from N in analysis)
• COPD population characteristic, if applicable	• Number of COPD patients, mean /median age or range of age, gender, BMI, smoke pack-year, exacerbation
• Results	• Overall results and specific ones in regard to our study
• Conclusions/Key findings	• Overall and specific to our study
• Research gaps	• As identified by author(s)
• Future recommended studies/research	• As suggested by the author(s)

FeNO: Fractional exhaled nitric oxide; COPD: Chronic obstructive pulmonary disease; BMI: Body mass index

Then the findings will be given in an explanatory and a narrative review and briefed in a table to make the comparisons of different studies easy. The replication of studies' results and their differences will be considered and reported. The results classification will be performed according to the studies' findings and other relevant indicators of interest. This scoping review will provide a comprehensive overview of FeNO utility and validity in describing patients with COPD and/or ACO. In addition, it will provide a new practical model to combine a variety of research articles specific to FeNO in COPD/ACO. We expect to report the results in early 2018. Reviewing and analyzing this large amount of peer-reviewed published literature as a scoping review may expose new needs and directions for FeNO research in COPD/ACO.

Quality Assessment of Included Studies

In accordance with scoping review guidance (32), we did not appraise methodological quality or risk of bias of the included articles. This approach is consistent with scoping reviews of clinical topics (218, 219).

Ethics and Dissemination

As this study is a scoping review, there will be no need for formal ethical review. The scoping review will be presented at a relevant conference and be published in a peer-reviewed journal.

CHAPTER 4: MANUSCRIPT 2: INVESTIGATING FRACTIONAL EXHALED NITRIC OXIDE (FENO) IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AND ASTHMA-COPD OVERLAP (ACO): A SCOPING REVIEW

The following manuscript is a scoping review that summarises the current evidence on the FeNO in COPD/ACO(S) patients. The goal of this scoping review is to investigate factors modifying FeNO measurement. As well to study FeNO associations, and its utility to differentiate COPD and ACO(S), anticipate treatment response, disease severity/progression in COPD/ACO(S) patients. This paper summarized results in qualitative form and identified gaps within the literature concerning different aspects of FeNO in a clinical setting or research. The manuscript is in preparation to be submitted to a very famous journal with high impact factor within November 2017. It has been formatted according to the journals' specification.

Title:

Investigating Fractional Exhaled Nitric Oxide (FeNO) in Chronic Obstructive Pulmonary Disease (COPD) and Asthma-COPD Overlap (ACO): A Scoping Review

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is the most common obstructive pulmonary disease identified by airflow limitation. COPD patients may demonstrate clinical features of both asthma and COPD in what has been called asthma-COPD overlap syndrome (ACOS), and more recently asthma-COPD overlap (ACO). Compared with asthma or COPD alone, ACO(S) patients are more severe and have a worse health-related quality of life. There is a need for a biomarker that could be used in clinical practice to differentiate ACO(S) from COPD. One of these promising biomarkers is fractional exhaled nitric oxide (FeNO). The exact role of FeNO in patients with ACO(S) and established COPD still remains unclear. Even though there are a number of preliminary studies regarding FeNO measurement in COPD, literature describing the role of FeNO and the practical cut-off value in patients with ACO(S) and COPD are minimal.

Aim: The goal of this scoping review is to investigate factors affecting FeNO measurement, FeNO associations, and its utility to differentiate COPD and ACO(S), anticipate treatment response and/or disease severity or progression in COPD/ACO(S) patients.

Methods: A structured comprehensive literature search was performed in major databases including Medline, EMBASE, CINAHL, Cochrane Library, Web of Science, and BIOSIS from 2005 onwards.

Results: Thirty-eight studies were retrieved after final review. From which, 24 articles on modifying factors in FeNO measurement, N=18 on FeNO in COPD or in COPD compared to healthy subjects, N=22 on FeNO and disease severity or progression, N=7 on FeNO and ACO(S), N=12 on FeNO and biomarkers, and N=8 on FeNO and treatment response.

Conclusion: FeNO measurement cannot be used alone in the clinical settings of COPD patients. Conducting more studies, especially randomized clinical trials with a large number of subjects is crucial to reach more precise results regarding the association between FeNO with exacerbations, eosinophils and treatment response, COPD/ACO(S) as well as defining the almost unique FeNO cut-off values for each of these associations.

Introduction

Chronic obstructive pulmonary disease (COPD) is the most common obstructive pulmonary disease (107, 220). The cause of COPD is chronic exposure to noxious particles or gases, mainly tobacco smoke and is not generally recognized below the age of 40 (1, 4, 220, 221). COPD is an inflammatory disease with persistent, progressive, and incomplete reversible airflow limitation, defined by the post-bronchodilator ratio forced expiratory volume in 1 second over forced vital capacity (FEV1/FVC) below 0.7, or below the lower limit of normal (202, 203). COPD is currently the fourth most common specific cause of death globally and anticipated to be the third by 2030 (14, 16, 222). According to the Global Burden of Disease Study 2010, COPD led to about 5% of global disability-adjusted life years– DALYs (76.7 million) – and 5% of total deaths (2.9 million) (12, 15).

Individuals may present with clinical features of both asthma and COPD. This condition is called asthma-COPD overlap syndrome, as reflected in the Global Initiative for Asthma/Global Initiative for Chronic Obstructive Lung Disease (GINA/GOLD) statement and (204) other guidelines (54, 203, 223). Recently it has been suggested to be called asthma-COPD overlap (ACO) (49). Prevalence of ACO(S) has been reported as low as 15% and as high as 60% according to different population samples, age groups, and definitions (203, 224). There is still no consensus on a definition of ACO(S). However, compared with asthma or COPD alone, there is an association between ACO(S) using different definitions and more frequent exacerbations (81, 225), worse health-related quality of life (81, 226), increased hospital admissions (225, 227), and higher health care costs (106). To date, the diagnosis of ACO(S) is based on questionnaires and doctor's personal opinion as well as defining some minor and major criteria, but there is no agreement regarding these criteria (54, 107, 203). Finally, we know very little about the treatment of ACO(S) since these patients have generally been excluded from both asthma and COPD studies (71, 203, 228) and drug trials (51, 107).

There is a need for a biomarker that could be used in clinical practice to differentiate ACO(S) from COPD. The measurement of fractional exhaled nitric oxide (FeNO) can assess airway inflammation, and thus, the American Thoracic Society clinical practice guideline recommends its use to manage and monitor asthma (3). However, the exact role of FeNO in patients with ACO(S) and COPD remains to be defined (4). FeNO is produced in the catalysis of nitric oxide synthase in different kinds of respiratory epithelial cells, inflammatory cells, and vascular endothelial cells. It is used as a known marker of airway hyperresponsiveness, the total number of inflammatory cells in the airways, eosinophilic airway inflammation, and T-helper cell 2

(Th2)-mediated airway inflammation (172, 229, 230). It is measured via a fast, noninvasive, reproducible, and easy way in close to real time by utilizing electrochemical detection, chemiluminescence, or laser spectroscopy devices (172, 206). A high level of FeNO is associated with eosinophilic inflammation (3, 188). Therefore, FeNO has been used clinically for detecting eosinophilic airway inflammation, monitoring airway inflammation in asthma, evaluating corticosteroid responsiveness, and as a management tool of asthma (3, 207). However, few studies have reported on the use of the FeNO level for monitoring ACO(S) patients undergoing inhaled corticosteroid (ICS) treatment (1). While there have been a number of preliminary studies on measuring FeNO in COPD, literature defining the role of FeNO and the practical cut-off value in patients with COPD and ACO(S) are minimal (2).

As this topic of FeNO in COPD covers a wide range of research questions and because of its exploratory nature, we decided to conduct a scoping review. The goal of this scoping review was to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize and aggregate findings from different studies. The research questions and the studies answering the related questions are presented in Table 1. The composition of research on this topic will help researchers to find out the current state of the evidence and determine areas for future research.

Methods

This study was conducted as per the methodology outlined in the Joanna Briggs Institute Reviewers'Manual (213) and reported as per the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement (214). It is also according to the Levac et al's (215) and Arksey et al's (208) framework for scoping review. According to Arksey's (208) recommendations the scoping review process included the following six key steps: 1) detection of the research question, 2) detection of relevant studies, 3) study selection, 4) charting the data, 5) gathering, summarizing, and reporting the results and 6) consultation which is optional.

Eligibility Criteria, Participants, and Type of Study

Studies of all languages were considered, from 2005 onwards and including n>10, those using FeNO measurement and having patients diagnosed as COPD and/or asthma-COPD overlap (ACO) Syndrome (ACOS). The randomized clinical trials (RCT), cohorts, cross-sectional, and longitudinal studies were only included.

Information Source (Databases), Literature Search and Search Strategy

A structured comprehensive literature search was conducted in major databases including Medline (via OvidSP), EMBASE (via OvidSP), CINAHL (via EBSCO host), Cochrane Library (via Wiley Online), Web of Science, BIOSIS Previews, BIOSIS Previews Archives on June 26, 2016. The inception of database searches was without date limitation but, then it was limited to the year 2005 onwards as this was the year when the ATS/ERS (American Thoracic Society/European Respiratory Society) guideline concerning FeNO and its measurement was published (183). We performed the new search on June 27, 2017, as updated literature search. No systematic and scoping reviews were retrieved through these database searches (previous and current search). We used a variety of keywords/text words and database subject heading such as [COPD OR Chronic Obstructive Lung Disease OR Emphysema OR Chronic Bronchitis OR ACOS OR Asthma-COPD Overlap Syndrome OR Concomitant asthma] AND [FeNO OR Fractional Exhaled Nitric Oxide]. Details of the search strategy are available as a supplementary file.

Study Selection

Two reviewers (SMY Mostafavi-Pour-Manshadi and Nafiseh Naderi) independently screened the title and abstract of retrieved articles from the database searches. Then, the full text of potential articles retrieved from first screening was investigated as the second screening. Discrepancies were solved by reaching the consensus between two reviewers according to the criteria eligibility. If the two reviewers could not reach the consensus concerning the specific article(s) or both were suspicious about including/excluding the articles, these papers were reviewed by the third reviewer (Jean Bourbeau) and the issue was solved.

Data Extraction

Data collection/extraction was done by using a designated data extraction form and gathered electronically. We used PICOS (214, 217) approach for designing the form and extracting data, which was developed from our research questions. The form was reviewed and revised by the reviewers after completing to reach the consensus among reviewers. Data extraction was independently done by two reviewers (SMY Mostafavi-Pour-Manshadi and Nafiseh Naderi).

The data included study title, first author's name, publication year, the country of conducted study, the name of the journal, the purpose of the study, FeNO measuring method, sample size, sample description, setting description, and outcomes. The information from the studies was summarized by producing descriptive summary tables.

Quality Assessment of Included Studies

We did not appraise methodological quality or risk of bias of the included articles due to nature of this work, which is scoping review (213). This approach is consistent with scoping reviews of clinical topics (218, 219).

Results

According to the PRISMA diagram (Figure 1), 2596 articles from 2005 onward were screened, 2516 articles were excluded due to irrelevant either topic or abstract or according to the exclusion criteria. Finally, 80 articles were selected for review of the full text and 38 articles were chosen to be included in the final scoping review. Based on the synthesis of the reviewed literature, six themes on FeNO and COPD emerged. Table 1 presents the themes, the specific review questions, and the included articles. Thirty-four articles covered more than one theme.

Factors Modifying FeNO Measurement

The studies (N=24, 63.15%) included in this theme are presented in Table 2. One of the most frequently tested factors was cigarette smoking. Most studies showed a decreased FeNO with current smoking (201, 231-237), only one study (238) reported an increased and one (239) showed no association. Relationship with inhaled corticosteroid (ICS)/systemic corticosteroid (GCS) (either intravenous or oral therapy) was also frequently tested. Five studies (1, 201, 240-242) showed a decreased FeNO and 5 studies (234, 237, 239, 243, 244) found no association. Two studies (245, 246) reported decreased FeNO with inhaled combination of ICS/ long-acting beta2 agonist (LABA) and one study (232) showed no association. Only one study (247) reported on exercise (6-minute walk test) showing that it is associated with a decreased FeNO. One study reported and showed that sodium bicarbonate rinse mouth could decrease FeNO (248). All the studies that assessed exacerbations (3/3) reported that exacerbation is associated with an increased FeNO (232, 242, 249). Cold weather and viral infection have been reported as potential factors to increase FeNO (232). Finally, two studies reported on sex and

FeNO, one showing a decreased FeNO in females (235) and one no association (201); and three studies reported on age and FeNO, one showing an increased FeNO with older age (242), one no association (234), and one negative association (163).

FeNO in COPD or in COPD Compared to Healthy Subjects

The studies (N=18, 47.36%) included in this theme are presented in Table 3. Thirteen studies reported an increased FeNO in COPD and/or ACO(S) (1, 2, 179, 206, 233, 237, 239, 242, 246-251), 2/2 studies in ACO(S) (1, 246), and 11/16 in COPD (2, 206, 233, 237, 239, 242, 247-251). In contrast, only one study (236) showed a reduction in FeNO levels in COPD patients compared to those with no airway disease and 4 studies (231, 234, 252, 253) reported no change/difference in FeNO levels among COPD patients or between COPD patients and age-matched healthy subjects. For most studies, COPD and/or ACO(S) patients were compared to healthy subjects. In one study (242), FeNO was compared within COPD GOLD groups showing an increase from GOLD A to GOLD D. There were no cut-off values assessed to differentiate COPD and healthy subjects.

FeNO and Disease Severity and/or Progression

The studies (N=22, 57.89%) included in this theme are presented in Table 4. Twelve studies were on disease severity, from which, 6 studies assessed the disease severity by GOLD (2, 4, 206, 207, 234, 252), 5 studies by exacerbations (232, 233, 243, 249, 253) and one by both (242).

For those studies using either GOLD airflow obstruction severity (I-IV) or GOLD 2011 risk assessment (ABCD), only one study (242) reported an association between GOLD 2011 (ABCD) (increased FeNO from GOLD A to D) and FeNO levels while 6 studies (2, 4, 206, 207, 234, 252) showed no association. Moreover, in the only study (242) showing an association with GOLD 2011 (ABCD), it was shown that COPD patients with high FeNO levels who were not on corticosteroid had a significant increase in hospital care (need for corticosteroids or bronchodilators or admission to the Intensive Care Unit) compared to those who had low FeNO levels. For the studies using exacerbations to evaluate the severity of the disease (6 studies), 4 studies (233, 242, 243, 249) showed that COPD patients with higher FeNO levels had an increased frequency or number of exacerbations per year. On the other hand, 2 studies (232, 253) showed no association between FeNO levels and exacerbations.

Fifteen studies assessed disease progression. These studies used pulmonary function tests to assess disease progression. Twelve studies reported no association between FeNO levels and pulmonary function tests (163, 207, 232-234, 240, 241, 245-247, 249, 254) while two studies showed an association (1, 250). One study (201) reported both the association and no association, i.e., an association between FeNO with FEV1 and FEV1% predicted while no association between FeNO and FVC (Table 4). From studies showing an association-only, one (1) study showed a negative association between FeNO with both FEV1% predicted and FEV1/FVC while another study (250) showed a negative association between FeNO levels and FEV1/FVC in COPD/ACO(S) patients.

FeNO and Asthma-COPD Overlap (ACO) Syndrome (ACOS)

The studies (N=7, 18.42%) included in this theme are presented in Table 5. Higher levels of FeNO in ACO(S) patients compared to those with COPD-only were observed in all studies. Four studies (4, 206, 244, 254) showed that FeNO can be useful to differentiate ACO(S) from COPD and introduced optimal cut-off values of 19, 22.5, 23, and 29 ppb with a sensitivity of 68, 70, 73, 80%, and specificity of 75, 75, 68.2, and 73%, respectively. The area under the curve (AUC) for the cut-off 19, 22.5, 23 and 29 ppb was 0.79, 0.78, 0.74, and 0.85, respectively (4, 206, 244, 254). On the other hand, one study (255) indicated that FeNO measurement cannot differentiate ACO(S) from COPD. In that study, although FeNO level was higher in ACO(S), especially in former smokers, compared to COPD alone, the AUC for the cut-off was 0.63. One study (207) used the cut-off value of 35 ppb (proposed by other studies) to determine the prevalence of ACO(S) among COPD patients. Using this cut-off value, the prevalence of 16.3% was reported for ACO(S) among COPD patients.

The studies used not only different cut-off values but also different FeNO measurement devices, sample size and definitions of ACO(S) subjects. Concerning ACO(S) definitions, history of asthma was included in all of these definitions, except one (207). Two studies used GINA definition (244, 254), from which, one (254) used the GINA-GOLD 2014 (204) and the other (244) used GINA-GOLD joint document (updated 2015) (205). Two studies used both major and minor criteria (206, 255). ACO(S) was diagnosed if there was either one major criterion or two minor criteria. Major criteria were defined in one of these studies (206) as a previous history of asthma/wheezing outside chest infections or an increased FEV1 >14% and >400 mL post-bronchodilator; in the other study (255) as a previous history of asthma and bronchodilator response (increased FEV1 ≥15% and 400 mL). Minor criteria (206, 255) were

as follows: positive bronchodilator response defined as ≥12% and/or 200 mL gain in FEV1, elevated blood eosinophil count, elevated IgE levels, history of atopy, hay fever or history of sensibilization to neumoalergens. Other studies (4, 256) used one minor criterion (positive bronchodilator test) and history/diagnosis of asthma or one minor criterion (elevated blood eosinophils) only (in diagnosed COPD) or chronic airflow limitation and a smoking history ≥ 20 pack-years (in diagnosed asthma patients) to define ACO(S). One study (207) used high levels of fractional exhaled nitric oxide (FeNO > 35 ppb) or elevated immunoglobulin E (IgE ≥ 173 IU/mL) as candidate markers of ACOS in COPD.

FeNO and Inflammatory Biomarkers (Sputum/Blood Eosinophils and IgE)

The studies (N=12, 31.57%) included in this theme are presented in table 6. Ten studies reported on sputum eosinophils and FeNO levels, 9/10 showing an increased FeNO (1, 163, 179, 235, 240, 246, 251, 257, 258) and one study showing no association (259) between COPD/ACO(S) patients with elevated sputum eosinophils and those without elevated sputum eosinophils. Elevated sputum eosinophils was defined in the studies as ≥ 2.5% (179) or ≥3% (258) or > 3% (257). Other studies did not define sputum eosinophilia (1, 163, 235, 240, 246, 251, 258). Four studies reported on blood eosinophils and FeNO, Two studies (163, 236) showed a relationship between FeNO levels with blood eosinophils (elevated blood eosinophil count not defined), and two studies (179, 256) reported no association (either elevated blood eosinophils ratio or count defined as blood eosinophil count ≥ 1% (179) or ≥200 eosinophils·μL^{-1}) (256). Four studies reported on IgE, all studies (1, 236, 246, 257) showed a relationship between IgE and FeNO levels.

Optimal cut-off was presented in three studies (179, 257, 258), 17.5, 19 and 23.5, to identify sputum eosinophilia (elevated sputum eosinophils). The area under the receiver operating characteristic (ROC) curve (AUC) for the cut-off 17.5 and 19 ppb was 0.61 and 0.89, respectively. There was no report of AUC for the cut-off 23.5 ppb. The cut-off values of 17.5, 18, and 23.5 had a sensitivity of 64.5, 90 and 62.1%, and specificity of 56.4, 74, and 70.5%, respectively. The study with the cut-off 23.5 showed that the exhaled nitric oxide (eNO) >23 ppb could be a good prediction of sputum eosinophilia (sputum eosinophil count > 3%).

FeNO and Treatment Response

The studies (N=8, 21.05%) included in this theme are presented in Table 7. Only one study was RCT (240). Treatment response was defined as an increase in FEV1 > 12% and 200 mL in two studies (201, 258), ≥ 200 mL in one study (241), and > 200 mL in another study (245). Three studies did not define the treatment response (1, 259, 260). Five of the eight studies reported an association between FeNO levels in COPD and response to treatments, one study (241) with ICS, one with GCS administered either intravenous or orally (240) and three with inhaled combination ICS/bronchodilator (201, 245, 258). One study (1) reported an association with FeNO levels in ACO(S) with ICS therapy. According to this study (1), no significant difference reported between mild and moderate ACO(S) with healthy subjects after 6 months ICS therapy while FeNO increased in severe and extremely severe ACO(S). ACO(S) and its severity were defined according to the GINA-GOLD 2014 (204) and GOLD 2011 report (GOLD stages ABCD) (261), respectively. Two studies (259, 260) reported no association between FeNO levels and FEV1 pre- and post-bronchodilator therapy without ICS.

According to the 5 studies on COPD patients (201, 240, 241, 245, 258) included in this theme that showed an association with the levels of FeNO, patients who had the higher levels of FeNO responded better to ICS/GCS with or without bronchodilator therapy than those who had the lower FeNO levels. Four of the five studies introduced an optimal cut-off value for the treatment response (201, 240, 245, 258). Two studies reported the same cut-off value for the FeNO level regarding treatment response, which was 26.8 ppb (201, 258). According to one of these two studies (201), the sensitivity and specificity of this cut-off value were 74 and 75%, respectively with the area under the ROC curve of 0.82. The other 2 studies (240, 245) proposed a cut-point of 50 and 35 ppb with a sensitivity of 29, 80% and specificity of 96, 66.7%, respectively. The area under the ROC curve for the cut-off 50 ppb was reported as 0.69 (240).

Discussion

To the best of our knowledge, this is the first systematic scoping review undertaken on FeNO and COPD. This scoping review started with a broad question: what do we know about FeNO and its use in patients with COPD? The search criteria and inclusion criteria were deliberately kept broad in order to facilitate the inclusion of the largest number of studies possible. This approach enabled the determination of 6 themes and 6 specific questions. This, in turn, allowed us to conduct a review on the existing evidence and to identify gaps in the literature and make recommendations for clinical use and research.

The most extensive covered theme, mentioned in more than 60% of the articles, was the factors modifying FeNO levels, followed by FeNO and disease severity or progression in 57.89%, FeNO in COPD compared to healthy subjects in 47.67%, FeNO and biomarkers in 31.57%, FeNO and treatment response in 21.05%, and FeNO in ACO/ACOS in 18.42%. All of the included studies in this review measured FeNO levels according to the ATS/ERS guideline 2005 (262).

We have seen from this review that when measuring FeNO, there is a need to account for some important factors that could influence the level of FeNO. Current cigarette smoking which is often present in COPD decreases FeNO level (201, 231-237) while COPD exacerbations increase FeNO level (232, 242, 249). Concerning ICS/GCS alone or combined with bronchodilators, studies yielded conflicting results. On one hand, 7 studies (1, 201, 240-242, 245, 246) showed a decrease of FeNO level with ICS/GCS while 6 studies (232, 234, 237, 239, 243, 244) showed no association. The difference in these results may be due to studies being underpowered, dealing with different COPD phenotypes (acute exacerbations, stable COPD and ACOS), and different devices being used for the measurement of FeNO. Many factors have not been studied enough, preventing us from being able to make a definitive recommendation on those specific factors. Cold weather and/or viral infection might increase FeNO level; sodium bicarbonate and exercise could decrease the FeNO level. Of importance, we should also take into consideration that differences of measurement in the studies could be related to the type of device/instrument used for measuring FeNO such as the chemiluminescence analyzer, electrochemical FeNO device, NioxMino/Vero analyzer, SV-02 NO Instrument, and portable analyzer of nitric oxide (HypAir-FeNO). Using different devices might induce variability and limit comparison between studies. What we take from this review, before patients are tested for FeNO, they should be advised not to smoke, to do exercise, and if possible to be off ICS or GCS. If the patient has an exacerbation and/or an acute respiratory infection such as a cold-like illness, FeNO testing should be postponed. Moreover, we recommend conducting more studies, in particular on factors that have not been covered or those only covered by one or two studies.

Although FeNO levels were generally higher in COPD compared to healthy individuals, none of the studies could propose a cut-off value to differentiate COPD from healthy subjects. Three studies (1, 4, 253) recommended conducting investigations with a large number of subjects to evaluate FeNO measurement. According to Chen et al. (4), in order to generalize utilization of FeNO measurement in the clinical setting, conducting prospective studies in large-scale would

be crucial. According to Beg et al. (250) development of new handheld analyzers may make the measurement easier, make the large comparative studies possible and lead to possible application in daily clinical setting. Until this is demonstrated or refuted, FeNO measurement is unlikely to be of clinical use to differentiate COPD from non-COPD individuals.

The review also addresses the question of FeNO and disease severity and progression. Studies reported an association with exacerbations, 4/6 studies (233, 242, 243, 249), but not with stage of disease defined by GOLD as ABCD or as stage I-IV, 6/7 (2, 4, 206, 207, 234, 252). This relationship with exacerbations, FeNO being elevated when patients have exacerbations may just be a consequence of the exacerbations. We still do not know if FeNO could be useful to diagnose different types of exacerbations to help guide therapy. FeNO does not appear to be associated with disease progression, defined by changes in pulmonary function tests (163, 201, 207, 232-234, 240, 241, 245-247, 249, 254).

Based on our review, FeNO could be useful to differentiate phenotypes of COPD, more particularly ACO(S). All studies demonstrated that FeNO has a higher level in ACO(S) patients than those with only COPD (4, 206, 207, 244, 254-256). However, different cut-off values have been proposed for differentiating ACO(S) from COPD which may be due to the use of different definitions of ACO(S) as well as using different devices to measure FeNO. There is still uncertainty in identifying ACO(S) among COPD patients, in particular defining the optimal cut-off value to be used in clinical practice. Accordingly, Chen et al. (4) and Goto et al. (255) suggested establishing further prospective studies with a large number of subjects to investigate and broaden the utilization of FeNO measurement in the clinical settings. Until we come up with a standard on which we can define and rely on a definition of ACO(S), it will be difficult to be definitive on a cut-off of FeNO even with large sample size study.

Another area of major focus is the identification of biomarkers that would be indicative of asthma, in particular, high sputum eosinophils, IgE and more recently the possibility of some threshold of blood eosinophils. FeNO can be measured via a fast, noninvasive, reproducible, and easy way in close to real time which would offer a significant advantage on measurement such as sputum induction. According to our scoping review, although there was a positive relationship between FeNO levels with sputum eosinophils (1, 163, 179, 235, 240, 246, 251, 257, 258) and serum IgE (1, 236, 246, 257), there was no unified cut-off of FeNO level. Studies used different definitions of high sputum eosinophilia. Finally, we cannot be definitive on the

relationship between FeNO level and blood eosinophils because of not enough evidence, two studies reported an association (163, 236) versus 2 studies showed no association (179, 256).

Aligned with the role that FeNO might play in monitoring airway inflammation in COPD, FeNO could predict corticosteroid responsiveness. COPD patients who had higher levels of FeNO at baseline (before treatment) demonstrated a better response to the medication therapy (ICS/GCS with or without bronchodilators) (201, 240, 241, 245, 258). However, studies used a different cut-off of FeNO levels from 26.8 (similar in two studies) (201, 258) to 35 (245) and 50 (240) ppb, for assessing treatment response. It is still unclear if FeNO could be used and which cut-off will have the best yield to predict treatment response to ICS/GCS. Moreover, there would be a need to conduct further investigations with well-designed longitudinal studies to determine if FeNO can reflect eosinophilic airway inflammation with enough precision to be used in a therapeutic decisional algorithm and/or as an alternative to sputum induction for guiding therapy.

This scoping review has the strength of selecting all the studies in the broad field of FeNO in COPD. Themes and questions were determined based on the existing studies and not excluding studies of a specific topic. Furthermore, there was no limitation of language as the review included all languages. Our scoping review like the other ones was not free of limitations. One of the limitations of our study was excluding the conference/meeting and no peer-reviewed abstracts as well as removing review articles. However, we searched major databases for this scoping review; there are other databases that were not searched. This scoping review was limited to the year 2005 onwards as this was the year that the first ATS/ERS guideline regarding FeNO measurement was published (262). Another limitation is related to the quality assessment of the studies included in the review. We did not perform quality assessment of the studies as it is usually not done in scoping review, according to the scoping review guidance (213) and consistent with scoping review of clinical topics (218, 219).

In conclusion, when measuring FeNO, there are several factors that can affect its measurement. This needs to be considered in clinical setting and research. The Evidence is still lacking preventing us from recommending the general use of FeNO in clinical practice for COPD patients. Although FeNO level is higher in ACO(S) patients than COPD-only, it is still unclear if there is a FeNO cut-off that can be used to make the diagnosis of ACO(S) and/or to guide therapy with ICS/GCS in COPD patients. The main focus of future research should be to

determine if FeNO could be part of a cascade of a therapeutic decisional algorithm and/or as an alternative to sputum induction for guiding COPD therapy.

Acknowledgement

We would like to thank our librarian at the McGill University Health Centre, Alex Amar, for his help in preparing search strategy document and searching databases as well as providing updated database search.

Tables and Figures

Table 1. Themes, research questions and the number of studies those were able to answer the related questions.

No.	Theme Title	Research Questions	Number of Studies (%)
1	Factors modifying FeNO Measurement	Which factors have been demonstrated to modify the level of FeNO in COPD patients?	24 (63.15)
2	FeNO in COPD or in COPD compared to Healthy Subjects	Does the FeNO level increase in patients with COPD and is there an optimal cut-off value that may be useful in COPD versus healthy subjects?	18 (47.36)
3	FeNO and disease severity and/or progression	Are FeNO values associated with disease severity and/or disease progression in patients with COPD?	22 (57.89)
4	FeNO and Asthma-COPD Overlap (ACO) Syndrome (ACOS)	Are there differences in FeNO levels between patients with asthma COPD overlap (ACO) syndrome (ACOS), i.e., with asthma features and patients with COPD-only?	7 (18.42)
5	FeNO and Inflammatory Biomarkers	Are FeNO values associated with changes in the level of inflammatory biomarkers such as blood/sputum eosinophils and/or IgE in patients with COPD?	12 (31.57)
6	FeNO and Treatment Response	Whether increased or decreased FeNO level has an influence on treatment response, especially ICS/GCS therapy with or without inhaled bronchodilators in patients with COPD.	8 (21.05)

FeNO: Fractional exhaled nitric oxide; COPD: Chronic obstructive pulmonary disease; ACO: Asthma-COPD overlap; ACOS: Asthma-COPD overlap syndrome; IgE: Immunoglobulin E; ICS: Inhaled corticosteroid; GCS: Glucocorticoid

Table 2. Factors Modifying Fractional Exhaled Nitric Oxide (FeNO) Measurement.*

Author (s) (Year)	Decreased FeNO	Increased FeNO	No Effect/Association	Comments
Bhowmik et al. (2005) (232)	Smoking	Cold weather/viral infection, exacerbation	ICS/LABA	Current COPD smokers versus ex-smokers. Increased FeNO in cold weather (October to December) could be due to viral infections.
Liu et al. (2007) (239)	-	-	Smoking, GCS	Smoking was reported in both COPD and healthy subjects.
de Laurentiis et al. (2008) (233)	Smoking	-	-	Current COPD smokers versus ex-smokers.
Kunisaki et al. (2008) (241)	ICS	-	-	ICS therapy to COPD patients was prescribed for 4 weeks.
Roy et al. (2009) (235)	Smoking, Sex (female)	-	-	COPD smoker versus COPD ex-smoker. COPD women versus COPD men
Dummer et al. (2009) (240)	GCS	-	-	Oral prednisone was prescribed for 3 weeks. Current smokers were excluded.
Antus et al. (2010) (201)	Smoking, GCS	-	Sex (male or female)	Exacerbated COPD smokers versus ex-smokers. Systemic (IV or oral) corticosteroids were prescribed in all exacerbated patients in the hospital. Tend to have decreased FeNO with GCS therapy at discharge.
Lehouck et al. (2010) (234)	Smoking	-	ICS, Age	Current smokers in both COPD patients and healthy controls. Prescribed ICS (39% of COPD patients) among different GOLD stages (I-IV).
Tilemann et al. (2011) (236)	Smoking	-	-	Current COPD smokers versus non-smokers (never smokers and ex-smokers).
Rouhos et al. (2011) (248)	Sodium bicarbonate	-	-	Both COPD and healthy subjects whom were prescribed sodium bicarbonate to rinse their mouth.
Bazeghi et al. (2011) (231)	Smoking	-	-	Current COPD smokers versus ex-smokers.
Akamatsu et al. (2011) (245)	ICS/LABA	-	-	ICS was prescribed twice daily for 12 weeks.
Antus et al. (2013) (243)	-	-	GCS, LABA, LAMA	Exacerbated COPD patients were prescribed systemic (IV or oral) corticosteroids and bronchodilators during hospital stay.

				No difference between low (<27 ppb) and high FeNO (≥ 27 ppb) regarding GCS and bronchodilator therapy.
Xia et al. (2014) (249)	-	Smoking, exacerbation	-	Current COPD smoker versus non-smoker. Exacerbation effect was considered in the mixed group of subjects including healthy, stable COPD, asthma and non-asthma.
Rawy et al. (2015) (163)	Age	-	-	Negative association between FeNO and age
Ishiura et al. (2015) (263)	-	-	ICS/LABA	ACOS patients were prescribed ICS/LABA for 12 weeks
Santini et al. (2016) (237)	Smoking	-	ICS	COPD current smokers versus COPD ex-smokers. No difference in FeNO values between COPD patients on ICS therapy and those not on ICS therapy.
Logotheti et al. (2016) (242)	ICS	Older age, exacerbation	-	COPD patients were prescribed an inhaled corticosteroid (ICS) for maintenance treatment. Older COPD patients (69±6 years) versus younger patients (66±7 years). A significant increase in exacerbations during the previous year in COPD patients with elevated FeNO levels.
Amer et al. (2016) (260)	-	Bronchodilator	-	FeNO was measured before and 15 minutes after short inhaled bronchodilator therapy.
Ji et al. (2016) (246)	ICS/LABA	-	-	ACOS patients were prescribed ICS/LABA for maintenance treatment.
Huang et al. (2016) (247)	6MWT (exercise)	-	-	FeNO was measured after exercise test in COPD patients.
Kobayashi et al. (2016) (244)	-	-	ICS	ACOS patients with prescribed ICS versus non-ACOS with prescribed ICS.
Feng et al. (2017) (1)	ICS	-	-	COPD patients were prescribed ICS, 3 times per day for 6 months. FeNO was measured before and after ICS therapy.
Zhao et al. (2017) (259)	-	-	Bronchodilator	FeNO was measured before and 15 minutes after inhaled short-acting bronchodilator.

FeNO: Fractional exhaled nitric oxide; ICS: Inhaled corticosteroid; LABA: Long-acting beta agonist; GCS: Glucocorticosteroid; COPD: Chronic obstructive pulmonary disease; IV: Intravenous; ppb: parts per billion; GOLD: Global Initiative for Chronic Obstructive Lung Disease; ACOS: Asthma-COPD overlap syndrome; 6MWT: 6-minute walk test

*- =Not mentioned

Table 3. Fractional exhaled nitric oxide (FeNO) in COPD or in COPD compared to Healthy Subjects.

Author (s) (Year)	FeNO Level in COPD or compared to healthy*	Optimal Cut-off Value**	Comments
Foschino Barbaro et al. (2007) (251)	Increase	-	COPD patients compared to healthy control subjects and reversible COPD compared to non-reversible COPD.
Liu et al. (2007) (239)	Increase	-	COPD patients compared to healthy subjects (eNO level)
de Laurentiis et al. (2008) (233)	Increase	-	COPD patients compared to healthy subjects: Not significant baseline mean level of FeNO but significant FeNO mean coefficient of variability (CoV)
Beg et al. (2009) (250)	Increase	-	COPD patients compared to healthy subjects
Lehouck et al. (2010) (234)	-	-	COPD patients compared to age-matched healthy control subjects
Tilemann et al. (2011) (236)	Decrease	-	COPD patients compared to subjects with no airway obstruction.
Rouhos et al. (2011) (248)	Increase	-	COPD patients compared to healthy subjects
Bazeghi et al. (2011) (231)	-	-	COPD patients only (Severe emphysema, chronic bronchitis, frequent exacerbations, low body mass)
Donohue et al. (2014) (2)	Increase	-	COPD patients with different phenotypes, especially those with concomitant asthmas
Xia et al. (2014) (249)	Increase	-	COPD patients compared to healthy subjects and also exacerbated COPD compared to stable COPD. No association in FeNO levels between stable COPD and healthy subjects
Durmaz et al. (2015) (253)	-	-	COPD patients at admission (exacerbation) or before discharge (eNO level)
Santini et al. (2016) (237)	Increase	-	COPD ex-smoker compared to healthy ex-smoker.
Arif et al. (2016) (252)	-	-	COPD patients (eNO)
Alcazar-Navarrete et al. (2016) (206)	Increase	-	COPD patients compared to non-smoker healthy controls.
Logotheti et al. (2016) (242)	Increase	-	COPD patients with GOLD stages (ABCD)
Ji et al. (2016) (246)	Increase	-	ACOS patients compared to healthy subjects, both before and after treatment (ICS/LABA).
Huang et al. (2016) (247)	Increase	-	COPD patients compared to healthy subjects
Feng et al. (2017) (1)	Increase	-	ACOS patients compared to healthy subjects

FeNO: Fractional exhaled nitric oxide; COPD: Chronic obstructive pulmonary disease; eNO: Exhaled nitric oxide; CoV: coefficient of variability; ppb: parts per billion; FEV1: Forced expiratory volume in one second; ICS: Inhaled corticosteroid; LABA: Long-acting beta agonist; ppb: parts per billion; GOLD: Global Initiative for Chronic Obstructive Lung Disease; ACOS: Asthma-COPD overlap syndrome
*Optimal cutoff value: - = Not mentioned,
**FeNO levels in COPD compared to healthy: - = No difference/change

Table 4. Fractional exhaled nitric oxide and disease severity and/or progression.*

Author (s) (Year)	Association			Comments**
	Disease Severity		Disease Progression	
	GOLD	Exacerbations	Pulmonary Function Tests	
Bhowmik et al. (2005) (232)	-	N	N	No association between eNO and FEV1, FVC or exacerbation frequency.
de Laurentiis et al. (2008) (233)	-	Y	N	No association between FeNO and FEV1; exacerbation rate was significantly associated with FeNO
Kunisaki et al. (2008) (241)	-	-	N	No association between FeNO and FEV1 or FVC.
Beg et al. (2009) (250)	-	-	Y	Negative association between FeNO and FEV1/FVC ratio
Dummer et al. (2009) (240)	-	-	N	No association between FeNO and FEV1.
Antus et al. (2010) (201)	-	-	Y/N	Positive association between FeNO levels at admission (acute exacerbation) and the post-treatment increase in FEV1 and FEV1% predicted. No association with FVC.
Lehouck et al. (2010) (234)	N	-	N	No association between FeNO and GOLD stages (I-IV). No association with FEV1.
Akamatsu et al. (2011) (245)	-	-	N	No association between FeNO and changes in FEV1 as well as other pulmonary physiological parameters.
Antus et al. (2013) (243)	-	Y	-	Association between low FeNO level and more exacerbations (increased number of exacerbation per patient-year) during the follow-up.
Donohue et al. (2014) (2)	N	-	-	No association between FeNO levels and GOLD stages (I-IV).
Xia et al. (2014) (249)	-	Y	N	Association between elevated FeNO and acute exacerbations (compared to stable COPD). No association with FEV1 and FEV1/FVC.
Tamada et al. (2015) (207)	N	-	N	No association between high and low FeNO levels with GOLD stages (I-IV). No association between low (≤ 35 ppb) and high FeNO (> 35 ppb) levels with pulmonary function tests (FVC, FEV1, and FEV1/FVC).
Rawy et al. (2015) (163)	-	-	N	No association between FeNO and FEV1/FVC.

Durmaz et al. (2015) (253)	-	N	-	No association between eNO level at presentation or before discharge and exacerbated patients
Arif et al. (2016) (252)	N	-	-	No association between eNO and GOLD stages (I-IV)
Alcazar-Navarrete et al. (2016) (206)	N	-	-	No association between FeNO and GOLD 2011 (ABCD).
Logotheti et al. (2016) (242)	Y	Y	-	Association between FeNO and GOLD 2011 (ABCD). Association with the increase in a number of exacerbations.
Chen et al. (2016) (4)	N	-	-	No association between FeNO and GOLD stages (I-IV).
Ji et al. (2016) (246)	-	-	N	No association between FeNO and FEV1% predicted in ACOS.
Huang et al. (2016) (247)	-	-	N	No association between eNO and FEV1% predicted or the FEV1 change secondary to 6MWT .
Feng et al. (2017) (1)	-	-	Y	Negative association between FeNO levels with FEV1% predicted and FEV1/FVC in ACOS patients.
Deng et al. (2017) (254)	-	-	N	No association between FeNO with FEV1% predicted and FEV1/FVC in COPD and ACOS.

GOLD: Global Initiative for Chronic Obstructive Lung Disease; eNO: Exhaled nitric oxide; FEV1: Forced expiratory volume in first second; FVC: Forced vital capacity; COPD: Chronic obstructive pulmonary disease; 6MWT: 6 minutes walk test; ACOS: Asthma-COPD overlap syndrome

*Disease severity and progression: Y=Yes, N=No, - = Not mentioned

**Subjects are COPD patients unless stated otherwise

Table 5. Fractional exhaled nitric oxide and Asthma-COPD overlap syndrome (ACO/ACOS).*

Author (s)	Optimal Cut-off Value	Utility	Comments
Tamada et al. (2015) (207)	-	Y	Higher FeNO values in COPD with asthma-like airway inflammation (ACOS) among COPD patients.
Alcazar-Navarrete et al. (2016) (206)	19 ppb	Y	Higher FeNO values in ACOS patients than those with other COPD phenotypes.
Chen et al. (2016) (4)	22.5 ppb	Y	Higher levels of FeNO in ACOS group than COPD group.
Goto et al. (2016) (255)	-	N	Higher levels of FeNO in ACOS than those with COPD alone.
Kobayashi et al. (2016) (244)	23 ppb	Y	Higher FeNO levels in ACOS than non-ACO(S) (among COPD patients).
Deng et al. (2017) (254)	29 ppb	Y	Higher levels of FeNO in ACOS than COPD patients.
Cosío et al. (2017) (256)	-	-	Higher levels of FeNO in ACOS than COPD patients

ppb: parts per billion; FeNO: Fractional exhaled nitric oxide; ACOS: Asthma-COPD overlap syndrome, COPD: Chronic obstructive pulmonary disease

*ACO(S) definition is different among the studies using major and minor criteria (206, 255) or either Global initiative for asthma (GINA)/Global Initiative for Chronic Obstructive Lung Diseases (GOLD) joint document 2014 (254) or 2015 (updated document) (244) or positive bronchodilator test and history of asthma (4) or history of asthma and smoking (≥ 20 pack-years) and chronic airflow limitation or diagnosed COPD and elevated blood eosinophil count (256) or high levels of fractional exhaled nitric oxide (FeNO > 35 ppb) or immunoglobulin E (IgE ≥ 173 IU/mL) in diagnosed COPD (207), Utility: Y=Yes, N=No, - = Not mentioned, Optimal cut-off value: - = Not mentioned

Table 6. Fractional exhaled nitric oxide and inflammatory biomarkers.*

Author (s) (Year)	Association			Optimal Cut-off Value	Comments‡
	Sputum Eosinophils**	Blood Eosinophil†	Serum IgE		
Foschino Barbaro et al. (2007) (251)	Y	-	-	-	COPD patients with airway reversibility
Roy et al. (2009) (235)	Y	-	-	-	Regardless of percentage differential or cell count.
Dummer et al. (2009) (240)	Y	-	-	-	Off-steroid FeNO and sputum eosinophil percentage
Tilemann et al. (2011) (236)	-	Y	Y	-	Mixed group of patients (COPD, asthma, and patient with partial reversibility)
Soter et al. (2013) (258)	Y	-	-	19 ppb	Regardless of percentage/number. Both at exacerbation and discharge
Rawy et al. (2015) (163)	Y	Y	-	-	Positive association, blood and sputum eosinophil percentage
Chou et al. (2015) (257)	Y	-	Y	23.5 ppb	Patients with sputum eosinophilia compared to those without sputum eosinophilia.
Ji et al. (2016) (246)	Y	-	Y	-	Pre and post treatment (ICS/LABA) FeNO levels with sputum eosinophils and serum total IgE.
Feng et al. (2017) (1)	Y	-	Y	-	ACOS patients
Cosío et al. (2017) (256)	-	N	-	-	Patients with elevated blood eosinophil compared to those without elevated blood eosinophil.
Gao et al. (2017) (179)	Y	N	-	17.5 ppb	Patients with elevated eosinophils either in sputum or in blood compared to those without elevated sputum/blood eosinophils
Zhao et al. (2017) (259)	N	-	-	-	Before and after bronchodilator inhalation

IgE: Immunoglobulin E; COPD: Chronic obstructive lung disease; FeNO: Fractional exhaled nitric oxide; ppb: parts per billion; eNO: Exhaled nitric oxide; ICS: Inhaled corticosteroid; LABA: Long-acting beta agonist; ACOS: Asthma-COPD overlap syndrome

*Y=Yes, N=NO, - = Not mentioned

**Sputum eosinophilia/elevated sputum eosinophils defiend as sputum eosinophil count ≥ 2.5% (179) or ≥ 3% (258) or > 3% (257) or no definition (1, 163, 235, 236, 240, 246, 251, 256, 259)

†Elevated blood eosinophils defined as blood eosinophil count ≥ 1% (179) or ≥200 eosinophils·μL^{-1} (256) or no definition (163, 236)

‡ Subjects/patients are COPD unless stated otherwise

Table 7. Fractional exhaled nitric oxide (FeNO) and treatment response.*

Author (s) (Year)	Treatment Response Influence/Association**	Optimal Cut-off Value	Comments†
Kunisaki et al. (2008) (241)	Y	-	Higher baseline FeNO levels in ICS responder than non-responders
Dummer et al. (2009) (240)	Y	50 ppb	Significant improvement in FEV1 from the lowest to the highest FeNO tertile using oral glucocorticoid
Antus et al. (2010) (201)	Y	26.8 ppb	Higher increase in FEV1 and FEV1% predicted at discharge in the exacerbated patients with FeNO > 26.8 ppb by administrating systemic (IV or orally) glucocorticoids and bronchodilators
Akamatsu et al. (2011) (245)	Y	35 ppb	Higher increase in FEV1 in patients with FeNO >35 ppb and atopy by adding ICS/LABA
Soter et al. (2013) (258)	Y	26.8 ppb	Higher increase in FEV1 at admission in exacerbated patients with FeNO >26.8 ppb by administrating systemic (IV or orally) glucocorticoids and bronchodilators
Amer et al. (2016) (260)	N	-	No association between FeNO and change in FEV1 after bronchodilator therapy
Feng et al. (2017) (1)	Y	-	Association between FeNO and ICS therapy (for 6 months) in ACOS. Lower FeNO in mild and moderate than severe and extremely severe ACOS‡
Zhao et al. (2017) (259)	N	-	No association between the FeNO and change in FEV1 after bronchodilator therapy

ICS: Inhaled corticosteroid; FeNO: Fractional exhaled nitric oxide; ppb: parts per billion; FEV1: Forced expiratory volume in first second; COPD: Chronic obstructive pulmonary disease; IV: Intravenous, ACOS: Asthma-COPD overlap syndrome; GINA: Global initiative for asthma; GOLD: Global initiative for chronic obstructive lung disease

* Treatment response influence/association: Y=Yes, N=No, Optimal cutoff value: - = Not mentioned

**Treatment response was defined as an increase in FEV1≥ 200mL (241) or > 200mL (245) or >12% and >200 mL (201, 258). Treatment response was not defined in three studies (1, 259, 260).

† Subjects/patients are COPD unless stated otherwise

‡ACOS and its severity was defined as GINA-GOLD joint document 2014 (204) and GOLD 2011 stages (ABCD) (261), respectively.

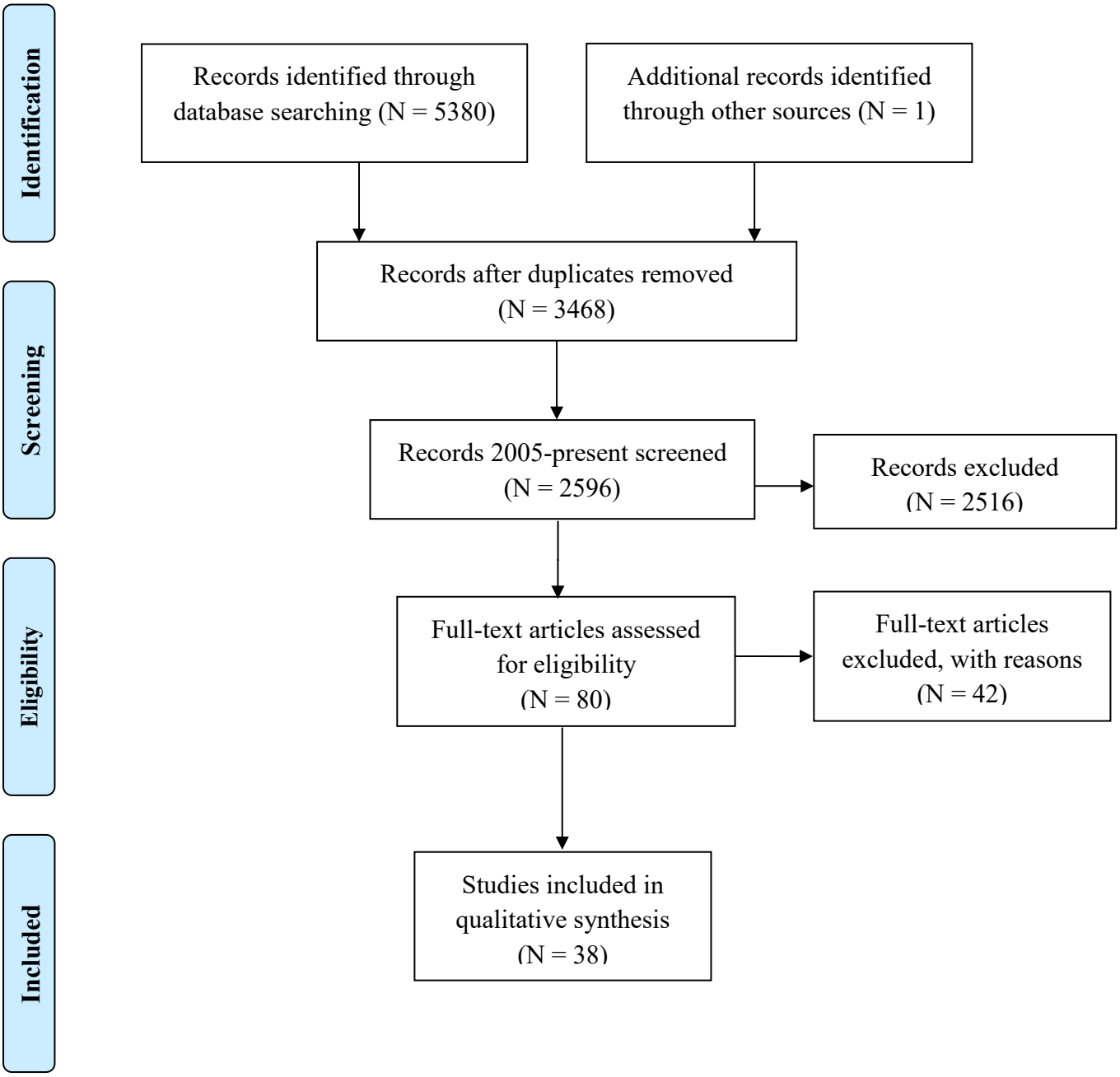

Figure 1. PRISMA flow diagram

CHAPTER 5: MANUSCRIPT 3: FRACTIONAL EXHALED NITRIC OXIDE (FENO) AS INFLAMMATORY BIOMARKER IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AND ASTHMA-COPD OVERLAP (ACO) IN CANADIAN COHORT OBSTRUCTIVE LUNG DISEASE (CANCOLD) POPULATION

The following manuscript is a prospective observational study with population sampling on fractional exhaled nitric oxide (FeNO) role in CanCOLD population, specifically in COPD patients from this specific population, who have extensive characterizations of COPD population. The main focus of this manuscript is on FeNO role in COPD and identified ACO patients according to most three common ACO definitions, including i) >12% and >200 ml of increment in the FEV1 post-bronchodilator; ii) Physician diagnosis of asthma; and iii) Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire). As well, this manuscript evaluates the ability of FeNO in differentiating ACO from non-ACO (COPD alone), which was the general of objective of this manuscript. The following manuscript presents details of the methods and the preliminary results of this research. This research is still ongoing and data gathering continues until the end of 2017. But for the sake of thesis, the data collection was closed on September 15, 2017. The manuscript is in preparation and will be submitted for publication with more subjects and may be different results (due to gathering more subjects) early 2018.

Introduction

Asthma and COPD are two common chronic inflammatory airway diseases that share some common clinical features. Asthma can present in all age groups; it is characterized by chronic airway inflammation with airway hyperresponsiveness (AHR) and complete airflow limitation reversibility (107, 220, 264). COPD is present in adult over 40 years old and most commonly in individuals who have been smoking; it is characterized by chronic inflammation of the small airways and the parenchyma, and persistent, progressive, and incomplete reversible airflow limitation. COPD is defined by spirometry with a post-bronchodilator ratio of forced expiratory volume in 1 second (FEV1) on forced vital capacity (FVC) <0.7 (220, 221, 265). Although COPD and asthma have distinct clinical and pathophysiological features, there is no definitive test allowing the clinician to separate the two diseases or to confirm when COPD is associated with concomitant asthma.

Airway inflammation is central to the pathogenesis of COPD and asthma. In patients with COPD, neutrophils, macrophages, and CD8+ T lymphocytes have been implicated (266). There is a relationship between T-cell, alveolar destruction, and the severity of airflow limitation (267). An increase in total leukocytes and in CD4+ and CD8+ lymphocytes in both peripheral airways and the lung parenchyma has been shown in patients with severe COPD (266). Asthma has been the dominant focus of airways research interest. With regards to a large number of eosinophils in the airways of people with asthma and data from murine models, asthma was long considered the hallmark T helper type 2 (TH2) disease of the airways (268). Although eosinophilic airway inflammation is usually considered a feature of asthma, it has been demonstrated in large and small airway tissue samples and in 20%–40% of induced sputum samples from patients with stable COPD. Thus, modifying eosinophilic inflammation may be a potential therapeutic target in COPD (269).

An important obstacle to assess COPD airway inflammation, it is that the most practical investigative techniques have important limitations. Resident cells such as airway, airway epithelial cells, circulatory endothelial cells, and trafficking inflammatory cells produce nitric oxide (NO) in both large and peripheral airways and alveoli (20). Endogenous NO may play a significant role in the physiological control of airway function and in the pathophysiology of airway diseases (163). Endogenous NO is synthesized by the conversion of the amino acid L-arginine to L-citrulline and NO by the enzyme NO synthase (NOS) of which three distinct isoforms exist, including nNOS, type I and eNOS, type III, both are calcium-dependent, and

iNOS, type II (calcium-independent) (162, 163, 172). In response to interleukin IL 4 and IL13 via the STAT-6 pathway nitric oxide gas is synthesized in the epithelial cells of the bronchial wall (2). Th2 allergic inflammation leads to an increase in the level of exhaled nitric oxide and often is related to eosinophilic inflammation in the airways (3).

Fractional exhaled nitric oxide (FeNO) is an easy, reproducible, sensitive, and noninvasive marker for identifying eosinophilic airway inflammation in asthma that is well established in research (2, 174, 179). The American Thoracic Society clinical practice guideline 2011(3) suggests using FeNO in asthma management as well as recognizing and monitoring eosinophilic airway inflammation and inhaled corticosteroid treatment response. Since some patients with COPD share features of asthma, which is called asthma–COPD overlap (ACO) (49) syndrome (ACOS) (48), it has been suggested that FeNO could be used to confirm the presence of concomitant asthma in these patients. There is no recommendation to general use of FeNO in clinical practice for COPD patients due to lack of evidence. Although FeNO level is higher in ACO(S) patients than COPD-only, it still remains unclear if there is a FeNO cut-off that can be used to diagnose ACO(S) and differ it from COPD-only and/or to guide inhaled corticosteroid (ICS)/glucocorticoid (GCS) therapy in COPD patients. As well, the exact role of FeNO in patients with ACO(S) and phenotyping COPD patients is still unclear. We hypothesized that FeNO as a surrogate marker of eosinophilic inflammation could be a useful non-invasive marker to identify COPD patients with concomitant asthma, i.e., ACO(S) and therefore, guiding treatment in these patients who would benefit from being prescribed inhaled corticosteroid (ICS). The present study embedded in a prospective cohort study, the Canadian Cohort Obstructive Lung Disease (CanCOLD) aimed: i) to determine COPD subjects' characteristics and FeNO in a COPD population sample; ii) to assess if a FeNO cut-off value could be used for differentiating COPD from ACO(S) (applying commonly used clinical definitions and; iii) to assess if FeNO used in COPD can predict risk of disease severity (lung function, exacerbations, and patient-reported outcomes) and disease progression (FEV1 annual decline).

Methods

Study Design

This study is embedded in the Canadian Cohort Obstructive Lung Disease (CanCOLD) study. CanCOLD is a prospective longitudinal cohort that includes individuals recruited in 9 centers across Canada from random sampling. Men and women, ≥40 years of age identified by random digit dialing from the general population and invited for a spirometry and then classified into four groups: healthy individuals, at risk of developing COPD (smokers or ex-smokers) and COPD individuals GOLD 1 or GOLD2+, that are followed every 18 months up to 3 years.

Study Population

We included all COPD subjects within the CanCOLD population, defined as a post-bronchodilator FEV1/FVC<0.70 (233). We identified ACO(S) among COPD subjects and compared characteristics of these two groups (COPD versus ACO(S)). Currently, there are no globally accepted criteria for identifying ACO(S), therefore, we defined subjects with ACO(S) by using three commonly used clinical definitions: i) >12% and >200 ml of increment in the FEV1 post-bronchodilator, iii) physician diagnosis of asthma; iii) atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire). However, there are a variety of definitions for identifying ACO(S), selecting the mentioned definitions comes from our unpublished study. According to our study (Miriam Barrecheguren et al, Identification of Asthma-COPD overlap individuals in the CanCOLD population through clinical definitions, In preparation), among 7 clinical definitions used in the literature on ACO(S), these 3 definitions were the most effective ones. The definition 1 (with reversibility) was included because it is often used in many criteria of ACO(S) diagnosis. The other two definitions (physician diagnosis of asthma with/without atopy) were the most stable definitions over time and differed most COPD-only from ACO patients. The protocol of this study was approved by the research ethics board (REB) of the research institute of McGill University health center (RI-MUHC), and all participants gave written informed consent.

Study Procedures and Measurements

Regular tests are performed in CanCOLD in all three visits (at baseline, 18 months and 3 years) include (270) blood tests (Hematology), biochemistry, lipid profile, DNA for genetic, RNA for transcriptomic profiling, St George's Respiratory Questionnaire (SGRQ) and COPD assessment test (CAT). For the purpose of this study, individuals had additional tests completed as part of a regular visit in CanCOLD or they had a separate visit. These additional tests

included FeNO, IgE and blood eosinophils, repeated pre- and post-bronchodilator spirometry and allergy skin tests. Socio-demographic and baseline characteristics were collected at the time of the visit. Acute exacerbations of COPD (AECOPD) were collected prospectively by scheduled phone calls/online or clinic visits every 3 months. AECOPD was defined as an event in the natural course of the disease characterized by a change in the patient's baseline symptoms including, dyspnea, cough, and/or sputum that is beyond normal day-to-day variations; regular medication; health care utilization, i.e., unplanned physician visits, emergency visits and hospitalizations. Severity was defined according level of FEV1, COPD Assessment Test (CAT), and St. George's Respiratory Questionnaire (SGRQ) as well as exacerbations. Progression was defined according to FEV1 annual decline, CAT and SGRQ. In addition, we used SGRQ and CAT score data for evaluating health status and quality of life. Data were retrieved from the CanCOLD database.

FeNO Measurement

FeNO was performed according to the American thoracic society (ATS)/European respiratory society (ERS) (183) guideline using the Niox Mino (Aerocrine, New York, USA). Patients were advised to avoid eating, drinking, smoking and doing exercise at least one hour before the test. We performed the test to obtain two quality measures with a difference no more than 10%. If subjects had a lower or upper respiratory infection, the FeNO measurement was deferred until recovery. Patients that were unable to provide two quality and reproducible FeNO measures were excluded from the study. Patients' respiratory medications were recorded. FeNO measurement was performed before spirometry tests as well as other respiratory maneuvers according to the ATS/ERS guideline (183). Subjects were instructed how to do the test. First, the subject exhales completely, then inhales NO-free air to their total lung capacity through the device and then exhales fully in a constant speed at a flow rate of 50 mL/s for 10 seconds (183). We used a mirror as a visual aid provided by Aerocrine to help subjects in order to keep their speed of exhalation constantly; furthermore, the device had a sound (as an aid) for this purpose as well. We recorded and reported the mean of two quality FeNO values (within 10%) for each subject.

Blood Biomarker Measurements

Blood samples were obtained on the same day as FeNO measurement to determine blood eosinophil count and percentage, and to quantify the level of serum IgE. The cut-off value of high blood eosinophil count and percentage were set at >0.45× 10^9 cells/L and > 4%, respectively, and the cut-off value of total serum IgE level was set at > 240 IU/mL according to the reference range of the clinical laboratory at the Royal Victoria Hospital, McGill University Health Centre, Montreal, Canada.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD) for quantitative variables, or median (interquartile range) for non-normal variables when appropriate, or number (%) of patients. Continuous variables were compared using the T-test for those that were normally distributed and/or Wilcoxon Mann Whitney for those that were not normally distributed. Chi-square or Fisher exact test was used to compare categorical variables. We tested different FeNO cut-off values retrieved from our scoping review as well as other studies regarding diagnosis and management of asthma. These cut-off values (3, 4, 179, 206, 244, 254, 257, 258, 271) are as follows: 17.5, 19, 22.5, 23, 23.5, 25, 29, 34, 36, and 50 ppb. To determine optimal cut-off value when ACO(S) was present, we performed constructing receiver operating characteristic (ROC) curve and measured the area under the curve (AUC). The optimal cut-off value was determined using Yuden Index, and sensitivity and specificity were calculated. Statistical significance was defined as $P < 0.05$. Analyses were performed using SAS version 9.4.

Results

A total of 172 CanCOLD subjects were invited to participate in the study (Figure 1). Of whom, 169 subjects completed but 3 subjects were not able to perform quality FeNO measurements; 95 COPD subjects were enrolled in the study.

The study subjects were comparable to those of the whole CanCOLD population except that they were younger, had lower pulmonary function tests (PFT), and on CT scan had a higher bronchiolitis score (Table 1s). The COPD subjects were also younger, more likely to be smokers, had lower PFT and worse health status (CAT and SGRQ) than COPD subjects from the whole CanCOLD population.

Baseline Characteristics and Biomarkers of Study Population

Table 1 shows the baseline characteristics among COPD, at risk and healthy subjects. They were similar in age and sex; more smokers in the COPD group than those at risk. COPD subjects, especially those with GOLD 2+ group had more dyspnea, lower PFT and health status, and more likely to be on a respiratory medication. Table 2 shows blood and exhaled breath biomarkers among study subjects. COPD subjects had a higher level of IgE and blood eosinophil count than those at risk and healthy subjects (not statistically significant). The GOLD2+ subjects had higher blood eosinophil count than those at risk and healthy subjects (statistically significant). Difference between mean FeNO levels among these groups [COPD (GOLD 1 and GOLD2+), healthy, and at risk] was not statistically significant. There were more COPD subjects with FeNO level 34>ppb ≥23.5 (third quartile) than those at risk or healthy subjects (p=0.04). There was a larger proportion of subjects with FeNO>50 ppb in GOLD2+ than GOLD1, at risk and healthy subjects (15% vs. 5.5, 4.3, 5.9%, respectively) (not statistically significant).

ACO Subjects and COPD-only (Non-ACO)

Table 3 shows the baseline characteristics and FeNO levels of subjects with ACO according to three pre-specified definitions (defs) and those with COPD-only (non-ACO COPD). The subjects with ACO compared to those with COPD-only were similar in age, more likely to be female, had lower lung function and higher FEV1 post-bronchodilator reversibility, lower emphysema score, higher dyspnea score and lower health status, and more were prescribed respiratory medications. The difference on IgE and blood eosinophil counts was not statistically significant. FeNO levels were higher in ACO subjects (all definitions) than COPD-only although it was not statistically significant (Figure 2, Table 3).

Table 3 shows that more subjects with ACO definition 1 compared to COPD-only had FeNO values ≥ 36 ppb (33% vs. 12%; p=0.046) with sensitivity, specificity, and area under the curve (AUC) of 39%, 88%, and 0.63, respectively (Table 2s); with ACO definition 2, no FeNO cut-off values were statistically significant; and with ACO definition 3, FeNO values ≥ 23.5 ppb (80% vs. 51%; p=0.047) with sensitivity, specificity, and area under the curve (AUC) of 80%, 50%, and 0.65 (Table 2s). Figures 3 and 4 present FeNO levels classified by quartile and according to the American Thoracic Society (ATS) 2011 guideline. FeNO levels ≥ 34 ppb

(fourth quartile) was observed more in ACO (all definitions) than COPD-only; it was statistically significant with ACO definition 1, p=0.028 (Figure 3). Fewer subjects with ACO definitions had FeNO < 25 than those with COPD-only; it was statistically significant with ACO definition 3, p=0.039 (Figure 4).

Figure 5a, b and c present the receiver operating curves (ROC) for an optimal cut-off value to differentiate ACO for each pre-specified definition and COPD-only. The best cut-off values were as follows: 33.5 (def 1 and def 2), and 22.5 ppb (def 3) with sensitivity of 44, 36, 56% and specificity of 85, 85, 80%, and AUC of 0.64, 0.55, and 0.63 for definition 1, 2, and 3, respectively. The sensitivity, specificity, and AUC of other FeNO cut-off values are presented in Table 2s.

FeNO levels and Disease Severity/Progression

Table 3 shows the relationship between FeNO with disease severity and progression. There was no statistically significant difference between FeNO for disease severity (FEV1, CAT, SGRQ, and exacerbations) and disease progression (annual FEV1 decline).

Discussion

Our results showed that using three common clinical definitions, ACO subjects are more frequently female, have more severe symptoms, worse lung function, and health status. Compared to COPD-only (non-ACO COPD), ACO individuals had higher mean FeNO levels but it did not reach statistical significance. ACO individuals had more frequently FeNO level ≥ 23.5 ppb or ≥ 36 ppb but only in two definitions (def 1 and 3); rarely the FeNO levels were <25ppb in ACO individuals. FeNO in COPD neither related to disease severity nor predicted disease progression.

There was no statistically significant difference in FeNO levels between COPD and healthy subjects that may be due to the neutrophilic predominance nature of COPD patients (2), while FeNO is a surrogate of eosinophilic airway inflammation (272). This result is in line with the result of one retrospective study (4) conducted on 689 patients including 500 asthmatics, 132 COPD, and 57 ACOS. Patients were divided into asthma alone group, COPD alone group, and ACOS group according to a clinical history, PFT values, and bronchial hyperresponsiveness or

bronchodilator test. In contrast, another study (206) reported a higher level of FeNO in COPD patients than non-smoker healthy subjects. They conducted a cross-sectional study on 192 patients including 103 with COPD; 16 healthy non-smokers; 30 healthy smokers; and 43 asthmatics. Patients' data were gathered on lung function, FeNO, CAT (COPD Assessment Test), and COPD clinical phenotype. No statistically significant difference was observed in FeNO levels between GOLD 1 and GOLD 2+, which is similar to the results from other studies (206, 273).

A recent study (2) reported that 8% of COPD patients had FeNO levels of 25-50 ppb, and 3% of them had FeNO levels of >50 ppb. Another study (274) conducted on 331 COPD patients showed that 20.6% of COPD patients had FeNO levels of 25-50 ppb, and 5.1% of them had FeNO levels of >50 ppb. In our current study, 40% of COPD patients had FeNO levels of 25-50 ppb and 9.5% of them had FeNO levels of >50 ppb. There is no clear explanation for this discrepancy, but one factor that can be considered is differences in age distribution among studies. The patients enrolled in one (2) of these studies had mean age ± SD of 63.9±11.34 years. In our present study, the mean age ± SD of enrolled COPD subjects was 67.5±2.4 years. Studies have reported that the prevalence of ACO tends to increase with age and it could be one possibility to have higher FeNO levels. In addition, most of our study subjects were not on ICS therapy that is another possibility to have a higher FeNO levels. Another factor can be having fewer smokers in our study population than another one (274), as cigarette smoke decreases the FeNO levels. Other factors that may result in this discrepancy can be the type of these studies and the selected subjects, as we used subjects in CanCOLD population to conduct this prospective study.

In accordance with previous studies (275, 276), in our population, ACO patients were more frequently female and had worse outcomes. These data show a crucial need to find a useful biomarker that is able to differentiate ACO from COPD-only. There are a number of preliminary studies that have measured FeNO in COPD, but literature explaining the exact role of FeNO in patients with ACO is limited. The present study showed that ACO subjects have a higher mean level of FeNO than COPD-only; however, it was not statistically significant. In contrast, one study (4) showed that ACO patients had a significantly higher level of FeNO than COPD ($p< 0.01$). One hundred eighty-nine COPD subjects (COPD-only and ACO) were included in this study. Moreover, another investigation (244) showed that ACO patients had a significantly higher level of FeNO than non-ACO (38.5 ppb vs. 20.3 ppb, $p<0.001$).

In this study, we tested different FeNO cut-off values (3, 4, 179, 206, 244, 254, 257, 258, 271) retrieved from our scoping review and asthma literature for differentiating ACO from COPD-only. In addition, we tried to find optimal cut-off values to differentiate ACO from COPD-only using the data from the study. We may consider FeNO as a weak but not a strong predictor to differ ACO from COPD with the AUC range of 0.55-0.66 found in our study. Significant cut-off values for this purpose were FeNO ≥ 36 ppb with the sensitivity of 39% and specificity of 88% for definition 1. If applied in practice, this will mean that when the test is positive we will mostly be right, but we will be able to identify less than 50% of the patients or missing more than 50%. For definition 3, FeNO ≥ 23.5 ppb with the sensitivity of 80% and specificity of 50%, we will identify 80% of the patients with the disease, i.e., ACO, be missing only 20% but not be right with the ACO diagnosis in about 50% of the patients. One study (4) conducted on 132 COPD and 57 ACO, showed that FeNO is a good predictor to differentiate ACO from COPD with AUC of 0.78. They introduced the cut-off value of 22.5 ppb with a sensitivity of 70% and a specificity of 75%. This variation in the results creates uncertainty and calls for studies to be done using same types of measuring tool for FeNO, describing the population selection and reference to a common definition of ACO.

Our study showed that FeNO is not able to predict disease severity/progression in COPD patients. Most studies are in line with our result regarding no association between FeNO and disease severity (decline in pulmonary function tests, especially FEV1) in COPD patients (163, 205, 277). With respect to exacerbations, one study (253) showed that there was no association between FeNO at presentation and after discharge for exacerbations while other studies have shown a relationship (242, 249).

Our study had strengths. One of them is using population-based sampling and longitudinal design (CanCOLD). Our COPD population is comparable to and representative of whole COPD population as well. We standardized the FeNO measurement according to the ATS/ERS guideline (183) and tried our best to avoid factors that may have an influence on FeNO measurement such as smoking, exercising, eating and drinking. We had an extensive assessment of disease severity and longitudinal follow-up for annual decline in FEV1.

Our study had some limitations that need to be addressed. Our current results are limited due to limited sample size, although the study is still ongoing, and we expect to increase the sample and therefore, enhance the statistical power of the study. We have not a unique definition neither the definition is validated for identifying ACO patients. Finally, although we have

seriously tried to standardize the method of FeNO measurement by using ATS/ERS guideline (183), we should be careful in inferring absolute values obtained in this research center to those obtained in other centers.

In conclusion, although FeNO may have the potential to be used as a biomarker for differentiating ACO from COPD-only, it is still too soon to be able to make a recommendation of using it in clinical practice. FeNO may have a place in a decision algorithm to help select patients who might need a further investigation that is more invasive or more definitive such as sputum induction. Further studies should be conducted using a validated definition of ACO, i.e., having a reference to the type of airway inflammation instead of relying on a clinical definition only.

Acknowledgement

I would like to thank Aerocrine for its support and providing the measurement instrument, Niox Mino, for my project. Also, I would like to thank all the current and past sponsors of CanCOLD, including Canadian Respiratory Research Network (CRRN), Astra Zeneca Canada Ltd, Boehringer Ingelheim Canada Ltd, GlaxoSmithKline Canada Ltd; Novartis, CIHR (CIHR/ Rx&D Collaborative Research Program Operating Grants- 93326), the Respiratory Health Network of the FRSQ. Finally, I would like to thank CanCOLD population to participate in this study.

Tables and Figures

Table 1. Baseline characteristics among all study subjects (COPD, healthy, and at risk).*

Baseline characteristics	Total N=169	Any COPD N=95	GOLD1 N=55	GOLD2+ N=40	At Risk N=51	Healthy N=23
Age, in year	66.8 ± 9.4	67.5 ± 9.4	68.3 ± 9.0	66.3 ± 9.8	66.2 ± 9.1	65.7 ± 10.4
Sex, male gender, n (%)	90 (53.3)	54 (56.8)	34 (61.8)	20 (50.0)	29 (56.9)	7 (30.4)
Smoking status, n (%)						
Never	41 (24.3)	18 (18.9)	13 (23.6)	5 (12.5)	-	23 (100.0)
Former	101 (59.8)	58 (61.1)	33 (60.0)	25 (62.5)	43 (84.3)	-
Current	27 (16.0)	19 (20.0)	9 (16.4)	10 (25.0)	8 (15.7)	-
MRC dyspnea scale score	1.3 ± 0.6	1.5 ± 0.6	1.3 ± 0.5	1.7 ± 0.7	1.2 ± 0.4	1.1 ± 0.3
Pulmonary Function Tests (PFT)						
FEV1, % predicted	90.8 ± 19.8	83.6 ± 19.0	97.3 ± 10.7	65.0 ± 9.5	98.3 ± 17.8	103.6 ± 14.3
FEV1/FVC, %	68.3 ± 10.4	61.4 ± 8.3	65.8 ± 3.9	55.4 ± 8.9	76.9 ± 4.3	77.3 ± 5.1
SGRQ-Total	12.5 ± 13.7	14.5 ± 15.3	10.0 ± 12.5	20.3 ± 16.8	9.3 ± 9.1	3.6 ± 3.5
CAT score	7.0 ± 6.0	8.2 ± 6.7	6.4 ± 5.7	10.7 ± 7.2	5.8 ± 5.0	4.5 ± 3.7
Emphysema score	1.4 ± 3.2	2.1 ± 4.0	1.0 ± 2.3	3.7 ± 5.2	0.4 ± 1.4	0.3 ± 1.0
Respiratory medication reported in the past 12 months, n (%)						

SABA	10 (5.9)	10 (10.5)	5 (9.1)	5 (12.5)	0 (0.0)	0 (0.0)
LABA or LAMA	3 (1.8)	3 (3.2)	2 (3.6)	1 (2.5)	0 (0.0)	0 (0.0)
ICS alone	9 (5.3)	7 (7.4)	2 (3.6)	5 (12.5)	2 (3.9)	0 (0.0)
ICS combined with LABA/LAMA	21 (12.4)	18 (18.9)	3 (5.5)	15 (37.5)	3 (5.9)	0 (0.0)
Any above medications	43 (25.4)	38 (40.0)	12 (21.8)	26 (65.0)	5 (9.8)	0 (0.0)

COPD: Chronic obstructive pulmonary disease; GOLD: Global initiative for chronic obstructive lung disease; MRC: Medical Research Council; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; SGRQ: St George respiratory questionnaire; CAT: COPD assessment test; SABA: Short-acting beta agonist; LABA: long-acting beta agonist; LAMA: Long-acting muscarinic antagonist; ICS: Inhaled corticosteroid;

*Data are presented as mean ± SD unless otherwise specified

Table 2. Baseline biomarkers among all study subjects (COPD, healthy, and at risk).*

Baseline biomarkers	**Total N=169**	**Any COPD N=95**	**GOLD 1 N=55**	**GOLD 2+ N=40**	**At Risk N=51**	**Healthy N=23**
IgE (N=100)	300.1 ± 594.4	331.6 ± 522.6	304.1 ± 574.5	361.0 ± 468.8	283.8 ± 877.2	173.8 ± 217.7
IgE<=240, n (%)	70 (70.0)	40 (62.5)	23 (69.7)	17 (54.8)	19 (82.6)	11 (84.6)
IgE>240, n (%)	30 (30.0)	24 (37.5)	10 (30.3)	14 (45.2)	4 (17.4)	2 (15.4)
Blood Eosinophil Percentage (N=97)	2.5 ± 1.5	2.6 ± 1.6	2.8 ± 1.7	2.4 ± 1.5	1.9 ± 1.2	2.5 ± 1.2
Eosinophil <=4, n (%)	83 (85.6)	51 (81.0)	25 (78.1)	26 (83.9)	20 (95.2)	12 (92.3)
Eosinophil >4, n (%)	14 (14.4)	12 (19.0)	7 (21.9)	5 (16.1)	1 (4.8)	1 (7.7)
Blood Eosinophil Count (N=98)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
Eosinophil <=0.45, n (%)	96 (98.0)	61 (96.8)	31 (96.9)	30 (96.8)	22 (100.0)	13 (100.0)
Eosinophil >0.45, n (%)	2 (2.0)	2 (3.2)	1 (3.1)	1 (3.2)	0 (0.0)	0 (0.0)
FeNO level (ppb)	26.6 ± 14.3	26.8 ± 14.5	25.6 ± 11.9	28.3 ± 17.5	25.6 ± 13.8	28.0 ± 15.1
FeNO classification by quartile, n (%)						
First quartile (ppb<15.5)	39 (23.1)	21 (22.1)	11 (20.0)	10 (25.0)	14 (27.5)	4 (17.4)
Second quartile (23.5>ppb ≥15.5)	44 (26.0)	22 (23.2)	16 (29.1)	6 (15.0)	16 (31.4)	6 (26.1)
Third quartile (34>ppb ≥23.5)	43 (25.4)	31 (32.6)	16 (29.1)	15 (37.5)	7 (13.7)	5 (21.7)
Forth quartile (ppb ≥34)	43 (25.4)	21 (22.1)	12 (21.8)	9 (22.5)	14 (27.5)	8 (34.8)
FeNO Classification by ATS, n (%)						
FeNO <25 ppb, n (%)	90 (53.3)	48 (50.5)	30 (54.5)	18 (45.0)	31 (60.8)	11 (47.8)
50ppb>=FeNO >= 25 ppb, n (%)	66 (39.1)	38 (40.0)	22 (40.0)	16 (40.0)	17 (33.3)	11 (47.8)
FeNO >50 ppb, n (%)	13 (7.7)	9 (9.5)	3 (5.5)	6 (15.0)	3 (5.9)	1 (4.3)

COPD: Chronic obstructive pulmonary disease; IgE: Immunoglobulin E; GOLD: Global Initiative for Chronic Obstructive Lung disease; FeNO: Fractional exhaled nitric oxide; ppb: parts per billion; ATS: American Thoracic Society

*Data are presented as mean ± SD unless otherwise specified

Table 3. Baseline characteristics and FeNO levels by ACO definitions.*

Baseline characteristics	**Definite Non-ACO (COPD-only)**	**Definition1**	**Definition2**	**Definition3**	**P. Value**		
	N=49	**N=18**	**N=36**	**N=15**	**Non-ACO vs. Definition 1**	**Non-ACO vs. Definition 2**	**Non-ACO vs. Definition 3**
Age, in year	67.8 ± 8.9	67.8 ± 10.8	67.4 ± 9.6	67.1 ± 9.5	0.982	0.825	0.773
Sex, male gender, n (%)	32 (65.3)	12 (66.7)	14 (38.9)	5 (33.3)	0.917	0.016**	0.028**
Smoking status, n (%)							
Never	7 (14.3)	5 (27.8)	9 (25.0)	7 (46.7)	0.202	0.212	0.008**
Former	32 (65.3)	9 (50.0)	22 (61.1)	7 (46.7)	0.254	0.691	0.195
Current	10 (20.4)	4 (22.2)	5 (13.9)	1 (6.7)	1.000	0.436	0.434
MRC Dyspnea scale Score	1.3 ± 0.5	1.6 ± 0.5	1.8 ± 0.7	1.6 ± 0.5	0.039**	0.002**	0.041**
Pulmonary Function Tests (PFT)							
FEV1, % predicted	91.4 ± 17.0	80.9 ± 17.3	74.0 ± 17.6	79.8 ± 15.9	0.032**	<0.001**	0.027**
FEV1/FVC, %	64.3 ± 6.0	59.8 ± 7.1	58.0 ± 9.7	61.4 ± 6.5	0.007**	0.001**	0.073
FEV1 reversibility, %	3.4 ± 5.5	20.5 ± 9.0	7.9 ± 11.5	9.5 ± 14.8	<0.001**	0.212	0.303
RV, % predicted	142.2 ± 39.5	163.8 ± 48.9	144.0 ± 39.3	143.9 ± 39.4	0.098	0.866	0.897
FRC, % predicted	132.6 ± 25.7	147.8 ± 38.0	131.5 ± 27.8	126.1 ± 27.5	0.190	0.880	0.457
DLCO, %predicted	120.7 ± 36.8	116.3 ± 27.5	110.3 ± 23.1	114.0 ± 21.2	0.749	0.332	0.689

SGRQ-Total	10.0 ± 10.7	14.0 ± 15.1	22.6 ± 18.4	19.7 ± 16.4	0.531	<0.001**	0.016**
CAT score	6.7 ± 5.5	8.6 ± 6.7	10.9 ± 7.9	9.4 ± 7.5	0.324	0.012**	0.224
Emphysema score	2.4 ± 4.3	1.4 ± 2.5	1.7 ± 3.9	0.4 ± 1.1	0.224	0.070	0.016**
Respiratory medications reported in the past 12 months, n (%)							
SABA	3 (6.1)	2 (11.1)	7 (19.4)	5 (33.3)	0.605	0.060	0.014**
LABA or LAMA	0 (0.0)	0 (0.0)	3 (8.3)	2 (13.3)	-	0.072	0.052
ICS alone	1 (2.0)	2 (11.1)	6 (16.7)	2 (13.3)	0.174	0.038**	0.134
ICS combined with LABA/LAMA	4 (8.2)	3 (16.7)	14 (38.9)	4 (26.7)	0.375	<0.001**	0.079
Any above medications	8 (16.3)	7 (38.9)	30 (83.3)	13 (86.7)	0.05	<0.001**	<0.001**
IgE	316.1 ± 593.6	330.4 ± 553.5	344.3 ± 357.4	407.3 ± 409.2	0.722	0.149	0.074
IgE<=250, n (%)	22 (71.0)	9 (64.3)	11 (47.8)	5 (41.7)	0.654	0.085	0.075
IgE>250, n (%)	9 (29.0)	5 (35.7)	12 (52.2)	7 (58.3)	0.654	0.085	0.075
Blood eosinophil percentage	2.4 ± 1.4	2.3 ± 1.3	3.2 ± 1.9	3.0 ± 2.1	0.821	0.151	0.486
Eosinophil percentage <=4, n (%)	25 (83.3)	11 (78.6)	17 (73.9)	10 (83.3)	0.695	0.402	1.000
Eosinophil percentage >4, n (%)	5 (16.7)	3 (21.4)	6 (26.1)	2 (16.7)	0.695	0.402	1.000
Blood eosinophil count	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.331	0.262	0.867
Eosinophil count <=0.45, n (%)	29 (96.7)	14 (100.0)	22 (95.7)	11 (91.7)	1.000	1.000	0.495
Eosinophil count >0.45, n (%)	1 (3.3)	0 (0.0)	1 (4.3)	1 (8.3)	1.000	1.000	0.495

FeNO level (ppb)	24.8 ± 11.5	32.1 ± 15.7	29.2 ± 18.2	30.0 ± 14.6	0.065	0.373	0.112
FeNO >= 17.5 ppb, n (%)	33 (67.3)	15 (83.3)	26 (72.2)	12 (80.0)	0.198	0.63	0.348
FeNO >= 19 ppb, n (%)	32 (65.3)	15 (83.3)	25 (69.4)	12 (80.0)	0.153	0.688	0.283
FeNO >= 22.5 ppb, n (%)	28 (57.1)	12 (66.7)	22 (61.1)	12 (80.0)	0.481	0.713	0.11
FeNO >= 23 ppb, n (%)	26 (53.1)	12 (66.7)	22 (61.1)	12 (80.0)	0.319	0.46	0.063
FeNO >= 23.5 ppb, n (%)	25 (51.0)	11 (61.1)	22 (61.1)	12 (80.0)	0.463	0.355	0.047**
FeNO >= 29 ppb, n (%)	16 (32.7)	9 (50.0)	14 (38.9)	7 (46.7)	0.193	0.552	0.322
FeNO >= 36 ppb, n (%)	6 (12.2)	6 (33.3)	10 (27.8)	4 (26.7)	0.046**	0.07	0.226

FeNO: Fractional exhaled nitric oxide; ACO: Asthma-COPD overlap; MRC: Medical Research Council; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; RV: Residual volume; FRC: Functional residual capacity; DLCO: Diffusing capacity of the lungs for carbon monoxide; SGRQ: St George respiratory questionnaire; CAT: COPD assessment test; SABA: Short-acting beta agonist; LABA: Long-acting beta agonist; LAMA: Long-acting muscarinic antagonist; ICS: Inhaled corticosteroid; IgE: Immunoglobulin E; FeNO: Fractional exhaled nitric oxide; ppb: parts per billion

*Data are presented as mean ± SD unless otherwise specified; P-value was obtained by performing Chi-square analysis for categorical variables, and T-test or Kruskal-Wallis Test for continuous variables. Definition1: >12% and >200 ml of increment in the FEV1 post-bronchodilator; Definition 2: Physician diagnosis of asthma; Definition 3: Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

**Statistically significant difference: $p< 0.05$

Table 4. The association between FeNO and disease severity/progression among all COPD subjects.*

	FeNO used as continuous variable- per 1 ppb increased		FeNO used as binary variable: the last quartile ≥34 ppb vs. <34 ppb		FeNO used as binary variable: FeNO ≥22.5 ppb vs. < 22.5 ppb	
Outcome	**β (95% CI)/RR (95% CI)**	**p Value**	**β (95% CI)/RR (95% CI)**	**p Value**	**β (95% CI)/RR (95% CI)**	**p Value**
Disease Severity						
FEV1, mL	0.03 (-9.51, 9.58)	0.995	11.84 (-306.92, 330.59)	0.941	49.48 (-222.58, 321.54)	0.719
SGRQ-Total	-0.09 (-0.32, 0.14)	0.457	-3.23 (-11.01, 4.54)	0.411	-1.10 (-7.79, 5.60)	0.746
CAT score	-0.04 (-0.14, 0.06)	0.417	-1.62 (-5.04, 1.80)	0.349	-0.74 (-3.65, 2.18)	0.617
Exacerbation rate	1.00 (0.98, 1.02)	0.913	1.50 (0.73, 3.12)	0.272	0.93 (0.48, 1.79)	0.829
Disease Progression						
FEV1 annual decline, rate, mL/year	-0.24 (-1.37, 0.89)	0.675	-8.64 (-46.38, 29.10)	0.65	-11.35 (-43.54, 20.83)	0.485

FeNO: Fractional exhaled nitric oxide; COPD: Chronic obstructive pulmonary disease; ppb: parts per billion; CI: confidence interval; RR: Risk ratio; mL: Millilitre; FEV1: Forced expiratory volume in 1 second; SGRQ: St George respiratory questionnaire; CAT: COPD assessment test

* Multiple Liner Regression Parameter Estimate β (95%CI) (p-value) adjusted for age, gender, and current smoking

**Multiple GEE models RRs [95%CI] adjusted for age, gender, and current smoking.

Table 1s. Characteristics of the COPD subjects who have FeNO results and those who do not have.

	CanCOLD Subjects with FeNO Level				CanCOLD Subjects without FeNO Level			
Baseline characteristics	**Total N=169**	**Healthy N=23**	**At Risk N=51**	**COPD N=95**	**Total N=851**	**Healthy N=178**	**At Risk N=228**	**COPD N=445**
Age, in year	66.8 ± 9.4‡	65.7 ± 10.4	66.2 ± 9.1	67.5 ± 9.4†	69.8 ± 9.8	69.1 ± 9.1	68.5 ± 10.7	70.7 ± 9.4
Sex, male gender, n (%)	90 (53.3)	7 (30.4)	29 (56.9)	54 (56.8)	464 (54.6)	79 (44.4)	125 (55.1)	260 (58.4)
Smoking status, n (%)								
Never	41 (24.3) ‡	23 (100.0)	-	18 (18.9)**	317 (37.3)	178 (100.0)	-	139 (31.2)
Former	101 (59.8)	-	43 (84.3)	58 (61.1)	442 (51.9)	-	199 (87.3)	243 (54.6)
Current	27 (16.0)	-	8 (15.7)	19 (20.0)	92 (10.8)	-	29 (12.7)	63 (14.2)
Pack years of cigarettes	17.3 ± 23.4	0.0 ± 0.0	16.0 ± 18.4	22.3 ± 26.5	15.0 ± 21.7	0.0 ± 0.0	16.5 ± 21.9	20.4 ± 23.1
MRC dyspnea scale Score	1.3 ± 0.6	1.1 ± 0.3	1.2 ± 0.4	1.5 ± 0.6	1.4 ± 0.6	1.2 ± 0.5	1.3 ± 0.5	1.5 ± 0.7
MRC dyspnea scale Score ≥ 3/5, n (%)	5 (3.1)	-	-	5 (5.4)	36 (4.8)	3 (2.0)	4 (1.9)	29 (7.5)
Pulmonary Function Tests (PFT)								
FEV1, L	2.5 ± 0.8	2.6 ± 0.6	2.8 ± 0.7	2.3 ± 0.8	2.4 ± 0.8	2.6 ± 0.8	2.7 ± 0.7	2.2 ± 0.7
FEV1, % predicted	90.8 ± 19.8	103.6 ± 14.3	98.3 ± 17.8	83.6 ± 19.0	90.9 ± 20.7	102.2 ± 17.1	99.3 ± 16.9	82.2 ± 19.9
FEV1/FVC, %	68.3 ± 10.4	77.3 ± 5.1	76.9 ± 4.3	61.4 ± 8.3	68.5 ± 10.5	77.8 ± 4.8	76.5 ± 4.7	60.9 ± 8.0
FEV1 reversibility, %	5.5 ± 7.7	3.2 ± 4.3	4.4 ± 4.5	6.7 ± 9.4	5.6 ± 7.5	3.3 ± 5.1	3.3 ± 5.3	7.6 ± 8.5
FEV1 annual decline, rate, ml/year	-27.2 ± 62.5†	-8.1 ± 45.6**	-13.4 ± 38.6‡	-39.2 ± 73.2	-44.1 ± 69.3	-38.3 ± 71.3	-39.6 ± 67.8	-48.6 ± 68.9

RV, % predicted	132.2 ± 40.5‡	118.8 ± 27.9	110.7 ± 26.6	147.1 ± 43.0‡	119.8 ± 34.7	114.3 ± 28.5	104.5 ± 25.7	128.4 ± 36.9
VC, % predicted	118.3 ± 18.2	121.7 ± 19.3	117.2 ± 17.9	118.0 ± 18.3	115.7 ± 23.2	119.5 ± 22.6	117.3 ± 23.4	114.2 ± 23.1
FRC, % predicted	125.3 ± 28.6‡	117.6 ± 20.2	110.8 ± 19.1†	135.2 ± 31.0‡	110.9 ± 26.7	108.0 ± 25.6	100.3 ± 23.6	116.8 ± 26.8
TLC, % predicted	123.3 ± 19.3‡	122.3 ± 17.1	114.9 ± 15.0	128.2 ± 20.4‡	116.7 ± 17.5	116.2 ± 21.1	112.0 ± 16.2	119.1 ± 16.9
DLCO, %predicted	115.9 ± 29.7‡	131.1 ± 23.0	109.8 ± 27.5	115.7 ± 31.3‡	105.2 ± 25.9	118.1 ± 27.1	107.8 ± 23.2	101.2 ± 26.0
CVD, n (%)	18 (10.7)**	2 (8.7)	3 (5.9)	13 (13.7)	146 (17.2)	28 (15.7)	33 (14.5)	85 (19.1)
Diagnosed COPD, n (%)	38 (22.5)	0 (0.0)	4 (7.8)	34 (35.8)	209 (24.6)	12 (6.7)	38 (16.7)	159 (35.7)
Physician diagnosed asthma, n (%)	46 (27.2)	0 (0.0)**	10 (19.6)	36 (37.9)	229 (26.9)	30 (16.9)	37 (16.3)	162 (36.4)
SGRQ-Total	12.5 ± 13.7	3.6 ± 3.5	9.3 ± 9.1	14.5 ± 15.3	12.4 ± 13.8	6.3 ± 10.7	9.8 ± 12.2	14.1 ± 14.4
CAT score	7.0 ± 6.0	4.5 ± 3.7	5.8 ± 5.0	8.2 ± 6.7**	6.3 ± 5.7	5.3 ± 4.8	5.8 ± 4.8	6.9 ± 6.4
Emphysema score	1.4 ± 3.2	0.3 ± 1.0	0.4 ± 1.4	2.1 ± 4.0	1.0 ± 2.3	0.2 ± 0.8	0.6 ± 1.4	1.6 ± 2.9
Emphysema (score ≥ 1)	47 (29.2)	2 (10.0)	8 (16.3)	37 (40.2)	221 (27.7)	17 (10.2)	44 (20.9)	160 (38.0)
Bronchiolitis (score ≥ 2)	36 (22.4)**	3 (15.0)	14 (28.6)	19 (20.7)	115 (14.4)	13 (7.8)	36 (17.1)	66 (15.7)
Respiratory medications reported in the past 12 months, n (%)								
SABA	10 (5.9)	0 (0.0)	0 (0.0)	10 (10.5)	53 (6.2)	3 (1.7)	9 (3.9)	41 (9.2)
LABA or LAMA	3 (1.8)	0 (0.0)	0 (0.0)	3 (3.2)	12 (1.4)	1 (0.6)	2 (0.9)	9 (2.0)
ICS alone	9 (5.3)	0 (0.0)	2 (3.9)	7 (7.4)	42 (4.9)	9 (5.1)	7 (3.1)	26 (5.8)
ICS combined with LABA/LAMA	21 (12.4)	0 (0.0)	3 (5.9)	18 (18.9)	107 (12.6)	8 (4.5)	13 (5.7)	86 (19.3)
Any above medications	43 (25.4)	0 (0.0)	5 (9.8)	38 (40.0)	214 (25.1)	21 (11.8)	31 (13.6)	162 (36.4)

Subjects with one or more symptoms-based exacerbation in year 1, n (%)	40 (27.2)	1 (4.8)	11 (25.6)	28 (33.7)	180 (23.4)	27 (16.2)	50 (25.3)	103 (25.6)
Subjects with one or more events-based exacerbation in year 1, n (%)	18 (12.2)	0 (0.0)	5 (11.6)	13 (15.7)	133 (17.3)	23 (13.8)	36 (18.2)	74 (18.4)
Exacerbation rate in year 1, person-year	0.4 ± 0.9	0.0 ± 0.2	0.4 ± 0.8	0.5 ± 1.0	0.3 ± 0.7	0.2 ± 0.4	0.3 ± 0.7	0.4 ± 0.8

COPD: Chronic obstructive pulmonary disease; FeNO: Fractional exhaled nitric oxide; CanCOLD: Canadian Cohort Obstructive Lung Disease; FeNO: Fractional exhaled nitric oxide; COPD: Chronic Obstructive Pulmonary Disease; MRC: Medical Research Council; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; RV: Residual volume; VC: Vital capacity; FRC: Functional residual capacity; TLC: Total lung capacity; DLCO: Diffusing capacity of the lungs for carbon monoxide; CVD: Cardiovascular disease; SGRQ: St George respiratory questionnaire; CAT: COPD assessment test; SABA: Short-acting beta agonist; LABA: Long-acting beta agonist; LAMA: Long-acting muscarinic antagonist; ICS: Inhaled corticosteroid

*Data are presented as mean ± SD unless otherwise specified

Table 2s. Criterion values for FeNO level to predict ACO and coordinates of the ROC curve.

Criterion for FeNO (ppb)	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV	AUC	95% CI
ACO Definition1*								
≥23.5	0.61	0.38 - 0.84	0.50	0.36 - 0.64	0.31	0.77	0.56	0.42 - 0.69
≥25	0.61	0.38 - 0.84	0.58	0.44 - 0.72	0.35	0.80	0.60	0.46 - 0.73
≥34	0.39	0.16 - 0.62	0.88	0.79 - 0.97	0.54	0.79	0.63	0.51 - 0.76
≥36	0.33	0.11 - 0.55	0.90	0.82 - 0.98	0.55	0.78	0.61	0.49 - 0.73
ACO Definition2**								
≥23.5	0.61	0.45 - 0.77	0.50	0.36 - 0.64	0.48	0.63	0.56	0.45 - 0.66
≥25	0.58	0.42 - 0.74	0.58	0.44 - 0.72	0.51	0.65	0.58	0.48 - 0.69
≥34	0.28	0.13 - 0.43	0.88	0.79 - 0.97	0.63	0.62	0.58	0.49 - 0.66
≥36	0.28	0.13 - 0.43	0.90	0.82 - 0.98	0.67	0.62	0.59	0.50 - 0.67
ACO Definition3†								
≥23.5	0.80	0.60 - 1.00	0.50	0.36 - 0.64	0.33	0.89	0.65	0.52 - 0.78
≥25	0.73	0.51 - 0.95	0.58	0.44 - 0.72	0.35	0.88	0.66	0.52 - 0.79
≥34	0.27	0.05 - 0.49	0.88	0.79 - 0.97	0.40	0.79	0.57	0.45 - 0.70
≥36	0.27	0.05 - 0.49	0.90	0.82 - 0.98	0.44	0.80	0.58	0.46 - 0.71

FeNO: Fractional exhaled nitric oxide; ACO: Asthma-COPD overlap; ROC: Receiving operative characteristic; ppb: parts per billion;

CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve

*>12% and >200 ml of increment in the FEV1 post-bronchodilator

**Physician diagnosis of asthma

†Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

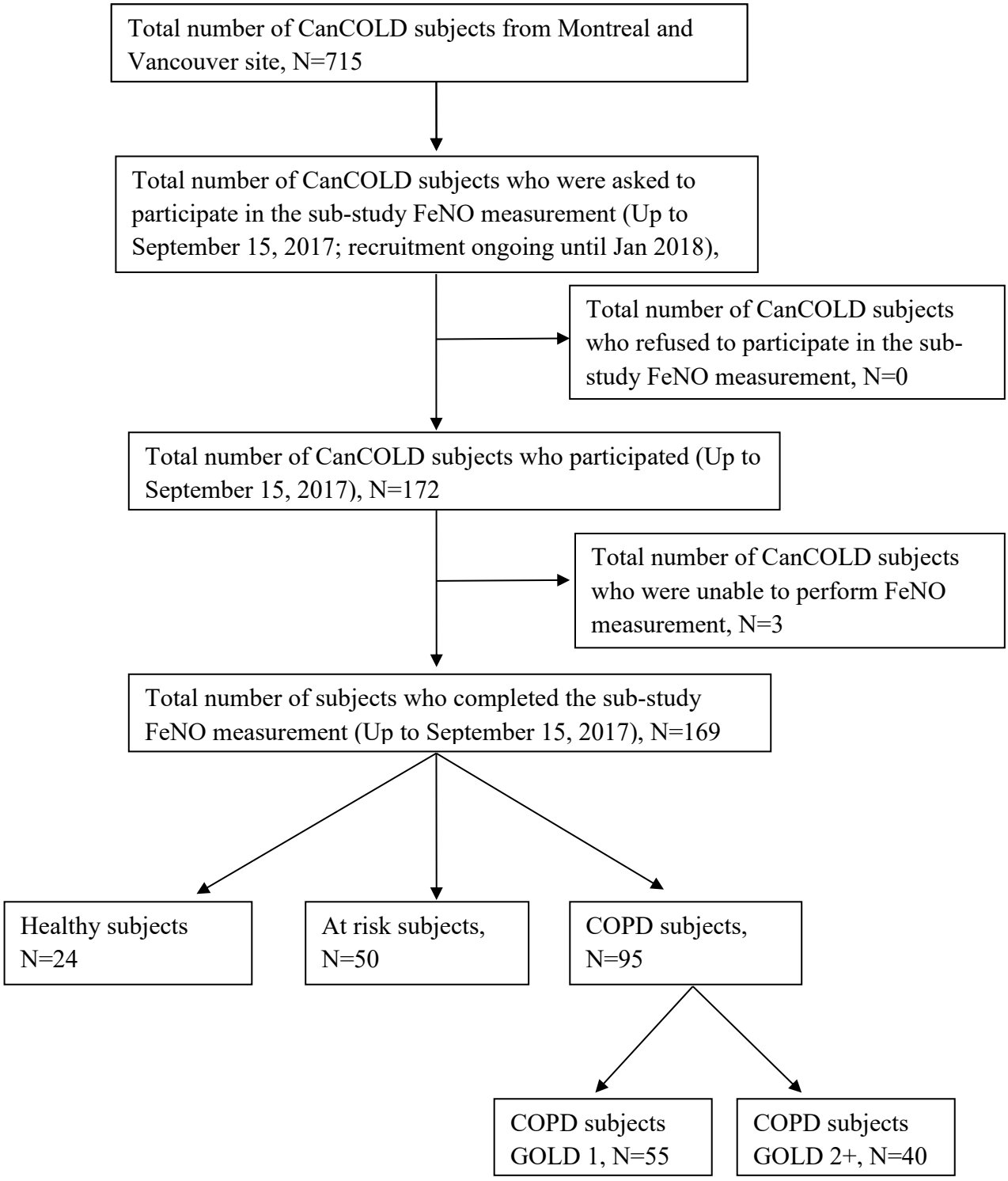

Figure 1. Study participant flow diagram.

CanCOLD: Canadian Cohort Obstructive Lung Disease; FeNO: Fractional exhaled nitric oxide; COPD: Chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease

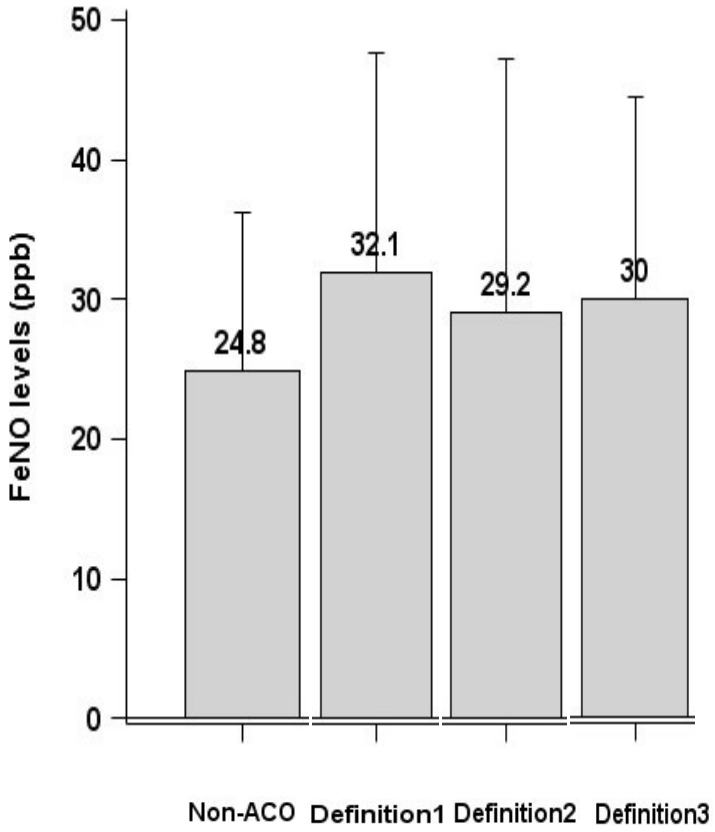

Figure 2. FeNO levels by ACO definitions.

FeNO: Fractional exhaled nitric oxide; ppb: parts per billion; ACO: Asthma-COPD overlap

Note: Definition1: >12% and >200 ml of increment in the FEV1 post-bronchodilator; Definition 2: Physician diagnosis of asthma; Definition 3: Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

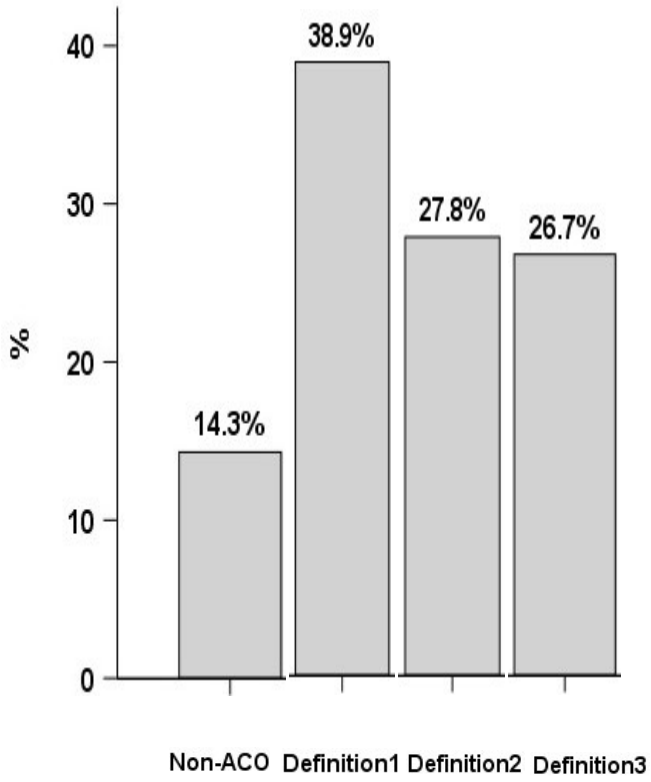

Figure 3. FeNO quartile by ACO definitions.

FeNO: Fractional exhaled nitric oxide; ACO: Asthma-COPD overlap

Note: The last quartile FeNO ≥ 34 parts per billion (ppb). Definition1: >12% and >200 ml of increment in the FEV1 post-bronchodilator; Definition 2: Physician diagnosis of asthma; Definition 3: Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

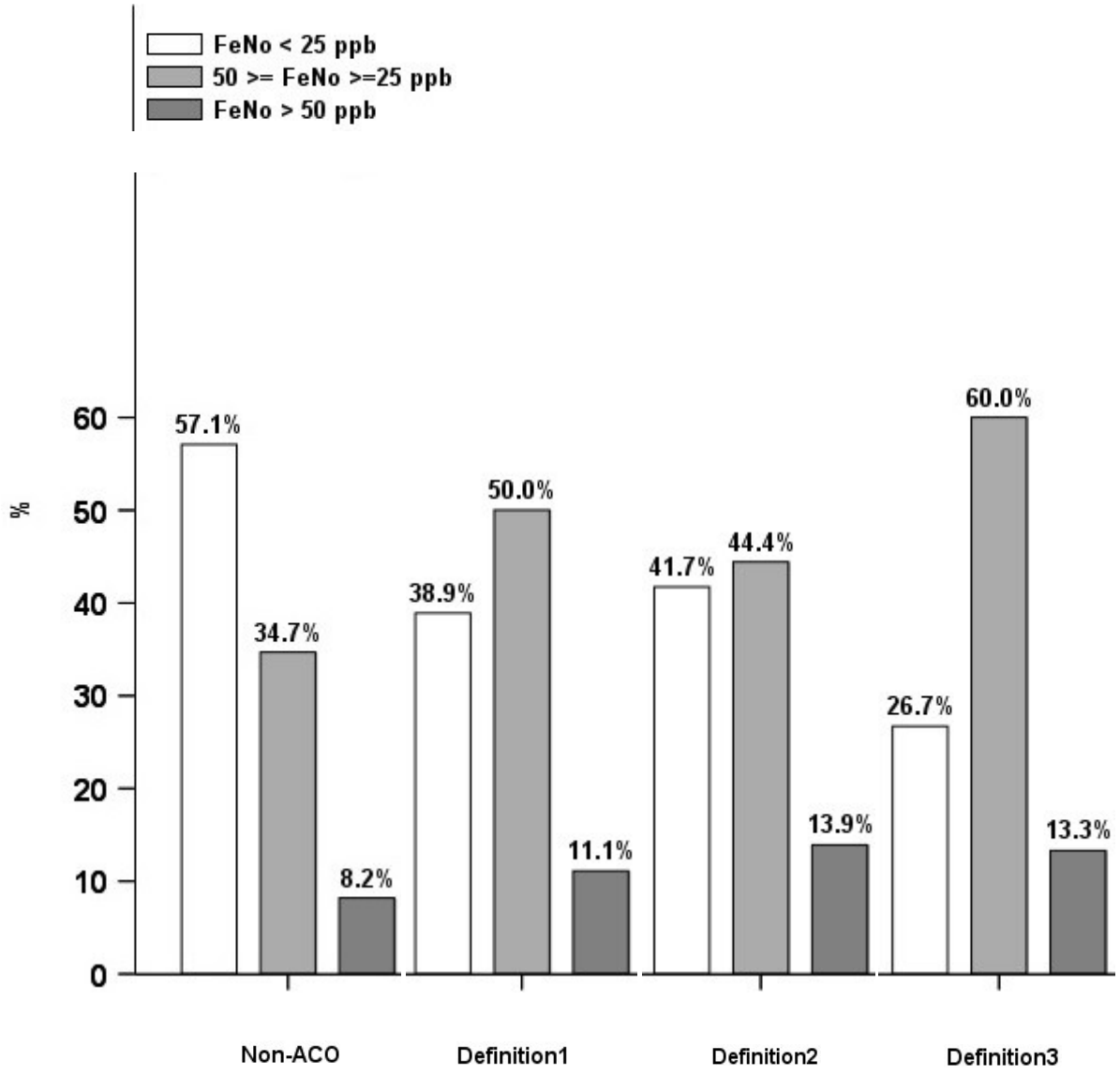

Figure 4. FeNO classification (ATS guideline 2011) by ACO definitions.

FeNO: Fractional exhaled nitric oxide; ppb: parts per billion; ACO: Asthma-COPD overlap

Note: Definition1: >12% and >200 ml of increment in the FEV1 post-bronchodilator; Definition 2: Physician diagnosis of asthma; Definition 3: Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

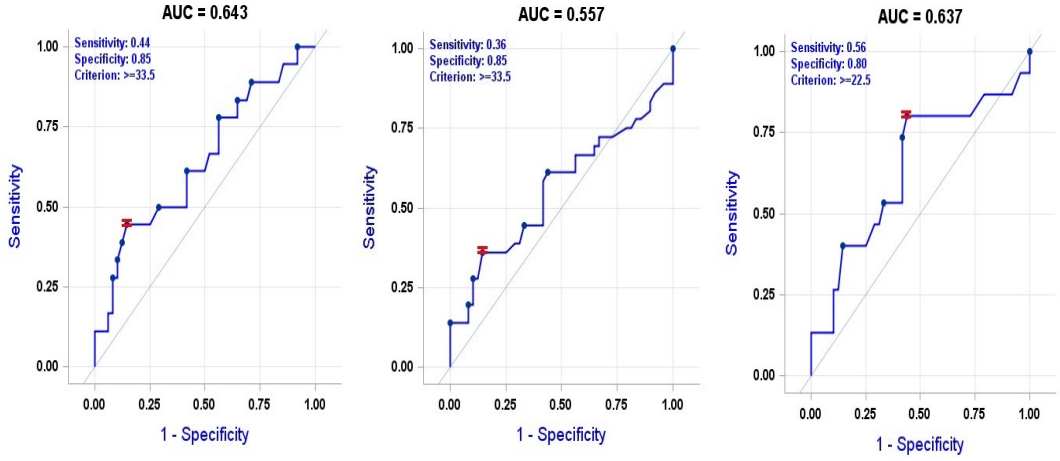

Figure 5a (Definition 1) **Figure 5b (Definition 2)** **Figure 5 c (Definition 3)**

Figure 5. ROC curve analysis of the sensitivity and specificity of FeNO for identifying ACO.

ROC: Receiving operative curve; FeNO: Fractional exhaled nitric oxide; ACO: Asthma-COPD overlap; AUC: Area under the curve

Note: Definition1: >12% and >200 ml of increment in the FEV1 post-bronchodilator; Definition 2: Physician diagnosis of asthma; Definition 3: Atopy and a physician diagnosis of asthma (as reported in a self-reported questionnaire).

CHAPTER 6: SUMMARY OF THE FINDINGS AND FINAL CONCLUSIONS

The major goal of this Master thesis was to investigate exact role of FeNO in COPD patients and find gaps in this research topic. We first attempted to systematically review the available literature in COPD/ACO(S) on FeNO (Manuscript 2-Scoping review). The intent of our scoping review was to present an overview of the existing literature in a field of interest, i.e., FeNO in COPD, and as well synthesize and aggregate findings from different studies. Then, the use of knowledge obtained from the scoping review to better define and understanding of FeNO role in Canadian Cohort Obstructive Lung Disease (CanCOLD) population, especially CanCOLD COPD subjects (Manuscript 3-Observational longitudinal study).

In the scoping review we have found that when measuring FeNO, there are several factors that can affect its measurement. This needs to be considered in clinical setting and research. A majority of studies reported an elevated level of FeNO in COPD/ACO(S) compared to healthy subjects. Unfortunately, no cut-off value could be identified to differentiate COPD/ACO(S) from healthy subjects. Regarding disease severity, most of the studies resulted in no association between FeNO and Global Initiative for Chronic Obstructive Lung Disease (GOLD) classifications (I-IV or ABCD). On the other hand, most of the studies resulted in an association between FeNO and patients having exacerbations. In regard to disease progression, most of the studies reported no association between FeNO pulmonary function tests (PFT). Regarding the differentiation of ACO(S) from COPD, most of the studies reported a higher level of FeNO in ACO than COPD patients. However, different cut-off values were reported which limited its use in clinical practice. None of the inflammatory biomarkers such as IgE and blood eosinophils were associated with FeNO measurements, but studies reported a positive association between FeNO and sputum eosinophils. Finally, in regard to treatment response, all of the studies reported a relationship between FeNO and ICS/GCS treatment response. The evidence is still lacking preventing us from recommending the general use of FeNO in clinical practice for COPD patients. Although FeNO level is higher in ACO(S) patients than COPD-only, it is still unclear if there is a FeNO cut-off that can be used to make the diagnosis of ACO(S) and/or to guide therapy with ICS/GCS in COPD patients.

The lack of studying in defining the role of FeNO in differentiating ACO(S) from COPD and not existing a unique cut-off value for this purpose suggested the need for a further prospective study on FeNO in COPD population. Thus, we have addressed this need with the investigation through the CanCOLD study, especially those with diagnosed mild (GOLD1) and moderate to

severe (GOLD 2+) COPD. According to our prospective CanCOLD study, there was no significant difference in mean FeNO levels among COPD (GOLD1 and GOLD2+), healthy and at risk subjects. In addition, no significant difference was observed in mean FeNO levels between COPD-only (non-ACO) and ACO (all 3 clinical definitions). Regarding FeNO cut-off, 80% of ACO patients (definition 3) had FeNO levels ≥23.5 ppb, which was statistically significant. Furthermore, 33.3% of ACO patients (definition 1) had FeNO ≥36 ppb, which was statistically significant as well. We identified cut-off values of 22.5 and 33.5 ppb for differentiating ACO from COPD-only. However, the area under the curve (AUC) for all identified cut-off values for this study was less than 0.70 demonstrating FeNO is not a good predictor for differentiating ACO from COPD-only. FeNO was not able to predict disease severity and progression.

In conclusion, these studies altogether have implications for the planning of future studies and in guiding diagnostic and therapeutic decisions in a clinical setting, informing on the most appropriate use of novel markers such as the use of FeNO in treatment, especially in the use of respiratory medications and guiding treatment decisions in COPD, especially for those who need the early use of inhaled corticosteroids, i.e. ACO patients. We recommend conducting other studies with higher number of COPD subjects and using all ACO definitions and also testing the potential utility of FeNO combined with other biomarkers in order to differentiate ACO from COPD-only. The main focus of future research should be to determine if FeNO could be part of a cascade of a therapeutic decisional algorithm and/or as an alternative to sputum induction for guiding COPD therapy.

CHAPTER 7: REFERENCES

1. Feng JX, Lin Y, Lin J, He SS, Chen MF, Wu XM, et al. Relationship between Fractional Exhaled Nitric Oxide Level and Efficacy of Inhaled Corticosteroid in Asthma-COPD Overlap Syndrome Patients with Different Disease Severity. J Korean Med Sci. 2017;32(3):439-47.
2. Donohue JF, Herje N, Crater G, Rickard K. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. International journal of chronic obstructive pulmonary disease. 2014;9:745-51.
3. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. American journal of respiratory and critical care medicine. 2011;184(5):602-15.
4. Chen FJ, Huang XY, Liu YL, Lin GP, Xie CM. Importance of fractional exhaled nitric oxide in the differentiation of asthma-COPD overlap syndrome, asthma, and COPD. Int J Chron Obstruct Pulmon Dis. 2016;11:2385-90.
5. Chen YW, Leung JM, Sin DD. A Systematic Review of Diagnostic Biomarkers of COPD Exacerbation. PloS one. 2016;11(7):e0158843.
6. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American journal of respiratory and critical care medicine. 2013;187(4):347-65.
7. Olloquequi J, Garcia-Valero J, Rodriguez E, Montero MA, Ferrer J, Montes JF. Lung CD57+ cell density is increased in very severe COPD. Histology and histopathology. 2012;27(1):39-47.
8. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. Clin Chest Med. 2014;35(1):71-86.
9. National Heart, Lung and Blood Institute, National of Health Institute. What is COPD? 2017. Available at: https://www.nhlbi.nih.gov/health/health-topics/topics/copd/. Accessed August 08, 2017.
10. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2017. Available at: http://goldcopd.org. Accessed August 08, 2017.

11. Silva R, Oyarzun M, Olloquequi J. Pathogenic mechanisms in chronic obstructive pulmonary disease due to biomass smoke exposure. Archivos de bronconeumologia. 2015;51(6):285-92.
12. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet (London, England). 2012;380(9859):2095-128.
13. Raherison C, Girodet PO. Epidemiology of COPD. European respiratory review : an official journal of the European Respiratory Society. 2009;18(114):213-21.
14. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. 2006;3(11):e442.
15. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet (London, England). 2012;380(9859):2197-223.
16. Adeloye D, Chua S, Lee C, Basquill C, Papana A, Theodoratou E, et al. Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. Journal of global health. 2015;5(2):020415.
17. Life and Breath: Respiratory Disease in Canada. 2007. Available at: https://www.canada.ca/en/public-health/services/reports-publications/2007/life-breath-respiratory-disease-canada-2007.html. Accesed August 07, 2017.
18. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, et al. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. Lancet (London, England). 2007;370(9589):741-50.
19. Evans J, Chen Y, Camp PG, Bowie DM, McRae L. Estimating the prevalence of COPD in Canada: Reported diagnosis versus measured airflow obstruction. Health reports. 2014;25(3):3-11.
20. Shirtcliffe P, Weatherall M, Marsh S, Travers J, Hansell A, McNaughton A, et al. COPD prevalence in a random population survey: a matter of definition. The European respiratory journal. 2007;30(2):232-9.
21. MacNee W. Pathology, pathogenesis, and pathophysiology. BMJ : British Medical Journal. 2006;332(7551):1202-4.

22. Pellegrino R, Brusasco V, Viegi G, Crapo RO, Burgos F, Casaburi R, et al. Definition of COPD: based on evidence or opinion? The European respiratory journal. 2008;31(3):681-2.
23. Angelis N, Porpodis K, Zarogoulidis P, Spyratos D, Kioumis I, Papaiwannou A, et al. Airway inflammation in chronic obstructive pulmonary disease. Journal of Thoracic Disease. 2014;6(Suppl 1):S167-S72.
24. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. The New England journal of medicine. 2011;365(17):1567-75.
25. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. American journal of respiratory and critical care medicine. 1995;152(5 Pt 2):S77-121.
26. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, et al. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. The European respiratory journal. 1995;8(8):1398-420.
27. Rennard SI. COPD: overview of definitions, epidemiology, and factors influencing its development. Chest. 1998;113(4 Suppl):235s-41s.
28. Rosenbloom J, Campbell EJ, Mumford R, Saldeen T, Senior RM, Starcher B, et al. Biochemical/immunologic markers of emphysema. Annals of the New York Academy of Sciences. 1991;624 Suppl:7-12.
29. O'Brien C, Guest PJ, Hill SL, Stockley RA. Physiological and radiological characterisation of patients diagnosed with chronic obstructive pulmonary disease in primary care. Thorax. 2000;55(8):635-42.
30. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. The New England journal of medicine. 2015;373(13):1241-9.
31. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA). 2017. Available at: www.ginasthma.org. Accessed August 08, 2017.
32. Siddiqui SH, Guasconi A, Vestbo J, Jones P, Agusti A, Paggiaro P, et al. Blood Eosinophils: A Biomarker of Response to Extrafine Beclomethasone/Formoterol in Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2015;192(4):523-5.
33. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol

in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. The Lancet Respiratory medicine. 2015;3(6):435-42.
34. Castaldi PJ, San Jose Estepar R, Mendoza CS, Hersh CP, Laird N, Crapo JD, et al. Distinct quantitative computed tomography emphysema patterns are associated with physiology and function in smokers. American journal of respiratory and critical care medicine. 2013;188(9):1083-90.
35. Jackson H, Hubbard R. Detecting chronic obstructive pulmonary disease using peak flow rate: cross sectional survey. BMJ (Clinical research ed). 2003;327(7416):653-4.
36. Willemse BW, Postma DS, Timens W, ten Hacken NH. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. The European respiratory journal. 2004;23(3):464-76.
37. Wu J, Sin DD. Improved patient outcome with smoking cessation: when is it too late? International Journal of Chronic Obstructive Pulmonary Disease. 2011;6:259-67.
38. Burge PS, Calverley PM, Jones PW, Spencer S, Anderson JA, Maslen TK. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. BMJ (Clinical research ed). 2000;320(7245):1297-303.
39. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. The New England journal of medicine. 2008;359(15):1543-54.
40. Melani AS. Long-acting muscarinic antagonists. Expert review of clinical pharmacology. 2015;8(4):479-501.
41. Ejiofor S, Turner AM. Pharmacotherapies for COPD. Clinical Medicine Insights Circulatory, Respiratory and Pulmonary Medicine. 2013;7:17-34.
42. Cazzola M, Molimard M. The scientific rationale for combining long-acting beta2-agonists and muscarinic antagonists in COPD. Pulmonary pharmacology & therapeutics. 2010;23(4):257-67.
43. Nannini LJ, Cates CJ, Lasserson TJ, Poole P. Combined corticosteroid and long-acting beta-agonist in one inhaler versus long-acting beta-agonists for chronic obstructive pulmonary disease. The Cochrane database of systematic reviews. 2007(4):Cd006829.
44. Nannini LJ, Poole P, Milan SJ, Kesterton A. Combined corticosteroid and long-acting beta(2)-agonist in one inhaler versus inhaled corticosteroids alone for chronic obstructive pulmonary disease. The Cochrane database of systematic reviews. 2013(8):Cd006826.

45. Wedzicha JA, Banerji D, Chapman KR, Vestbo J, Roche N, Ayers RT, et al. Indacaterol–Glycopyrronium versus Salmeterol–Fluticasone for COPD. New England Journal of Medicine. 2016;374(23):2222-34.
46. Mosenifar Z, Harrington A, Nikhanj N, Kamangar N. Chronic Obstructive Pulmonary Disease (COPD) Treatment & Management. Medscape. 2017.
47. Bonini M, Usmani OS. The importance of inhaler devices in the treatment of COPD. COPD Research and Practice. 2015;1(1):9.
48. Global Initiative for Asthma. Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS). A joint project of GINA and GOLD. 2015. Available at: www.ginasthma.org. Accessed August 12, 2017. .
49. Barnes PJ. Asthma-COPD Overlap. Chest. 2016;149(1):7-8.
50. Barrecheguren M, Esquinas C, Miravitlles M. The asthma-COPD overlap syndrome: a new entity? COPD Research and Practice. 2015;1(1):8.
51. Gibson PG, Simpson JL. The overlap syndrome of asthma and COPD: what are its features and how important is it? Thorax. 2009;64(8):728-35.
52. van den Berge M, Aalbers R. The asthma-COPD overlap syndrome: how is it defined and what are its clinical implications? Journal of asthma and allergy. 2016;9:27-35.
53. Koblizek V, Chlumsky J, Zindr V, Neumannova K, Zatloukal J, Zak J, et al. Chronic Obstructive Pulmonary Disease: official diagnosis and treatment guidelines of the Czech Pneumological and Phthisiological Society; a novel phenotypic approach to COPD with patient-oriented care. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2013;157(2):189-201.
54. Soler-Cataluna JJ, Cosio B, Izquierdo JL, Lopez-Campos JL, Marin JM, Aguero R, et al. Consensus document on the overlap phenotype COPD-asthma in COPD. Archivos de bronconeumologia. 2012;48(9):331-7.
55. Standardization of Spirometry, 1994 Update. American Thoracic Society. American journal of respiratory and critical care medicine. 1995;152(3):1107-36.
56. GINA-GOLD Diagnosis of disease of chronic airflow limitation: Asthma, COPD and asthma-COPD overlap syndrome (ACOS). 2014.
57. Calverley PM, Burge PS, Spencer S, Anderson JA, Jones PW. Bronchodilator reversibility testing in chronic obstructive pulmonary disease. Thorax. 2003;58(8):659-64.
58. Orie NGM SH, de Vries K, Tammeling GJ, Witkop J. The host factor in bronchitis. The host factor in bronchitis. In: Orie NGM, Sluiter HJ, editors. Bronchitis. Assen, The Netherlands: Royal Van Gorcum. 1961:43-59.

59. Barnes PJ. Against the Dutch hypothesis: asthma and chronic obstructive pulmonary disease are distinct diseases. American journal of respiratory and critical care medicine. 2006;174(3):240-3; discussion 3-4.
60. Ghebre MA, Bafadhel M, Desai D, Cohen SE, Newbold P, Rapley L, et al. Biological clustering supports both "Dutch" and "British" hypotheses of asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2015;135(1):63-72.
61. Kostikas K, Clemens A, Patalano F. The asthma-COPD overlap syndrome: do we really need another syndrome in the already complex matrix of airway disease? Int J Chron Obstruct Pulmon Dis. 2016;11:1297-306.
62. Alshabanat A, Zafari Z, Albanyan O, Dairi M, FitzGerald JM. Asthma and COPD Overlap Syndrome (ACOS): A Systematic Review and Meta Analysis. PloS one. 2015;10(9):e0136065.
63. Mirabelli MC, Beavers SF, Chatterjee AB. Active asthma and the prevalence of physician-diagnosed COPD. Lung. 2014;192(5):693-700.
64. Tai A, Tran H, Roberts M, Clarke N, Wilson J, Robertson CF. The association between childhood asthma and adult chronic obstructive pulmonary disease. Thorax. 2014;69(9):805-10.
65. Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest. 2004;126(1):59-65.
66. de Marco R, Marcon A, Rossi A, Anto JM, Cerveri I, Gislason T, et al. Asthma, COPD and overlap syndrome: a longitudinal study in young European adults. The European respiratory journal. 2015;46(3):671-9.
67. Golpe R, Perez de Llano L. Are the Diagnostic Criteria for Asthma-COPD Overlap Syndrome Appropriate in Biomass Smoke-Induced chronic obstructive pulmonary disease? Archivos de bronconeumologia. 2016;52(2):110.
68. Miravitlles M, Soler-Cataluna JJ, Calle M, Molina J, Almagro P, Quintano JA, et al. Spanish COPD Guidelines (GesEPOC): pharmacological treatment of stable COPD. Spanish Society of Pulmonology and Thoracic Surgery. Archivos de bronconeumologia. 2012;48(7):247-57.
69. Bobolea I, Pérez de Llano LA. Asthma-COPD Overlap Syndrome (ACOS): Current Understanding and Future Perspectives, Asthma - From Childhood Asthma to ACOS Phenotypes, Prof. Celso Pereira (Ed.), InTech. 2016.

70. Verkindre C, Bart F, Aguilaniu B, Fortin F, Guerin JC, Le Merre C, et al. The effect of tiotropium on hyperinflation and exercise capacity in chronic obstructive pulmonary disease. Respiration. 2006;73(4):420-7.
71. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, et al. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. The European respiratory journal. 2016;48(3):664-73.
72. Brightling CE, McKenna S, Hargadon B, Birring S, Green R, Siva R, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. Thorax. 2005;60(3):193-8.
73. Fujimoto K, Kubo K, Yamamoto H, Yamaguchi S, Matsuzawa Y. Eosinophilic inflammation in the airway is related to glucocorticoid reversibility in patients with pulmonary emphysema. Chest. 1999;115(3):697-702.
74. Kitaguchi Y, Komatsu Y, Fujimoto K, Hanaoka M, Kubo K. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. Int J Chron Obstruct Pulmon Dis. 2012;7:283-9.
75. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. J Allergy Clin Immunol. 2014;133(6):1557-63.e5.
76. Ravensberg AJ, Slats AM, van Wetering S, Janssen K, van Wijngaarden S, de Jeu R, et al. CD8(+) T cells characterize early smoking-related airway pathology in patients with asthma. Respir Med. 2013;107(7):959-66.
77. Proklou A, Soulitzis N, Neofytou E, Rovina N, Zervas E, Gaga M, et al. Granule cytotoxic activity and oxidative DNA damage in smoking and nonsmoking patients with asthma. Chest. 2013;144(4):1230-7.
78. Carpagnano G, Lacedonia D, Malerba M, Palmiotti G, Cotugno G, Carone M, et al. Analysis of mitochondrial DNA alteration in new phenotype ACOS. BMC pulmonary medicine. 2016;16(1):31.
79. Hardin M, Cho M, McDonald ML, Beaty T, Ramsdell J, Bhatt S, et al. The clinical and genetic features of COPD-asthma overlap syndrome. The European respiratory journal. 2014;44(2):341-50.
80. Bujarski S, Parulekar AD, Sharafkhaneh A, Hanania NA. The Asthma COPD Overlap Syndrome (ACOS). Current allergy and asthma reports. 2015;15(3):7.
81. Hardin M, Silverman EK, Barr RG, Hansel NN, Schroeder JD, Make BJ, et al. The clinical features of the overlap between COPD and asthma. Respir Res. 2011;12:127.

82. Marsh SE, Travers J, Weatherall M, Williams MV, Aldington S, Shirtcliffe PM, et al. Proportional classifications of COPD phenotypes. Thorax. 2008;63(9):761.
83. Menezes AMB, Montes de Oca M, Perez-Padilla R, Nadeau G, Wehrmeister FC, Lopez-Varela MV, et al. Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma. Chest. 2014;145(2):297-304.
84. Louie S, Zeki AA, Schivo M, Chan AL, Yoneda KY, Avdalovic M, et al. The asthma-chronic obstructive pulmonary disease overlap syndrome: pharmacotherapeutic considerations. Expert review of clinical pharmacology. 2013;6(2):197-219.
85. Miravitlles M. The overlap syndrome between asthma and COPD: implications for management. Hot Topics Respir Med. 2011;6(16):15-20.
86. Louie S, Zeki AA, Schivo M, Chan AL, Yoneda KY, Avdalovic M, et al. The asthma–chronic obstructive pulmonary disease overlap syndrome: pharmacotherapeutic considerations. Expert review of clinical pharmacology. 2013;6(2):197-219.
87. Miravitlles M, Huerta A, Fernandez-Villar JA, Alcazar B, Villa G, Forne C, et al. Generic utilities in chronic obstructive pulmonary disease patients stratified according to different staging systems. Health Qual Life Outcomes. 2014;12:120.
88. Miravitlles M, Soriano JB, Ancochea J, Munoz L, Duran-Tauleria E, Sanchez G, et al. Characterisation of the overlap COPD-asthma phenotype. Focus on physical activity and health status. Respir Med. 2013;107(7):1053-60.
89. Han MK, Agusti A, Calverley PM, Celli BR, Criner G, Curtis JL, et al. Chronic obstructive pulmonary disease phenotypes: the future of COPD. American journal of respiratory and critical care medicine. 2010;182(5):598-604.
90. de Marco R, Pesce G, Marcon A, Accordini S, Antonicelli L, Bugiani M, et al. The coexistence of asthma and chronic obstructive pulmonary disease (COPD): prevalence and risk factors in young, middle-aged and elderly people from the general population. PloS one. 2013;8(5):e62985.
91. Soriano JB, Davis KJ, Coleman B, Visick G, Mannino D, Pride NB. The proportional Venn diagram of obstructive lung disease: two approximations from the United States and the United Kingdom. Chest. 2003;124(2):474-81.
92. Gibson PG, McDonald VM. Asthma-COPD overlap 2015: now we are six. Thorax. 2015;70(7):683-91.
93. GINA Report, Global Strategy for Asthma Management and Prevention. 2015. Available at: www.ginasthma.org. Accessed August 15, 2017.

94. Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2003;167(3):418-24.
95. Tashkin DP, Celli B, Decramer M, Liu D, Burkhart D, Cassino C, et al. Bronchodilator responsiveness in patients with COPD. The European respiratory journal. 2008;31(4):742-50.
96. Weatherall M, Travers J, Shirtcliffe PM, Marsh SE, Williams MV, Nowitz MR, et al. Distinct clinical phenotypes of airways disease defined by cluster analysis. The European respiratory journal. 2009;34(4):812-8.
97. Plaza V, Alvarez F, Calle M, Casanova C, Cosio BG, Lopez-Vina A, et al. Consensus on the Asthma-COPD Overlap Syndrome (ACOS) Between the Spanish COPD Guidelines (GesEPOC) and the Spanish Guidelines on the Management of Asthma (GEMA). Archivos de bronconeumologia. 2017;53(8):443-9.
98. Ding B, Enstone A. Asthma and chronic obstructive pulmonary disease overlap syndrome (ACOS): structured literature review and physician insights. Expert Rev Respir Med. 2016;10(3):363-71.
99. Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:479-87.
100. Zeki AA, Schivo M, Chan A, Albertson TE, Louie S. The Asthma-COPD Overlap Syndrome: A Common Clinical Problem in the Elderly. Journal of allergy. 2011;2011:861926.
101. Bujarski S, Parulekar AD, Sharafkhaneh A, Hanania NA. The asthma COPD overlap syndrome (ACOS). Current allergy and asthma reports. 2015;15(3):509.
102. Hardin M, Silverman EK, Barr RG, Hansel NH, Schroeder JD, Make BJ. The clinical features of overlap between COPD and asthma. Respir Res. 2011;12.
103. Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, et al. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2000;162(5):1773-7.
104. Miravitlles M, Soriano JB, Ancochea J, Muñoz L, Duran-Tauleria E, Sánchez G, et al. Characterisation of the overlap COPD–asthma phenotype. Focus on physical activity and health status. Respiratory Medicine. 2013;107(7):1053-60.

105. Caillaud D, Chanez P, Escamilla R, Burgel PR, Court-Fortune I, Nesme-Meyer P, et al. Asthma-COPD overlap syndrome (ACOS) vs 'pure' COPD: a distinct phenotype? Allergy. 2017;72(1):137-45.
106. Rhee CK, Yoon HK, Yoo KH, Kim YS, Lee SW, Park YB, et al. Medical utilization and cost in patients with overlap syndrome of chronic obstructive pulmonary disease and asthma. Copd. 2014;11(2):163-70.
107. Kiljander T, Helin T, Venho K, Jaakkola A, Lehtimaki L. Prevalence of asthma-COPD overlap syndrome among primary care asthmatics with a smoking history: a cross-sectional study. NPJ primary care respiratory medicine. 2015;25:15047.
108. Gerhardsson de Verdier M, Andersson M, Kern DM, Zhou S, Tunceli O. Asthma and Chronic Obstructive Pulmonary Disease Overlap Syndrome: Doubled Costs Compared with Patients with Asthma Alone. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2015;18(6):759-66.
109. Gao Y, Zhai X, Li K, Zhang H, Wang Y, Lu Y, et al. Asthma COPD Overlap Syndrome on CT Densitometry: A Distinct Phenotype from COPD. Copd. 2016;13(4):471-6.
110. Chiba S, Tsuchiya K, Nukui Y, Sema M, Tamaoka M, Sumi Y, et al. Interstitial Changes in Asthma-COPD Overlap Syndrome (ACOS). The clinical respiratory journal. 2016.
111. Suzuki T, Tada Y, Kawata N, Matsuura Y, Ikari J, Kasahara Y, et al. Clinical, physiological, and radiological features of asthma–chronic obstructive pulmonary disease overlap syndrome. International Journal of Chronic Obstructive Pulmonary Disease. 2015;10:947-54.
112. Leung JM, Sin DD. Asthma-COPD overlap syndrome: pathogenesis, clinical features, and therapeutic targets. BMJ (Clinical research ed). 2017;358:j3772.
113. Miravitlles M, Alcázar B, Alvarez FJ, Bazús T, Calle M, Casanova C, et al. What pulmonologists think about the asthma–COPD overlap syndrome. International journal of chronic obstructive pulmonary disease. 2015;10:1321.
114. O'donnell DE, Aaron S, Bourbeau J, Hernandez P, Marciniuk DD, Balter M, et al. Canadian Thoracic Society recommendations for management of chronic obstructive pulmonary disease–2007 update. Canadian Respiratory Journal. 2007;14(Suppl B):5B-32B.
115. Nagai A, Aizawa H, Aoshiba K, Asano K, Hirata K, Ichinose M, et al. Guidelines for the diagnosis and treatment of COPD. Japanise Respiratory Society. 2009;2(3):56-8.
116. Koblizek V, Chlumsky J, Zindr V, Neumannova K, Zatloukal J, Jaroslav Z, et al. Chronic obstructive pulmonary disease: official diagnosis and treatment guidelines of the

Czech Pneumological and Phthisiological Society; a novel phenotypic approach to COPD with patient-oriented care. Biomedical Papers. 2013;157(2):189-201.
117. Kankaanranta H, Harju T, Kilpelainen M, Mazur W, Lehto JT, Katajisto M, et al. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the finnish guidelines. Basic & clinical pharmacology & toxicology. 2015;116(4):291-307.
118. Brightling CE, Bleecker ER, Panettieri RA, Jr., Bafadhel M, She D, Ward CK, et al. Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study. The Lancet Respiratory medicine. 2014;2(11):891-901.
119. De Gruttola VG, Clax P, DeMets DL, Downing GJ, Ellenberg SS, Friedman L, et al. Considerations in the evaluation of surrogate endpoints in clinical trials. summary of a National Institutes of Health workshop. Controlled clinical trials. 2001;22(5):485-502.
120. Leung JM, Sin DD. Biomarkers in airway diseases. Canadian Respiratory Journal : Journal of the Canadian Thoracic Society. 2013;20(3):180-2.
121. Jones PW. Health status and the spiral of decline. Copd. 2009;6(1):59-63.
122. Platts-Mills TAE. The Role of Immunoglobulin E in Allergy and Asthma. American journal of respiratory and critical care medicine. 2001;164(supplement_1):S1-S5.
123. Nair P, O'Byrne PM. Measuring Eosinophils to Make Treatment Decisions in Asthma. Chest. 2016;150(3):485-7.
124. Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood Eosinophil Count Is a Useful Biomarker to Identify Patients with Severe Eosinophilic Asthma. Annals of the American Thoracic Society. 2014;11(4):531-6.
125. Paone G, Leone V, Conti V, De Marchis L, Ialleni E, Graziani C, et al. Blood and sputum biomarkers in COPD and asthma: a review. European review for medical and pharmacological sciences. 2016;20(4):698-708.
126. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology (Carlton, Vic). 2006;11(1):54-61.
127. Seys SF, Grabowski M, Adriaensen W, Decraene A, Dilissen E, Vanoirbeek JA, et al. Sputum cytokine mapping reveals an 'IL-5, IL-17A, IL-25-high' pattern associated with poorly controlled asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2013;43(9):1009-17.
128. Tsilogianni Z, Hillas G, Bakakos P, Aggelakis L, Konstantellou E, Papaioannou AI, et al. Sputum interleukin-13 as a biomarker for the evaluation of asthma control. Clinical and

experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2016;46(7):923-31.
129. Dasgupta A, Nair P. When are biomarkers useful in the management of airway diseases? Polskie Archiwum Medycyny Wewnetrznej. 2013;123(4):183-8.
130. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2):115-20.
131. Nair P, Kraft M. Serum periostin as a marker of T(H)2-dependent eosinophilic airway inflammation. J Allergy Clin Immunol. 2012;130(3):655-6.
132. Ahmad Al Obaidi AH, Mohamed Al Samarai AG, Yahya Al Samarai AK, Al Janabi JM. The Predictive Value of IgE as Biomarker in Asthma. Journal of Asthma. 2008;45(8):654-63.
133. Pelaia G, Terracciano R, Vatrella A, Gallelli L, Busceti MT, Calabrese C, et al. Application of proteomics and peptidomics to COPD. BioMed research international. 2014;2014:764581.
134. Shaw JG, Vaughan A, Dent AG, O'Hare PE, Goh F, Bowman RV, et al. Biomarkers of progression of chronic obstructive pulmonary disease (COPD). J Thorac Dis. 2014;6(11):1532-47.
135. Faner R, Tal-Singer R, Riley JH, Celli B, Vestbo J, MacNee W, et al. Lessons from ECLIPSE: a review of COPD biomarkers. Thorax. 2014;69(7):666-72.
136. Celli BR. Predictors of mortality in COPD. Respir Med. 2010;104(6):773-9.
137. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nature reviews Immunology. 2008;8(3):183-92.
138. Cazzola M, Novelli G. Biomarkers in COPD. Pulmonary pharmacology & therapeutics. 2010;23(6):493-500.
139. Hacievliyagil SS, Mutlu LC, Temel I. Airway inflammatory markers in chronic obstructive pulmonary disease patients and healthy smokers. Nigerian journal of clinical practice. 2013;16(1):76-81.
140. Sapey E, Ahmad A, Bayley D, Newbold P, Snell N, Rugman P, et al. Imbalances between interleukin-1 and tumor necrosis factor agonists and antagonists in stable COPD. Journal of clinical immunology. 2009;29(4):508-16.
141. Koutsokera A, Kostikas K, Nicod LP, Fitting JW. Pulmonary biomarkers in COPD exacerbations: a systematic review. Respir Res. 2013;14:111.

142. Stockley RA. Biomarkers in chronic obstructive pulmonary disease: confusing or useful? Int J Chron Obstruct Pulmon Dis. 2014;9:163-77.
143. Ravi AK, Khurana S, Lemon J, Plumb J, Booth G, Healy L, et al. Increased levels of soluble interleukin-6 receptor and CCL3 in COPD sputum. Respir Res. 2014;15:103.
144. Kaur M, Singh D. Neutrophil chemotaxis caused by chronic obstructive pulmonary disease alveolar macrophages: the role of CXCL8 and the receptors CXCR1/CXCR2. The Journal of pharmacology and experimental therapeutics. 2013;347(1):173-80.
145. Costa C, Rufino R, Traves SL, Lapa ESJR, Barnes PJ, Donnelly LE. CXCR3 and CCR5 chemokines in induced sputum from patients with COPD. Chest. 2008;133(1):26-33.
146. Comandini A, Rogliani P, Nunziata A, Cazzola M, Curradi G, Saltini C. Biomarkers of lung damage associated with tobacco smoke in induced sputum. Respir Med. 2009;103(11):1592-613.
147. Zanini A, Chetta A, Imperatori AS, Spanevello A, Olivieri D. The role of the bronchial microvasculature in the airway remodelling in asthma and COPD. Respir Res. 2010;11:132.
148. Zhu A, Ge D, Zhang J, Teng Y, Yuan C, Huang M, et al. Sputum myeloperoxidase in chronic obstructive pulmonary disease. European journal of medical research. 2014;19:12.
149. Ilumets H, Rytila P, Demedts I, Brusselle GG, Sovijarvi A, Myllarniemi M, et al. Matrix metalloproteinases -8, -9 and -12 in smokers and patients with stage 0 COPD. Int J Chron Obstruct Pulmon Dis. 2007;2(3):369-79.
150. Tufvesson E, Ekberg M, Bjermer L. Inflammatory biomarkers in sputum predict COPD exacerbations. Lung. 2013;191(4):413-6.
151. Dickens JA, Miller BE, Edwards LD, Silverman EK, Lomas DA, Tal-Singer R. COPD association and repeatability of blood biomarkers in the ECLIPSE cohort. Respir Res. 2011;12:146.
152. Lomas DA, Silverman EK, Edwards LD, Locantore NW, Miller BE, Horstman DH, et al. Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD. The European respiratory journal. 2009;34(1):95-102.
153. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, Tal-Singer R, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. The New England journal of medicine. 2010;363(12):1128-38.
154. Duvoix A, Dickens J, Haq I, Mannino D, Miller B, Tal-Singer R, et al. Blood fibrinogen as a biomarker of chronic obstructive pulmonary disease. Thorax. 2013;68(7):670-6.

155. Engstrom G, Segelstorm N, Ekberg-Aronsson M, Nilsson PM, Lindgarde F, Lofdahl CG. Plasma markers of inflammation and incidence of hospitalisations for COPD: results from a population-based cohort study. Thorax. 2009;64(3):211-5.
156. Valvi D, Mannino DM, Mullerova H, Tal-Singer R. Fibrinogen, chronic obstructive pulmonary disease (COPD) and outcomes in two United States cohorts. Int J Chron Obstruct Pulmon Dis. 2012;7:173-82.
157. Montano M, Sansores RH, Becerril C, Cisneros J, Gonzalez-Avila G, Sommer B, et al. FEV1 inversely correlates with metalloproteinases 1, 7, 9 and CRP in COPD by biomass smoke exposure. Respir Res. 2014;15:74.
158. Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. Respir Med. 2009;103(8):1231-8.
159. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. American journal of respiratory and critical care medicine. 2011;184(6):662-71.
160. Price D, Rigazio A, Postma D, Papi A, Guy B, Agusti A, et al. Blood eosinophilia and the number of exacerbations in COPD patients. European Respiratory Journal. 2014;44(Suppl 58).
161. Barnes NC, Sharma R, Lettis S, Calverley PM. Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. The European respiratory journal. 2016;47(5):1374-82.
162. Malerba M, Radaeli A, Olivini A, Damiani G, Ragnoli B, Montuschi P, et al. Exhaled nitric oxide as a biomarker in COPD and related comorbidities. BioMed research international. 2014;2014:271918.
163. Rawy AM, Mansour AI. Fraction of exhaled nitric oxide measurement as a biomarker in asthma and COPD compared with local and systemic inflammatory markers. Egyptian Journal of Chest Diseases and Tuberculosis. 2015;64(1):13-20.
164. Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. Immunology today. 1995;16(3):128-30.
165. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. The Journal of biological chemistry. 1994;269(19):13725-8.
166. Yates DH. Role of exhaled nitric oxide in asthma. Immunol Cell Biol. 2001;79(2):178-90.

167. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. Physiological reviews. 2004;84(3):731-65.
168. Ward JK, Barnes PJ, Springall DR, Abelli L, Tadjkarimi S, Yacoub MH, et al. Distribution of human i-NANC bronchodilator and nitric oxide-immunoreactive nerves. American journal of respiratory cell and molecular biology. 1995;13(2):175-84.
169. Barnes PJ, Kharitonov SA. Exhaled nitric oxide: a new lung function test. Thorax. 1996;51(3):233-7.
170. Abba AA. Exhaled nitric oxide in diagnosis and management of respiratory diseases. Annals of Thoracic Medicine. 2009;4(4):173-81.
171. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respiratory Medicine. 2014;108(6):830-41.
172. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respiratory medicine. 2014;108(6):830-41.
173. Ludviksdottir D, Diamant Z, Alving K, Bjermer L, Malinovschi A. Clinical aspects of using exhaled NO in asthma diagnosis and management. The clinical respiratory journal. 2012;6(4):193-207.
174. Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide. Eur Respir Monogr 2010;49:1-31.
175. Mahr TA, Malka J, Spahn JD. Inflammometry in pediatric asthma: A review of fractional exhaled nitric oxide in clinical practice. Allergy and Asthma Proceedings. 2013;34(3):210-9.
176. Pérez-de-Llano LA, Carballada F, Castro Añón O, Pizarro M, Golpe R, Baloira A, et al. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. European Respiratory Journal. 2010;35(6):1221.
177. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, et al. Exhaled Nitric Oxide. American journal of respiratory and critical care medicine. 2005;172(4):453-9.
178. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. Journal of Allergy and Clinical Immunology. 2000;106(4):638-44.

179. Gao J, Zhang M, Zhou L, Yang X, Wu H, Zhang J, et al. Correlation between fractional exhaled nitric oxide and sputum eosinophilia in exacerbations of COPD. Int J Chron Obstruct Pulmon Dis. 2017;12:1287-93.
180. Maniscalco M, Vitale C, Vatrella A, Molino A, Bianco A, Mazzarella G. Fractional exhaled nitric oxide-measuring devices: technology update. Medical Devices (Auckland, NZ). 2016;9:151-60.
181. Hahn Y-S. Measurements of fractional exhaled nitric oxide in pediatric asthma. Korean Journal of Pediatrics. 2013;56(10):424-30.
182. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: Comparison with the "gold standard" technique. Chest. 2007;131(2):410-4.
183. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. American journal of respiratory and critical care medicine. 2005;171(8):912-30.
184. Olin A-C, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, Age, and Atopy Are Associated With Fraction of Exhaled Nitric Oxide in a Large Adult General Population Sample. Chest. 2006;130(5):1319-25.
185. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62(12):1043-9.
186. Rodway GW, Choi J, Hoffman LA, Sethi JM. Exhaled nitric oxide in the diagnosis and management of asthma: clinical implications. Chronic respiratory disease. 2009;6(1):19-29.
187. Dweik RA, Sorkness RL, Wenzel S, Hammel J, Curran-Everett D, Comhair SAA, et al. Use of Exhaled Nitric Oxide Measurement to Identify a Reactive, at-Risk Phenotype among Patients with Asthma. American journal of respiratory and critical care medicine. 2010;181(10):1033-41.
188. Essat M, Harnan S, Gomersall T, Tappenden P, Wong R, Pavord I, et al. Fractional exhaled nitric oxide for the management of asthma in adults: a systematic review. The European respiratory journal. 2016;47(3):751-68.
189. Osoata GO, Hanazawa T, Brindicci C, Ito M, Barnes PJ, Kharitonov S, et al. Peroxynitrite Elevation in Exhaled Breath Condensate of COPD and Its Inhibition by Fudosteine. Chest. 2009;135(6):1513-20.

190. Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, et al. Clinical Use of Noninvasive Measurements of Airway Inflammation in Steroid Reduction in Children. American journal of respiratory and critical care medicine. 2005;171(10):1077-82.
191. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. American journal of respiratory and critical care medicine. 2011;184(5):602-15.
192. Sterk PJ, De Gouw HW, Ricciardolo FL, Rabe KF. Exhaled nitric oxide in COPD: glancing through a smoke screen. Thorax. 1999;54(7):565-7.
193. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. American journal of respiratory and critical care medicine. 1995;152(2):609-12.
194. Clini E, Cremona G, Campana M, Scotti C, Pagani M, Bianchi L, et al. Production of endogenous nitric oxide in chronic obstructive pulmonary disease and patients with cor pulmonale. Correlates with echo-Doppler assessment. American journal of respiratory and critical care medicine. 2000;162(2 Pt 1):446-50.
195. Agusti AG, Villaverde JM, Togores B, Bosch M. Serial measurements of exhaled nitric oxide during exacerbations of chronic obstructive pulmonary disease. The European respiratory journal. 1999;14(3):523-8.
196. Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov SA, Barnes PJ. Exhaled nitric oxide in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1998;157(3):998-1002.
197. PAPI A, ROMAGNOLI M, BARALDO S, BRACCIONI F, GUZZINATI I, SAETTA M, et al. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2000;162(5):1773-7.
198. Kunisaki KM, Rice KL, Janoff EN, Rector TS, Niewoehner DE. Exhaled nitric oxide, systemic inflammation, and the spirometric response to inhaled fluticasone propionate in severe chronic obstructive pulmonary disease: A prospective study. Therapeutic Advances in Respiratory Disease. 2008;2(2):55-64.
199. Dummer JF, Epton MJ, Cowan JO, Cook JM, Condliffe R, Landhuis CE, et al. Predicting Corticosteroid Response in Chronic Obstructive Pulmonary Disease Using Exhaled Nitric Oxide. American journal of respiratory and critical care medicine. 2009;180(9):846-52.

200. de Laurentiis G, Maniscalco M, Cianciulli F, Stanziola A, Marsico S, Lundberg JO, et al. Exhaled nitric oxide monitoring in COPD using a portable analyzer. Pulmonary pharmacology & therapeutics. 2008;21(4):689-93.
201. Antus B, Barta I, Horvath I, Csiszer E. Relationship between exhaled nitric oxide and treatment response in COPD patients with exacerbations. Respirology (Carlton, Vic). 2010;15(3):472-7.
202. Aggarwal AN, Gupta D, Agarwal R, Jindal SK. Comparison of the lower confidence limit to the fixed-percentage method for assessing airway obstruction in routine clinical practice. Respir Care. 2011;56(11):1778-84.
203. Cataldo D, Corhay JL, Derom E, Louis R, Marchand E, Michils A, et al. A Belgian survey on the diagnosis of asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2017;12:601-13.
204. Hanania NA, Wittman R, Kesten S, Chapman KR. Medical personnel's knowledge of and ability to use inhaling devices. Metered-dose inhalers, spacing chambers, and breath-actuated dry powder inhalers. Chest. 1994;105.
205. Lavorini F, Ninane V, Haughney J, Bjermer L, Molimard M, Dekhuijzen RP. Switching from branded to generic inhaled medications: potential impact on asthma and COPD. Expert Opin Drug Deliv. 2013;10.
206. Alcazar-Navarrete B, Romero-Palacios PJ, Ruiz-Sancho A, Ruiz-Rodriguez O. Diagnostic performance of the measurement of nitric oxide in exhaled air in the diagnosis of COPD phenotypes. Nitric oxide : biology and chemistry. 2016;54:67-72.
207. Tamada T, Sugiura H, Takahashi T, Matsunaga K, Kimura K, Katsumata U, et al. Biomarker-based detection of asthma-COPD overlap syndrome in COPD populations. Int J Chron Obstruct Pulmon Dis. 2015;10:2169-76.
208. Arksey H OML. Scoping studies: towards a methodological framework. Int J Soc Res Methodol. 2005;8:19-32.
209. Pham MT, Rajić A, Greig JD, Sargeant JM, Papadopoulos A, McEwen SA. A scoping review of scoping reviews: advancing the approach and enhancing the consistency. Research synthesis methods. 2014;5(4):371-85.
210. Brien SE, Lorenzetti DL, Lewis S, Kennedy J, Ghali WA. Overview of a formal scoping review on health system report cards. Implementation science : IS. 2010;5:2.
211. Cahill J, Barkham M, Hardy G, Gilbody S, Richards D, Bower P, et al. A review and critical appraisal of measures of therapist-patient interactions in mental health settings. Health technology assessment (Winchester, England). 2008;12(24):iii, ix-47.

212. Thorsson L, Edsbacker S. Lung deposition of budesonide from a pressurized metered-dose inhaler attached to a spacer. The European respiratory journal. 1998;12.
213. Peters MD, Godfrey CM, Khalil H, McInerney P, Parker D, Soares CB. Guidance for conducting systematic scoping reviews. International journal of evidence-based healthcare. 2015;13(3):141-6.
214. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic reviews. 2015;4:1.
215. Levac D, Colquhoun H, O'Brien KK. Scoping studies: advancing the methodology. Implementation science : IS. 2010;5:69.
216. Heyder J. Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery. Proc Am Thorac Soc. 2004;1.
217. Stone PW. Popping the (PICO) question in research and evidence-based practice. Applied nursing research : ANR. 2002;15(3):197-8.
218. Tricco AC, Lillie E, Zarin W, O'Brien K, Colquhoun H, Kastner M, et al. A scoping review on the conduct and reporting of scoping reviews. BMC medical research methodology. 2016;16:15.
219. Pham MT, Rajic A, Greig JD, Sargeant JM, Papadopoulos A, McEwen SA. A scoping review of scoping reviews: advancing the approach and enhancing the consistency. Research synthesis methods. 2014;5(4):371-85.
220. Burgel PR, Paillasseur JL, Roche N. Identification of clinical phenotypes using cluster analyses in COPD patients with multiple comorbidities. BioMed research international. 2014;2014:420134.
221. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). The European respiratory journal. 2008;31(4):869-73.
222. Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS, et al. Chronic obstructive pulmonary disease: current burden and future projections. The European respiratory journal. 2006;27(2):397-412.
223. The Global Strategy for Diagnosis, Management, and Prevention of COPD. 2015. Available at: http://www.goldcopd.org/. Accessed July 07, 2017. .

224. Bateman ED, Reddel HK, van Zyl-Smit RN, Agusti A. The asthma-COPD overlap syndrome: towards a revised taxonomy of chronic airways diseases? The Lancet Respiratory medicine. 2015;3(9):719-28.
225. Menezes AM, Montes de Oca M, Perez-Padilla R, Nadeau G, Wehrmeister FC, Lopez-Varela MV, et al. Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma. Chest. 2014;145(2):297-304.
226. Kauppi P, Kupiainen H, Lindqvist A, Tammilehto L, Kilpelainen M, Kinnula VL, et al. Overlap syndrome of asthma and COPD predicts low quality of life. The Journal of asthma : official journal of the Association for the Care of Asthma. 2011;48(3):279-85.
227. Andersen H, Lampela P, Nevanlinna A, Saynajakangas O, Keistinen T. High hospital burden in overlap syndrome of asthma and COPD. The clinical respiratory journal. 2013;7(4):342-6.
228. Miravitlles M, Calle M, Soler-Cataluna JJ. Clinical phenotypes of COPD: identification, definition and implications for guidelines. Archivos de bronconeumologia. 2012;48(3):86-98.
229. Hart CM. Nitric oxide in adult lung disease. Chest. 1999;115(5):1407-17.
230. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signaling in inflammation. Current drug targets Inflammation and allergy. 2005;4(4):471-9.
231. Bazeghi N, Gerds TA, Budtz-Jorgensen E, Hove J, Vestbo J. Exhaled nitric oxide measure using multiple flows in clinically relevant subgroups of COPD. Respiratory medicine. 2011;105(9):1338-44.
232. Bhowmik A, Seemungal TA, Donaldson GC, Wedzicha JA. Effects of exacerbations and seasonality on exhaled nitric oxide in COPD. The European respiratory journal. 2005;26(6):1009-15.
233. de Laurentiis G, Maniscalco M, Cianciulli F, Stanziola A, Marsico S, Lundberg JO, et al. Exhaled nitric oxide monitoring in COPD using a portable analyzer. Pulmonary pharmacology & therapeutics. 2008;21(4):689-93.
234. Lehouck A, Carremans C, De Bent K, Decramer M, Janssens W. Alveolar and bronchial exhaled nitric oxide in chronic obstructive pulmonary disease. Respiratory medicine. 2010;104(7):1020-6.
235. Roy K, Smith J, Kolsum U, Borrill Z, Vestbo J, Singh D. COPD phenotype description using principal components analysis. Respiratory research. 2009;10:41.

236. Tilemann L, Gindner L, Meyer F, Szecsenyi J, Schneider A. Differences in local and systemic inflammatory markers in patients with obstructive airways disease. Primary care respiratory journal : journal of the General Practice Airways Group. 2011;20(4):407-14.
237. Santini G, Mores N, Shohreh R, Valente S, Dabrowska M, Trove A, et al. Exhaled and non-exhaled non-invasive markers for assessment of respiratory inflammation in patients with stable COPD and healthy smokers. Journal of breath research. 2016;10(1):017102.
238. Ziora D, Dworniczak S, Kozielski J. Induced sputum metalloproteinases and their inhibitors in relation to exhaled nitrogen oxide and sputum nitric oxides and other inflammatory cytokines in patients with chronic obstructive pulmonary disease. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2008;59 Suppl 6:809-17.
239. Liu J, Sandrini A, Thurston MC, Yates DH, Thomas PS. Nitric oxide and exhaled breath nitrite/nitrates in chronic obstructive pulmonary disease patients. Respiration; international review of thoracic diseases. 2007;74(6):617-23.
240. Dummer JF, Epton MJ, Cowan JO, Cook JM, Condliffe R, Landhuis CE, et al. Predicting corticosteroid response in chronic obstructive pulmonary disease using exhaled nitric oxide. American journal of respiratory and critical care medicine. 2009;180(9):846-52.
241. Kunisaki KM, Rice KL, Janoff EN, Rector TS, Niewoehner DE. Exhaled nitric oxide, systemic inflammation, and the spirometric response to inhaled fluticasone propionate in severe chronic obstructive pulmonary disease: a prospective study. Therapeutic advances in respiratory disease. 2008;2(2):55-64.
242. Logotheti H, Pourzitaki C, Tsaousi G, Aidoni Z, Vekrakou A, Ekaterini A, et al. The role of exhaled nitric oxide in patients with chronic obstructive pulmonary disease undergoing laparotomy surgery - The noxious study. Nitric oxide : biology and chemistry. 2016;61:62-8.
243. Antus B, Barta I. Relationship between exhaled nitric oxide and the frequency of severe acute exacerbation of COPD: 3-year follow-up. Acta physiologica Hungarica. 2013;100(4):469-77.
244. Kobayashi S, Hanagama M, Yamanda S, Ishida M, Yanai M. Inflammatory biomarkers in asthma-COPD overlap syndrome. International journal of chronic obstructive pulmonary disease. 2016;11:2117-23.
245. Akamatsu K, Matsunaga K, Sugiura H, Koarai A, Hirano T, Minakata Y, et al. Improvement of Airflow Limitation by Fluticasone Propionate/Salmeterol in Chronic

Obstructive Pulmonary Disease: What is the Specific Marker? Frontiers in pharmacology. 2011;2:36.
246. Ji Z, Pan X, Ji F, Ni DT, Shang Y, Bai C. Fractional exhaled nitric oxide detection in treatment of asthma-chronic obstructive pulmonary disease overlap syndrome. [Chinese]. Academic Journal of Second Military Medical University. 2016;37(10):1250-5.
247. Huang SY, Chou PC, Wang TY, Lo YL, Joa WC, Chen LF, et al. Exercise-Induced Changes in Exhaled NO Differentiates Asthma With or Without Fixed Airway Obstruction From COPD With Dynamic Hyperinflation. Medicine. 2016;95(15):e3400.
248. Rouhos A, Kainu A, Piirila P, Sarna S, Lindqvist A, Karjalainen J, et al. Repeatability of exhaled nitric oxide measurements in patients with COPD. Clinical physiology and functional imaging. 2011;31(1):26-31.
249. Xia Q, Pan P, Wang Z, Lu R, Hu C. [Fractional exhaled nitric oxide in bronchial inflammatory lung diseases]. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences. 2014;39(4):365-70.
250. Beg MF, Alzoghaibi MA, Abba AA, Habib SS. Exhaled nitric oxide in stable chronic obstructive pulmonary disease. Ann Thorac Med. 2009;4(2):65-70.
251. Foschino Barbaro MP, Carpagnano GE, Spanevello A, Cagnazzo MG, Barnes PJ. Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease. International journal of immunopathology and pharmacology. 2007;20(4):753-63.
252. Arif AA, Mitchell C. Use of Exhaled Nitric Oxide as a Biomarker in Diagnosis and Management of Chronic Obstructive Pulmonary Disease. Journal of primary care & community health. 2016;7(2):102-6.
253. Durmaz D, Goksu E, Kilic T, Ozbudak O, Eray O. The role of nitric oxide in predicting revisit of patients with exacerbated chronic obstructive pulmonary disease. The Journal of emergency medicine. 2015;48(2):247-53.
254. Deng DD, Zhou AY, Shuang QC, Chen P. [The value of fractionated exhaled nitric oxide in the diagnosis of asthma-chronic obstructive pulmonary disease overlap syndrome]. Chung Hua Chieh Ho Ho Hu Hsi Tsa Chih. 2017;40(2):98-101.
255. Goto T, Camargo CA, Jr., Hasegawa K. Fractional exhaled nitric oxide levels in asthma-COPD overlap syndrome: analysis of the National Health and Nutrition Examination Survey, 2007-2012. International journal of chronic obstructive pulmonary disease. 2016;11:2149-55.

256. Cosio BG, Perez de Llano L, Lopez Vina A, Torrego A, Lopez-Campos JL, Soriano JB, et al. Th-2 signature in chronic airway diseases: towards the extinction of asthma-COPD overlap syndrome? The European respiratory journal. 2017;49(5).
257. Chou KT, Su KC, Huang SF, Hsiao YH, Tseng CM, Su VY, et al. Exhaled nitric oxide predicts eosinophilic airway inflammation in COPD. Lung. 2014;192(4):499-504.
258. Soter S, Barta I, Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. Inflammation. 2013;36(5):1178-85.
259. Zhao H, Li R, Lv Y, Dong H, Yao L, Wu Y, et al. Albuterol inhalation increases FeNO level in steroid-naive asthmatics but not COPD patients with reversibility. The clinical respiratory journal. 2017;11(3):328-36.
260. Amer M, Cowan J, Gray A, Brockway B, Dummer J. Effect of Inhaled beta2-Agonist on Exhaled Nitric Oxide in Chronic Obstructive Pulmonary Disease. PloS one. 2016;11(6):e0157019.
261. Osterberg L, Blaschke T. Adherence to medication. The New England journal of medicine. 2005;353.
262. Rees J. Methods of delivering drugs. BMJ (Clinical research ed). 2005;331.
263. Ishiura Y, Fujimura M, Shiba Y, Ohkura N, Hara J, Kasahara K. A comparison of the efficacy of once-daily fluticasone furoate/vilanterole with twice-daily fluticasone propionate/salmeterol in asthma-COPD overlap syndrome. Pulmonary pharmacology & therapeutics. 2015;35:28-33.
264. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. The European respiratory journal. 2014;43(2):343-73.
265. Feng J-X, Lin Y, Lin J, He S-S, Chen M-F, Wu X-M, et al. Relationship between Fractional Exhaled Nitric Oxide Level and Efficacy of Inhaled Corticosteroid in Asthma-COPD Overlap Syndrome Patients with Different Disease Severity. Journal of Korean Medical Science. 2017;32(3):439-47.
266. Di Stefano A, Caramori G, Ricciardolo FL, Capelli A, Adcock IM, Donner CF. Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2004;34(8):1156-67.
267. MacNee W. Pathogenesis of Chronic Obstructive Pulmonary Disease. Proceedings of the American Thoracic Society. 2005;2(4):258-66.

268. George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. Therapeutic advances in chronic disease. 2016;7(1):34-51.
269. Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. Int J Chron Obstruct Pulmon Dis. 2006;1(1):39-47.
270. Bourbeau J, Tan WC, Benedetti A, Aaron SD, Chapman KR, Coxson HO, et al. Canadian Cohort Obstructive Lung Disease (CanCOLD): Fulfilling the need for longitudinal observational studies in COPD. Copd. 2014;11(2):125-32.
271. Heffler E, Guida G, Marsico P, Bergia R, Bommarito L, Ferrero N, et al. Exhaled nitric oxide as a diagnostic test for asthma in rhinitic patients with asthmatic symptoms. Respiratory medicine. 2006;100(11):1981-7.
272. Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Toren K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest. 2006;130(5):1319-25.
273. Donohue JF, Herje N, Crater G, Rickard K. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. International Journal of Chronic Obstructive Pulmonary Disease. 2014;9:745-51.
274. Tamada T, Sugiura H, Takahashi T, Matsunaga K, Kimura K, Katsumata U, et al. Biomarker-based detection of asthma–COPD overlap syndrome in COPD populations. International Journal of Chronic Obstructive Pulmonary Disease. 2015;10:2169-76.
275. Wurst KE, St Laurent S, Hinds D, Davis KJ. Disease Burden of Patients with Asthma/COPD Overlap in a US Claims Database: Impact of ICD-9 Coding-based Definitions. Copd. 2017;14(2):200-9.
276. Joo H, Han D, Lee JH, Rhee CK. Heterogeneity of asthma–COPD overlap syndrome. International Journal of Chronic Obstructive Pulmonary Disease. 2017;12:697-703.
277. Deng DD, Zhou AY, Shuang QC, Chen P. [The value of fractionated exhaled nitric oxide in the diagnosis of asthma-chronic obstructive pulmonary disease overlap syndrome]. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases. 2017;40(2):98-101.

CHAPTER 8: APPENDIX

8.1 Sample of Search Strategies of Major Databases for Scoping Review

Embase via OvidSP

Embase Classic+Embase 1947 to 2016 June 28		
#	Searches	Results
1	chronic obstructive lung disease/	90816
2	chronic bronchitis/	13971
3	exp lung emphysema/	25119
4	obstructive airway disease/	1876
5	bronchus obstruction/	4097
6	airway obstruction/	30149
7	(obstructive adj2 (pulmonary or lung$ or respirat$ or air$)).tw.	62353
8	(chronic air$ adj2 (obstruction$ or limitation$ or occlusion$)).tw.	1831
9	(chronic bronch$ adj2 (obstruction$ or limitation$ or occlusion$)).tw.	159
10	(chronic$ adj2 bronch$).tw.	20615
11	COPD.tw.	58043
12	COAD.tw.	292
13	emphysema$.tw.	37016
14	(acos and asthm*).tw.	128
15	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	202126
16	nitric oxide/	131877
17	(feno and (fraction* or exhal* or nitric)).tw.	2503
18	((fe adj no) and (fraction* or exhal* or nitric)).tw.	261
19	nitric oxid*.tw.	150577
20	16 or 17 or 18 or 19	184366
21	15 and 20	2049
22	remove duplicates from 21	2024
23	from 22 keep 1-1000	1000
24	from 22 keep 1001-2000	1000
25	from 22 keep 2001-2024	24

Medline via OvidSP

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily 1946 to 2016 June 28		
#	Searches	Results
1	("10712994" or "10907593" or "11296168" or "11413349" or "15817806" or "15939243" or "16289590" or "16646959" or "17426212" or "18460522" or "18547853" or "19124359" or "19401794" or "19820080" or "19881162" or "20210889" or "21143751" or "21530214" or "23445725" or "23509896" or "23681903" or "23989961" or "24013942" or "24719850" or "24929061" or "25053884" or "26252571" or "26372312" or "26491283" or "26496331" or "26497109" or "26814886" or "26916083" or "26952317" or "27142135" or "27209003").ui.	36
2	exp Pulmonary Disease, Chronic Obstructive/	43308
3	Lung Diseases, Obstructive/	18072
4	exp Pulmonary Emphysema/	14866
5	(obstructive adj2 (pulmonary or lung$ or respirat$ or air$)).tw,kf.	44058
6	(chronic air$ adj2 (obstruction$ or limitation$ or occlusion$)).tw,kf.	1428
7	(chronic bronch$ adj2 (obstruction$ or limitation$ or occlusion$)).tw,kf.	95
8	(chronic$ adj2 bronch$).tw,kf.	12908
9	COPD.tw,kf.	32628
10	COAD.tw,kf.	222
11	emphysema$.tw,kf.	24011
12	(acos and asthm*).tw,kf.	86
13	or/2-12	99543
14	Nitric Oxide/	78284
15	(feno and (fraction* or exhal* or nitric)).tw,kf.	1099
16	(fe no and (fraction* or exhal* or nitric)).tw,kf.	401
17	nitric oxid*.tw,kf.	126245
18	or/14-17	140975
19	13 and 18	750
20	1 and 19	32
21	1 not 20	4

Cochrane Library via Wiley Online

ID	Search	Hits
#1	(obstructive near/2 (pulmonary or lung* or respirat* or air*)):ti,ab,kw	7664
#2	("chronic air*" near/2 (obstruction* or limitation* or occlusion*)):ti,ab,kw	286
#3	("chronic bronch*" near/2 (obstruction* or limitation* or occlusion*)):ti,ab,kw	16
#4	(chronic* near/2 bronch*):ti,ab,kw	2028
#5	COAD:ti,ab,kw	47
#6	COPD:ti,ab,kw	8954
#7	emphysema*:ti,ab,kw	909
#8	acos:ti,ab,kw and asthm*:ti,ab,kw	2
#9	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8	14399
#10	feno:ti,ab,kw and (fraction* or exhal* or nitric):ti,ab,kw	189
#11	(fe N1 no):ti,ab,kw and (fraction* or exhal* or nitric):ti,ab,kw	0
#12	nitric oxid*:ti,ab,kw	4652
#13	#10 or #11 or #12	4661
#14	#9 and #13	91

Cochrane Reviews (2)
Trials (89)

CINAHL via EBSCOhost

#	Query	Results
S19	S13 AND S18	158
S18	S14 OR S15 OR S16 OR S17	9,826
S17	TI nitric oxid* OR AB nitric oxid*	7,415
S16	TI ((fe N1 no and (fraction* or exhal* or nitric))) OR AB ((fe N1 no and (fraction* or exhal* or nitric)))	44
S15	TI ((feno and (fraction* or exhal* or nitric))) OR AB ((feno and (fraction* or exhal* or nitric)))	157
S14	(MH "Nitric Oxide")	6,342
S13	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12	25,637
S12	TI ((acos and asthm*)) OR AB ((acos and asthm*))	12
S11	TI emphysema* OR AB emphysema*	2,329
S10	TI COAD OR AB COAD	24
S9	TI COPD OR AB COPD	9,032
S8	TI chronic* N2 bronch* OR AB chronic* N2 bronch*	936
S7	TI ("chronic bronch*" N2 (obstruction* OR limitation* OR occlusion*)) OR AB ("chronic bronch*" N2 (obstruction* OR limitation* OR occlusion*))	12
S6	TI ("chronic air*" N2 (obstruction* OR limitation* OR occlusion*)) OR AB ("chronic air*" N2 (obstruction* OR limitation* OR occlusion*))	136
S5	TI (obstructive N2 (pulmonary OR lung* OR respirat* OR air*)) OR AB (obstructive N2 (pulmonary OR lung* OR respirat* OR air*))	9,228
S4	MH "Emphysema+"	2,522
S3	(MH "Respiratory Tract Diseases")	3,304
S2	(MH "Lung Diseases, Obstructive")	3,603
S1	MH "Pulmonary Disease, Chronic Obstructive+"	12,513

8.2 Study Characteristics and Key Findings of Studies Included in Scoping Review

Author (s) (Year)	Title	Country	Study type/intervention (if applicable)	FeNO measurement	Setting/Sample	Key findings, including N analyzed if different from N in sample
Bhowmik et al. (2005) (232)	Effects of exacerbations and seasonality on exhaled nitric oxide in COPD	UK	Prospective cohort study	eNO was measured using a chemiluminescence analyser (Model LR 2000; Logan Research, Rocheste, UK)	N=98, COPD patients included in this study were volunteers from the outpatient clinics at the London Chest Hospital (London, UK)	N=79 (Nineteen patients could not perform an adequate baseline exhalation: they were older and with lower FEV1 and FVC) Lower eNo in current smokers than non-smokers. No association between eNO with FEV1, FVC or exacerbation frequency. No significant difference between patients who took ICS and/or inhaled long acting beta-agonist and who not (n=8, n=19 respectively). Higher level of FeNO from October to December perhaps due to viral infection. Higher levels of FeNO in exacerbation, N=38
Foschino Barbaro et al. (2007) (251)	Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease	Italy	Not mentioned	FeNO was measured using a rapid-response chemiluminescence NO analyzer (model 280; Sievers Instruments; Boulder, Colorado, USA)	N=27 mild stable ex-smoker COPD, and N=15 healthy smokers were recruited from the Respiratory Disease Institute, University of Foggia	Higher FeNO in COPD patients compared to control subjects and in reversible compared to non-reversible COPD. COPD patients with airway reversibility showed increased sputum eosinophils and exhaled NO.
Liu et al. (2007) (239)	Nitric Oxide and Exhaled Breath Nitrite/Nitrates in	Australia	Cross-sectional	eNO was measured offline by using a closed circuit which was connected to a	N=96 COPD and N=80 healthy subjects were recruited from the community, including	No effect of smoking status (even in control group) or glucocorticosteroid (GCS) treatment on eNO levels in COPD patients.

	Chronic Obstructive Pulmonary Disease Patients			chemiluminescent NO analyzer for NO determinations (Dasibi Environmental Corp., Glendale, Calif., USA)	hospital staff and their friends, and the respiratory outpatient clinics of the Prince of Wales Hospital and St. Vincent's Hospital	Higher eNO levels in COPD patients than normal subjects.
de Laurentiis et al. (2008) (233)	Exhaled nitric oxide monitoring in COPD using a portable analyzer	Italy	Cohort, Prospective	FeNO was measured using electrochemical FeNO device (NIOX MINO, Aerocrine, Sweden) and the chemiluminescence analyzer (NOA, Sensormedics, Italy)	N=59 COPD patients visiting department as outpatients	Higher mean coefficient of variability (CoV) FeNO in COPD than healthy group. Lower FeNO levels in COPD current smokers than COPD ex-smoker. No association between FeNO and FEV1. Significant association between individual exacerbations and FeNO.
Kunisaki et al. (2008) (241)	Exhaled nitric oxide, systemic inflammation, and the spirometric response to inhaled fluticasone propionate in severe chronic obstructive pulmonary disease: A prospective study	USA	A single-arm, open-label prospective study/Salmeterol, fluticasone propionate	FeNO was measured online (realtime) with a chemiluminescence device (Sievers NOA 280i, GE Analytical Instruments, Boulder, CO)	N=73 ex-smoker severe COPD were recruited from the Minneapolis Veterans Affairs Medical Center	N=60 (Thirteen subjects were withdrawn: they were similar in COPD severity but used more medications (antibiotics, prednisolone, inhaled corticosteroid before study participation)). No association between baseline (pre-ICS) FeNO with FEV1 or FVC. FeNO significantly decreased after four weeks of ICS therapy. Significant difference in baseline FeNO levels between ICS responders and non-responders, higher baseline FeNO levels compared with non-responders.
Beg et al. (2009) (250)	Exhaled Nitric Oxide in Chronic Obstructive	Saudi Arabia	Cross-sectional	FeNO was measured using NOX EVA 4000 chemiluminescence	N=14 COPD ex-smokers, N=25 patients with bronchial/naïve steroid asthma, and N=25 healthy	Significant higher FeNO in COPD patients than healthy.

	Pulmonary Disease			analyzer (SERES-FRANCE)	recruited from pulmonary clinic at the King Khalid University Hospital, Riyadh, Saudi Arabia	Negative association between FEV1/FVC and the FeNO levels among COPD patients.
Roy et al. (2009) (235)	COPD phenotype description using principal components analysis	UK	Single study visit	FeNO was measured using Niox chemiluminescence on-line analyzer (Aerocrine, Solna, Sweden)	N=127 COPD patients were recruited from primary care by media advertising	Significant association between FeNO and sputum eosinophils, regardless of whether the data were expressed as percentage differential or cell count. Lower levels of FeNO in COPD smokers and women.
Dummer et al. (2009) (240)	Predicting Corticosteroid Response in Chronic Obstructive Pulmonary Disease Using Exhaled Nitric Oxide	New Zealand	Randomized double-blind, placebo-controlled, crossover trial/ Oral Prednisone	FeNO was measured using an on-line chemiluminescence analyzer (Aerocrine AB, Solna, Sweden)	N=82 COPD patients were recruited from the research database and respiratory clinics	N=62 (Thirteen patients were symptomatic after withdrawal of ICS, 2 had a too busy schedule to continue, 1 performed inadequate FeNO technique, and 1 had an unrelated illness, 2 patients were excluded because of nonadherence and 1 was excluded because of a new diagnosis of angina). FeNO decreased after prednisone in the case group. A significant association between off-steroid FeNO and sputum eosinophil percentage. Significant association between baseline FeNO and the FEV1. A significant improvement in FEV1 from the lowest to the highest FeNO tertile. There was a significant predictive value of baseline FeNO for an increase of 0.2 L in FEV1 with prednisone with an optimum FeNO cut-point of 50 ppb,

						area under the curve (AUC) 0.69, sensitivity 29%, and specificity 96%).
Antus et al. (2010) (201)	Relationship between exhaled nitric oxide and treatment response in COPD patients with exacerbation	Hungary	Longitudinal study	FeNO was measured using a chemiluminescence analyzer (Model LR2000, Logan Research, Rochester, UK)	N=58 COPD patients with exacerbations referred to the 3rd Pulmonary Department at National Koranyi Institute for TB and Pulmonology were recruited	Lower FeNO levels in smokers at admission compared with ex-smokers. Reduced FeNO levels in patients receiving ICS therapy compared with those not taking ICS at admission. Similar FeNO level in men and women. Significant positive association between FeNO levels at admission and the post-treatment increases in FEV1 and FEV1% predicted. No association between an increase in FVC and FeNO levels at admission or with changes in FeNO levels. FeNO was a good anticipator of a significant post-treatment increase in FEV1. The optimum cut point for FeNO was 26.8 ppb with the sensitivity and specificity of 74 and 75%, respectively with the area under the ROC curve of 0.82. FEV1 and FEV1% predicted increased significantly at discharge in the group with high FeNO levels (> 26.8 ppb) (n = 24).
Lehouck et al. (2010) (234)	Alveolar and bronchial exhaled nitric oxide in chronic obstructive pulmonary disease	Belgium	Case-control	Exhaled NO was measured by a chemiluminescence analyzer (NIOX Flex;	N=28 healthy ex-smokers, N=39 healthy smokers, N=55 COPD ex-smokers, and N=29 COPD smokers were recruited during the	No significant difference in FeNO levels between COPD patients and age-matched healthy control.

				AerocrineAB, Stockholm, Sweden).	stable clinical conditions from the neighborhood Leuven(Belgium) via the service of Respiratory Medicine at the University Hospital of Leuven	Significant reduced FeNO in both current smoker COPD patients and healthy controls. No significant difference in FeNO levels between the GOLD stages. No significant association between FEV1 and FeNO. No significant difference in FeNO and use of ICS (39% of COPD patients). No association between FeNO value measurements and age.
Tilemann et al. (2011) (236)	Differences between local and systemic inflammatory markers in patients with obstructive airways disease	Germany	Not mentioned	FENO was measured using a NioxMino® analyzer (Aerocrine AG, Solna, Sweden)	N=86 asthmatics, N=36 COPD, N=13 subjects with partial reversibility, and N=75 subjects with no obstructive airway diseases from adults presenting to their general practitioners for the first time with complaints suggestive of obstructive airway disease were consecutively included	Higher level of FeNO in current non-smokers (never smokers and ex-smokers) than in current smokers. Significant lower level of FeNO in COPD patients compared to subjects with no airway obstruction. Association between FeNO with blood eosinophils and IgE levels.
Rouhos et al. (2011) (248)	Repeatability of exhaled nitric oxide measurements in patients with COPD	Finland	Not mentioned	FeNO was measured using chemiluminescence analyzer (Sievers 270B, Boulder, CO, USA) by using computer software specially developed for this purpose	N=20 COPD patients were recruited from the outpatient department of the Division of Respiratory Diseases and from the Research Unit for Respiratory Diseases of the Helsinki University Central Hospital, N=20	N=19 COPD (One subject was unable to perform acceptable FeNo measure), N=18 for FeNO measurement (one subject did not return for second study day). Higher FeNO at baseline in COPD patients than healthy subjects.

					healthy subjects were recruited from the hospital staff and their relatives	Higher FeNO in both COPD and healthy subjects when the subjects did not use sodium bicarbonate.
Bazeghi et al. (2011) (231)	Exhaled nitric oxide measure using multiple flows in clinically relevant subgroups of COPD	Denmark	Cohort database study, ECLIPES substudy	FeNO was measured using a Niox chemiluminescence online analyzer (Aerocrine, Solna, Sweden)	N=91 COPD recruited for the ECLIPSE study. (ECLIPSE sub-study, using data of ECLIPSE database)	Significant lower FeNO levels in active smokers than in ex-smokers.
Akamatsu et al. (2011) (245)	Improvement of air flow limitation by fluticasone propionate/salmeterol in chronic obstructive pulmonary disease: what is the specific marker?	Japan	Not mentioned/ Fluticasone (FP), Salmeterol (SAL)	FeNO was measured using a chemiluminescence analyzer (modified NA-623N®; Chest, Inc.,Tokyo,Japan)	N=14 stable COPD patients receiving long-acting muscarinic receptor antagonist(tiotropium18μg /day) were consecutively enrolled from the outpatient clinic of Wakayama Medical University Hospital	Significant decrease in FeNO levels by the treatment with FP/SAL. No association between the baseline FeNO level and the changes in FEV1 as well as other pulmonary physiological parameters. To identify subjects with significant improvement in FEV1, a baseline FeNO level >35 ppb is useful. With the sensitivity of 80 and specificity of 66.7% Improvement in FEV1 by adding treatment of FP/SAL in COPD subjects with FeNO >35 ppb.
Antus et al. (2013) (243)	Relationship between exhaled nitric oxide and the frequency of severe acute exacerbation of COPD: 3-year follow-up	Hungary	Retrospective pilot study	Levels of FeNO were recorded using a chemiluminescence analyzer (Model LR2000, Logan Research Rochester, UK) at hospital admission	N=58 COPD patients referred to the National Korányi Institute of TB and Pulmonology with an acute exacerbation of the disease were recruited	No association between FeNO and inhaled corticosteroid (ICS), long-acting β2-agonist (LABA) and long-acting muscarinic agonist (LAMA) therapy on FeNO. More exacerbations in COPD patients with low FeNO level. Administrating antibiotics 18% more frequently in COPD subjects with low

						FeNO level than subjects in the high FeNO level group.
Soter et al. (2013) (258)	Predicting Sputum Eosinophilia in Exacerbations of COPD using Exhaled Nitric Oxide	Hungary	Prospective study	FeNO was measured using a chemiluminescence analyzer (Model LR2500, Logan Research, Rochester, UK)	N=49 COPD patients referred to the National Koranyi Institute of TB and Pulmonology with an acute exacerbation of the disease were recruited consecutively for the study	FeNO cut point: 26.8 parts per billion (ppb) was used for the estimation of the treatment response. Patients with FeNO levels of >26.8 ppb had a greater increase in FEV1 compared to those with FeNO levels of <26.8 ppb at admission. Significant association between the percentage/number of sputum eosinophils and FeNO levels, both at exacerbation and discharge. To identify sputum eosinophilia in COPD patients with acute exacerbations, the optimum cut point of 19 ppb with AUC of 0.089 and sensitivity of 90% and specificity of 74% is useful.
Donohue et al. (2014) (2)	Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study	USA	A pilot, observational (no treatment allocation), multicenter, single-visit	FeNO was measured using FeNO analyzer (NIOX MINO®; Aero-crineAB)	N=200 COPD outpatients aged 40 years and older were recruited at two sites within the University of North Carolina, Chapel Hill, North Carolina, Department of Respiratory and Critical Care Medicine	N=191(Nine patients were excluded due to inability to perform FeNO and/or spirometry or having asthma without COPD). Increased FeNO level in COPD patients. No association between FeNO levels and GOLD stages (I-IV).
Xia et al. (2014) (249)	Fractional exhaled nitric oxide in bronchial inflammatory lung diseases	China	Not mentioned	FeNO was measured using SV-02 NO Instrument made in Wuxi Shangwo	N=38 COPD, N=57 suspected asthmatics, N=26 healthy subjects were recruited from those who had an outpatient visit	Significant higher level of FeNO in COPD patients than healthy subjects. Significant higher level of FeNO in COPD patients with exacerbations

				Biological Technology Co., Ltd	at Central South University, Xiangya Hospital	(N=25) compared to stable COPD subjects (N=13). No association between FeNO with FEV1 and FEV1/FVC level in COPD patients. Higher FeNO levels in smoking group (N=29) than non-smoker (N=34) (Mix subjects: stable COPD (N=13), healthy (N=26), non-asthmatic (N=24).
Rawy et al. (2015) (163)	Fraction of exhaled nitric oxide measurement as a biomarker in asthma and COPD compared with local and systemic inflammatory markers	Egypt	Not mentioned	FeNO was measured using Niox Mino analyzer (Aerocrine AG, Solna, Sweden)	N=60 COPD, N=90 asthmatic, and N=30 control group with no airway obstruction were recruited from attended pulmonary outpatient clinic	Positive association between FeNO with sputum and blood eosinophil percentage. Negative association between FeNO and age. No association between FeNO and FEV1/FVC.
Tamada et al. (2015) (207)	Biomarker-based detection of asthma-COPD overlap syndrome in COPD populations	Japan	Multicenter, cross-sectional study	FENO was measured using the NIOX MINO® device (Aerocrine, Morrisville, NC, USA)	N=331 COPD outpatients were enrolled from Tohoku University Hospital, Sendai, Japan, and five hospitals (Tohoku University Hospital, Sendai, Japan; NTT East Tohoku Hospital, Sendai, Japan; Wakayama Medical University Hospital, Kimiidera, Japan; Hiraka General Hospital, Yokote,	High FeNO in COPD with asthma-like airway inflammation (ACOS) among COPD patients. No association between low (≤ 35 ppb) and high FeNO (> 35 ppb) levels with pulmonary function tests (FVC, FEV1, and FEV1/FVC). No association between high and low FeNO levels with GOLD stages (I-IV).

					Japan; Iwate Prefectural Isawa Hospital, Oshu, Japan)	
Durmaz et al. (2015) (253)	The role of nitric oxide in predicting revisit of patients with exacerbated chronic obstructive pulmonary disease	Turkey	Prospective cohort study	NO was measured using a hand-held analyzer device (NIOX MINO, Aerocrine, Solna, Sweden)	N=92 COPD patients presented to the emergency department for the treatment of acute exacerbation	No significant difference in eNO level at presentation or before discharge between the groups.
Ishiura et al. (2015) (263)	A comparison of the efficacy of once-daily fluticasone furoate (FF)/ vilanterol (VI) with twice-daily fluticasone propionate (FP)/salmeterol (SAL) in asthma-COPD overlap syndrome	Japan	Randomized, open-label cross-over study/ fluticasone furoate(FF)/vilanterole (VI), fluticasone propionate (FP)	FeNO was measured using NIOX MINO™, Aerocrine, Stockholm, Sweden	N=16 stable ACOS	No significant difference in FeNO levels, among the run-in, FP/SAL treatment, and FF/VI treatment periods.
Chou et al. (2015) (257)	Exhaled Nitric Oxide Predicts Eosinophilic Airway Inflammation in COPD	Taiwan	Not mentioned	eNO levels were measured using hand-held analyzer (NIOX MINO, Aerocrine)	N=90 COPD were enrolled from outpatient clinics in Taipei Veterans General Hospital, a tertiary medical center and a university-affiliated teaching hospital in Taiwan	Higher levels of eNO in patients with sputum eosinophilia (N=29) compared to those without eosinophilia (N=61). Significant association between levels of sputum eosinophils, eNO, and serum IgE in the COPD patients. To predict sputum eosinophilia, use of eNO at the cut-off of 23.5 ppb with a sensitivity of 62.1 % and a specificity of 70.5 % is useful.

Santini et al. (2016) (237)	Exhaled and non-exhaled non-invasive markers for assessment of respiratory inflammation in patients with stable COPD and healthy smokers	Italy	Multicentre, observational, cross-sectional study	FENO was measured with the NIOX system (Aerocrine, Stockholm, Sweden) with a single breath on-line method	N=48 stable COPD ex-smokers, N=17 stable COPD current smokers, N=12 healthy current smokers, and N=12 healthy ex-smokers	N=47 stable COPD ex-smokers Lower FeNO levels in COPD current smokers compared to COPD ex-smokers. Higher FeNO in COPD ex-smoker compared to healthy ex-smoker. No difference in FeNO values between COPD patients on ICS therapy and those not on ICS therapy.
Arif et al. (2016) (252)	Use of Exhaled Nitric Oxide as a Biomarker in Diagnosis and Management of Chronic Obstructive Pulmonary Disease	USA	Secondary data from the National Health and Nutrition Examination Survey 2007 to 2010	Not mentioned	N=10214 individuals 30 years or older from the National Health and Nutrition Examination	No association between eNO and COPD or between COPD severity (GOLD stage I-IV) and eNO.
Alcazar-Navarrete et al. (2016) (206)	Diagnostic performance of the measurement of nitric oxide in exhaled air in the diagnosis of COPD phenotypes	Spain	Cross-sectional observational study	FeNO was measured using chemiluminescence analyzer of nitric oxide (HypAirFeNO®, Medisoft, Belgium)	N=103 COPD, N=16 healthy nonsmokers, N=30 healthy smokers, and N=43 asthmatics patients who received assistance consecutively in an outpatient pulmonary care facility were enrolled	Higher levels of FeNO in COPD than non-smoking healthy controls. No differences in FeNO levels between the GOLD 2011 groups. Significant higher FeNO levels in ACOS patients (N=22) than other COPD phenotypes (Non-exacerbators (N=34), frequent exacerbators with emphysema (N=13), frequent exacerbators with chronic bronchitis (N=34)). To diagnose ACOS use of 19 ppb as the optimal cut off value of FeNO with a

						sensitivity of 0.68 and specificity of 0.75 and AUC of 0.79.
Logotheti, et al. (2016) (242)	The role of exhaled nitric oxide in patients with chronic obstructive pulmonary disease undergoing laparotomy surgery e The noxious study	Greece	A prospective, observational study	FeNO measurement using a portable analyzer of nitric oxide (HypAir FeNO®, Medisoft, Belgium)	N=70 COPD smoker who were scheduled for major abdominal surgeries	Higher FeNO in older COPD patients compared to younger patients. Lower FeNO in COPD patients under ICS than those who were not under ICS. Association between GOLD category 2011 (ABCD) and the elevated FeNO. Significant increase in exacerbations in COPD patients with elevated FeNO levels. Association between elevated FeNO and extra hospital care.
Amer, et al. (2016) (260)	Effect of Inhaled β2-Agonist on Exhaled Nitric Oxide in Chronic Obstructive Pulmonary Disease	New Zealand	Not mentioned/ Salbutamol (bronchodilator)	FeNO was measured using chemiluminescence nitric oxide analyzer (NOA 280i; Sievers, Boulder, CO)	N=24 stable COPD were recruited	N=21 (Three subjects were not able to do acceptable maneuver). Increased level of FeNO in COPD subjects after bronchodilator therapy. No association between the change in FeNO and change in FEV1.
Chen, et al. (2016) (4)	Importance of fractional exhaled nitric oxide in the differentiation of asthma-COPD overlap syndrome, asthma, and COPD	China	Not mentioned	FeNO was measured using a NO analyzer (NIOX MINO Analyzer; Aerocrine AB, Solna, Sweden)	N=132 COPD, N=500 asthmatics, and N=57 ACOS visiting the First Affiliated Hospital of Sun Yet-Sen University were retrospectively enrolled in this study	Significant higher level of FeNO in ACOS than COPD group. No differences in FeNO levels among the GOLD groups (stage I-IV). To differentiate ACOS from COPD the optimal FeNO cut-off value was 22.5 ppb with 70% sensitivity and 75% specificity and AUC of 0.78.
Ji, et al. (2016) (246)	Fractional exhaled nitric oxide detection in treatment of	China	Not mentioned/ ICS/LABA	FeNO was measured using NIOX MINO Aerocribe AB, Sweden	N=28 ACOS and N=28 healthy subjects were recruited from Kowloon	Significant decrease in the level of FeNO among ACOS subjects after treatment.

	asthma-chronic obstructive pulmonary disease overlap syndrome				Hospital outpatient or ward	Higher level of FeNO in ACOS group than healthy subjects both before and after treatment. Positive association between pre-and post-treatment FeNO levels with sputum eosinophils and serum total IgE. No association between FeNO levels of pre-and post treatment with FEV1% predicted.
Goto, et al. (2016) (255)	Fractional exhaled nitric oxide levels in asthma–COPD overlap syndrome: analysis of the National Health and Nutrition Examination Survey, 2007–2012	USA	Cross-sectional analysis of the National Health and Nutrition Examination Survey (NHANES)	FeNO was measured using Aerocrine NIOX MINO ® (Aerocrine AB, Solna, Sweden)	Data of N=197 COPD patient from National Health and Nutrition Examination Survey (NHANES)	Higher levels of FeNO in subjects with ACOS (N=48 from 197 COPD) compared to those with COPD alone.
Huang, et al. (2016) (247)	Exercise-Induced Changes in Exhaled NO Differentiates Asthma with or Without Fixed Airway Obstruction From COPD With Dynamic Hyperinflation	Taiwan	Control, prospective study	eNO was measured by NIOX MINO (Aerocrine AB, Sweden), a hand-held device	N=62 COPD, N=60 asthma, and N=27 healthy subjects were recruited from outpatient clinics of Chang Gung Memorial Hospital, Linkuo Medical Center in Taiwan	Higher levels of FeNO were in COPD patients compared to healthy subjects at the baseline. Significant decrease in the change of eNO level after 6MWT in patients with COPD. No association between the percentage of eNO change and the % predicted value of FEV1 at baseline or the percent change of FEV1 in COPD patients.

Kobayashi, et al. (2016) (244)	Inflammatory biomarkers in asthma-COPD overlap syndrome	Japan	Cross-sectional study	FENO level was measured using the NIOX MINO device (Aerocrin, Morrisville, NC, USA)	N=257 COPD patients, data were collected from prospectively consecutively scheduled visits or newly registered patients from the Ishinomaki COPD Network (ICON) registry	Higher FeNO levels in ACOS compared to non-ACOS. No association between FeNO levels and ICS therapy in neither ACOS nor non-ACOS group. To diagnose ACOS the best cutoff value of FeNO was 23 ppb with AUC 0.74, the sensitivity of 73%, and specificity of 68.2%.
Feng et al. (2017) (1)	Relationship between Fractional Exhaled Nitric Oxide Level and Efficacy of Inhaled Corticosteroid in Asthma-COPD Overlap Syndrome Patients with Different Disease Severity	China	Not mentioned/ ICS (budesonide inhalation suspension)	The FeNO levels were measured using a nitric oxide analyzer (NIXO; Aerocrine AB, Solna, Sweden)	N=127 ACOS and N=131 healthy subjects were enrolled	Higher FeNO levels in ACOS patients than healthy subjects at baseline (before ICS therapy). Decrease in FeNO levels in all ACOS patients compared to pre-treatment levels after ICS therapy. Positive association between FeNO levels with total serum IgE and sputum eosinophil. Negative association between FeNO levels with FEV1%pred and FEV1/FVC.
Cosío, et al. (2017) (256)	Th-2 signature in chronic airway diseases: towards the extinction of Asthma-COPD overlap syndrome?	Spain	Cross-sectional, observational, multicentre study	Not mentioned	N=89 COPD, N=94 asthmatics, and N=109 ACOS recruited from 23 outpatient clinics based in tertiary hospitals in Spain	Higher FeNO in ACOS than COPD No significant difference in FeNO between COPD subjects with eosinophilia and those without eosinophilia.
Deng, et al. (2017) (254)	The value of fractionated exhaled nitric oxide in the diagnosis of	China	Not mentioned	FeNO was measured using Naku Lun breath analyzer	N=82 COPD, N=76 asthma, N=81 ACOS, and N=39 healthy non-smoker subjects were recruited from those who had an	Higher FeNO levels in ACOS patients than COPD patients. No association between FeNO and FEV1% predicted and FEV1/FVC in COPD and ACOS.

	asthma-chronic obstructive pulmonary disease overlap syndrome				outpatient visit at Central South University, Xiangya Hospital	To differentiate ACOS form COPD patients the best cutoff value was 29 ppb with a sensitivity 80% and specificity 73%.
Gao et al. (2017) (179)	Correlation between fractional exhaled nitric oxide and sputum eosinophilia in exacerbations of COPD	China	Cross-sectional study	FeNO was measured using NO electrochemical equipment (NIOX Vero; Aerocrine AB, Solna, Sweden)	N=68 COPD patients diagnosed to have acute exacerbations visiting the Third People's Hospital of Guangzhou Medical College in Huizhou, China	Elevated FeNO levels in patients with sputum eosinophilia compared to patients without eosinophilia. Significant association between sputum eosinophils and FeNO levels. No significant association between FeNO levels and blood eosinophil ratio. To diagnose sputum eosinophilia the best FeNO cut-off value was 17.5 ppb with a sensitivity of 64.5%, specificity of 56.4%, AUC 0.617.
Zhao, et al. (2017) (259)	Albuterol inhalation increases FeNO level in steroid-naive asthmatics but not COPD patients with reversibility	China	Observational, prospective study/ Albuterol (bronchodilator)	FeNO was measured using a portable nitric oxide analyzer (NIOX MINO; Aerocrine AB, Solna, Sweden)	N=30 steroid-naive asthma, N=25 ICS treated asthma, and N=20 COPD outpatients selected from patients at the Department of Respiratory Medicine at a hospital	No significant change in FeNO after albuterol inhalation in COPD patients. No significant associations between sputum eosinophils and FeNO levels both before and after bronchodilator inhalation. No association between the FeNO and change in FEV1 after bronchodilator therapy.

COPD: Chronic obstructive lung disease; UK: United Kingdom; eNO: exhaled nitric oxide; FEV1: Forced expiratory volume in first second; FVC: Forced vital capacity; ICS: Inhaled corticosteroid; FeNO: Fractional exhaled nitric oxide; USA: United States of America; GCS: Glucocorticoid, CoV: Coefficient of variation; AUC: Area under the curve; ROC: Receiver operative characteristics; ppb: parts per billion; GOLD: Global Initiative for Chronic Obstructive Lung Disease; BMI: Body mass index; IgE: Immunoglobulin E; FP: Fluticasone; SAL: Salmeterol; TB: Tuberculosis; LABA: Long-acting beta agonist; LAMA: Long-acting muscarinic antagonists; ACOS: Asthma-COPD overlap syndrome; FF: Fluticasone furoate;VI: vilanterol; NHANES: National Health and Nutrition Examination Survey; 6MWT: 6-minute walk test; Th-2: T-helper 2