ASSESSMENT OF HUMORAL IMMUNE FUNCTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is an irreversible lung disease, characterized by chronic inflammation which compromises immune responses and facilitates acute exacerbations of COPD (AECOPD). One notable issue is that some patients continue to experience frequent AECOPD despite receiving the maximum available therapies. Although there is a substantial gap in our understanding of the underlying factors contributing to AECOPD, it is well-established that patients with AECOPD and humoral immunodeficiency are predisposed to similar recurrent, respiratory infections; indicating that humoral immune (HI) dysfunction may underlie AECOPD. We hypothesize that HI dysfunction, defined by impaired antibody production to a polysaccharide vaccine, is present in a subset of COPD patients and associated with higher AECOPD frequency.

This thesis aimed to assess the relationship between HI dysfunction in patients with COPD and mean rate of AECOPD in the past year, using a Typhim Vi (polysaccharide) vaccine response test to measure HI function. A prospective cohort study of COPD patients with at least moderate airflow obstruction (FEV1<80% predicted) was conducted. Pre- and post-immunization anti-Typhi Vi IgG titers were quantified in serum samples by the VaccZyme^{TM} *Salmonella* anti-Typhi Vi IgG ELISA kit in 44 patients.

HI dysfunction was observed in a subset of COPD patients, specifically those with frequent AECOPD in the past year. Age, quality of life scores, smoking history, cellular components and immunoglobulin levels were not associated with HI dysfunction in COPD. Overall, the findings suggest that the presence of HI dysfunction, defined by Typhoid vaccine response, may be an innovative biomarker in clinical practice to phenotype individuals at high-risk of AECOPD.

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ABBREVIATIONS

AECOPD – acute exacerbation of chronic obstructive pulmonary disease

APRIL – A proliferation inducing ligand

BAFF – B cell activating factor of tumor necrosis factor family

BMI – body mass index

CAT - Chronic Obstructive Pulmonary Disease Assessment Test

CI – confidence interval

CIU – Clinical Investigation Unit

COPD – chronic obstructive pulmonary disease

CRP – C-reactive protein

CT – computed tomography

ED – emergency department

FEV1 – forced expiratory volume in the first second

FVC – forced vital capacity

GOLD – Global Initiative for Chronic Obstructive Lung Disease

HI dysfunction - humoral immune dysfunction

ICS – inhaled corticosteroid

Ig – immunoglobulin

Mod. – moderate

Ns – not significant

NTHI – non-typeable *Haemophilus influenza*

OHRI – Ottawa Hospital Research Institute

Pneumovax - pneumococcal polysaccharide vaccine (23-valent)

Prevnar 13 - pneumococcal conjugate vaccine (13-valent)

Pts – patients

RCT – randomized controlled trial

SD – standard deviation

Sev. – severe

SGRQ - St. George's Respiratory Questionnaire

TOH – The Ottawa Hospital

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CHAPTER ONE: INTRODUCTION

1.1 Thesis overview

This thesis consists of four chapters: *Chapter One (Introduction)* provides a thorough background and literature review on chronic obstructive pulmonary disease (COPD), acute exacerbations of COPD (AECOPD) risk factors, a rationale for the presence of humoral immune (HI) dysfunction in COPD, the hypothesis and the study objectives. *Chapter Two (Methods)* presents the methodology and materials required for the study protocol, experimental procedures and data analysis to be performed. *Chapter Three (Results)* presents the main findings from the AiCOP study on the association between humoral immune dysfunction and AECOPD frequency, and other outcome measures. *Chapter Four (Discussion)* summarizes and interprets the main findings in the context of previous literature, identifies strengths and limitations. The significance of the study including clinical and research implications was also discussed, followed by concluding remarks.

1.2 Definition - Chronic Obstructive Pulmonary Disease (COPD)

COPD is an irreversible lung disease, ranked as the third-leading cause of death worldwide, accounting for over 3.22 million deaths in 2019[1],[2]. It is characterized by increased airway inflammation, chronic expiratory airflow limitation and high respiratory symptom burden with dyspnea, cough, chest tightness and/or sputum production[3]. These symptoms are persistent and progressive as a result of significant lung damage and abnormalities in the airways[2],[4]. The cause of COPD is multifactorial, resulting from various gene-environment interactions over the lifetime of an individual that causes reduced lung function and damage[5],[6]. With environmental

exposures such as tobacco smoke, air pollution and inhalation of toxic chemicals contributing significantly to an increased risk of COPD[7].

In clinical context, diagnosis of COPD is confirmed by the presence of non-fully reversible airflow obstruction (FEV1/FVC < 0.7 post-bronchodilation) measured by post-bronchodilator spirometry[8],[9]. Individuals with respiratory symptoms, structural lung lesions (e.g. emphysema as observed on a computed tomography scan) and physiological abnormalities in the absence of airflow obstruction are diagnosed with 'Pre-COPD[10]. Sub-classification into mild, moderate, severe and very severe is achieved by assessing FEV1, as percentage of predicted value, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria as seen in Table 1[8], [9] , [11]. Further diagnostic measures and assessment of the complexity of COPD involves taking thorough clinical history, physical examination, and chest radiography[12]. In addition to the respiratory symptoms above, COPD patients typically complain of chest tightness, wheezing, fatigue and acute events with worsening of respiratory symptoms called acute exacerbation (AECOPD). Also, COPD is commonly associated with other concomitant chronic conditions that influence disease progression and treatment strategy[8].

Table 1: *GOLD classification (I, II, III, IV) of the severity of airflow obstruction in COPD (based on post-bronchodilator FEV1).*

GOLD classification	**Airflow limitation**	**FEV1, L (%) predicted value**
I	Mild	≥ 80% predicted
II	Moderate	≥ 50% and < 80% predicted
III	Severe	≥ 30% and < 50% predicted
IV	Very severe	< 30% predicted

1.3 Epidemiology and burden of COPD

1.3.1 Prevalence, morbidity and mortality

It is estimated that the global prevalence of COPD is approximately 15.17% from 2020-2022[13]. With the prevalence of COPD being significantly higher in smokers (21.51%) and ex-smokers (7.55%) compared to non-smokers, in those ≥40 years of age (12.64%) and in men (15.47%) compared to women[13],[14],[8]. This prevalence increases steeply with age, from 4.37% in those aged 40-49 years to 24.03% in those >70 years. With an ageing population, increased prevalence of smoking and continued exposure to COPD risk factors, the prevalence of COPD is expected to increase over the coming decades[15]. Specifically, in Canada, COPD is the fifth leading cause of death, with a prevalence of 16.3% in individuals (≥40 years) in 2021[16],[17].

To date, studies indicate that morbidity due to COPD will increase with age and development of comorbidities such as lung cancer, cardiovascular disease, metabolic syndrome, diabetes, osteoporosis, depression and much more[18],[19],[20],[21],[22]. As stated, COPD is one of the three leading causes of death in most countries[13],[23]. Between 2009-2019, the mortality rate of COPD increased by 35.4%[13]. This increase in COPD-related mortality has been driven by the epidemic of smoking, ageing of the world population, and scarcity of effective alternative therapies[24]. Lower BMI (underweight) indicative of malnutrition and active smoking status was also associated with increased mortality rates; overweight individuals had a 48% lower risk of death due to respiratory complications[25,26]. It can be approximated that there are over 3 million deaths annually due to COPD, this number is only expected to rise by 2060 to over 5.6 million deaths[27],[28,29].

1.3.2 Economic burden

As one of the leading causes of mortality, COPD is major economic and social burden. In Canada, the annual societal cost of COPD was $4.52 billion Canadian dollars in 2011[30]. These indirect and direct costs were related to emergency department admissions, hospitalization, medications, early retirement, absence from work and comorbidities. A 2003 Canadian survey determined that the total annual costs were $3,196 per patient, with direct costs at $1,998 and indirect costs $1,198 per patient[31]. There is a direct relationship between the severity and progression of COPD and cost of care including increased hospitalization and use of ambulatory oxygen; highlighting the significant burden on the economy[32].

1.4 Pathophysiology

As described in the GOLD 2024 report, "COPD is the end-result of complex, cumulative and dynamic gene-environment interactions over the lifetime that can damage the lungs and/alter their normal developmental or ageing processes"[8]. Pathological effects induce physiological changes including significant narrowing of the airways, specifically the bronchioles[33]. COPD is commonly associated with emphysema and chronic bronchitis[33]. Emphysema involves permanent and gradual degradation of the normal elastic recoil of the parenchyma and consequent destruction of the tiny air sacs (alveoli) in the lungs[34]. While, chronic bronchitis is defined by inflammation and remodeling of the bronchioles which results in a chronic cough and mucus hyper-secretion[1],[35],[36].

1.4.1 Pathobiology

COPD develops as a result of sustained inflammatory and structural changes in the airways, lung parenchyma and vasculature [5,37]. The lungs are a complex organ with numerous

cells that respond to exposures such as infectious agents, cigarette smoking and pollutants[38]. The disruption of homeostasis during this normal inflammatory response to these chronic irritants leads to the development of irreversible morphological and functional changes in the lungs known as COPD. This abnormal inflammatory process involves the interplay of oxidative stress, pro-inflammatory cells, cytokines and protease-antiprotease imbalances that amplify inflammatory mechanisms; further contributing to a chronic inflammatory lung environment[2],[34,37]. An inflammatory lung environment induces lung damage in patients, and compromises immune responses when encountering respiratory pathogen[5],[39],[3]. The degree of lung inflammation and disease progression varies between individuals based on age, sex, comorbidities, genetics, history of infections, socioeconomic status and continued exposure to harmful particles[8].

Molecular pathology involves both innate and adaptive immunity. Activation of innate immunity causes a key structural change associated with COPD, known as emphysema. This process involves recruitment of epithelial cells and alveolar macrophages which release pro-inflammatory cytokines and chemokines[37]. Neutrophils and alveolar macrophages also release multiple inflammatory mediators such as oxidants and proteases which cause elastin degradation that damages the alveolar walls[37]. Destruction of the alveoli leads to airway collapse during exhalation, decreased airflow and impaired gas exchange. Additional effects involve gas-trapping from airway collapse during exhalation called hyper-inflation which leads to decreased inspiratory capacity, dyspnea and decreased exercise tolerance. Impaired gas exchange further results in hypoxemia which causes vasoconstriction of the pulmonary arteries, and airflow obstruction[40],[41].

Further damage to the lung tissue induces the release of antigens which are recognized by dendritic cells and presented to T-lymphocytes, resulting in the activation of adaptive immunity[42]. Upregulation of autoreactive B cells and regulatory T cells contributes to inflammation, lung tissue destruction and COPD progression[43]. Previous studies have shown a correlation between the number of T and B lymphocytes in the lung parenchyma and severity of COPD[34]. Patients with severe COPD have also reported a higher number of autoreactive B cells that may induce autoimmune effects or immune dysfunction in COPD patients[38]. Overall, the inflammatory response and structural alterations cause increased reduction of air flow which can be measured by forced expiratory volume in one second, FEV1, using spirometry[2].

1.4.2 Risk factors

Cigarette smoking is the most significant environmental factor for COPD; the effects of smoking on lung function abnormalities and severe respiratory symptoms have been well-documented[44],[45]. Long-term smokers have a 50% probability of developing COPD with an accelerated decline in lung function and higher mortality rates[46],[47]. While smoking is highly associated with COPD, fewer than 50% of cases are related to non-smoking factors[7]. These other environmental exposures include second-hand smoking, biomass exposure such as air pollution from burning coal/wood for cooking/heating in enclosed spaces, outdoor pollution, and occupational exposures including chemical agents, dusts and toxins[7,8,48]. Additionally, a genetic risk of airflow obstruction has also been observed in people who smoke and are siblings of patients with severe COPD[49]. There are several studies being conducted to identify possible genetic markers associated with a greater risk of COPD and poor lung function[50–54]. Other risk factors include age, asthma, chronic bronchitis, history of severe childhood respiratory infections, and lower socioeconomic status[14,55,56].

1.4.3 Management of stable COPD

The primary objectives of COPD management are to control symptoms, improve quality of life and reduce exacerbations[57]. Pharmacological interventions are initially based on the patient's GOLD classification and exacerbation severity as seen in Table 1 and 2[8]. Common pharmacotherapy includes: short-acting and/or long-acting (muscarinic antagonist/beta2-agonist) bronchodilators (mono or dual combination therapy), inhaled corticosteroids (or triple therapy with bronchodilators), macrolide antibiotics such as azithromycin, oxygen therapy and vaccinations (i.e. influenza, pneumococcal, COVID-19)[58–70]. Each patient's treatment plan is reviewed after a suitable interval to consider modifications based on their current symptoms, treatment adherence, and exacerbation frequency. Non-pharmacological approaches involve smoking cessation programs which incorporate behavioural therapy, social support counseling, patient education, financial incentives and pharmacological interventions to overcome nicotine addiction[71]. Pulmonary rehabilitation is a comprehensive program which involves supervised exercise training, education sessions and group therapy to improve the physical and psychological well-being of patients with COPD[72,73]. The goal of these interventions is to develop long-term behavioural changes to improve overall quality of life[57]. Several questionnaires such as the COPD Assessment Test (CAT, an 8-item questionnaire) (Figure S1) and the St George Respiratory Questionnaire (SGRQ) (Figure S2) are used to assess health status and respiratory symptom burden[74–76].

1.5 Acute exacerbations of COPD (AECOPD)

COPD patients often experience recurrent and acute exacerbations of COPD (AECOPD), which are defined as events characterized by worsening of respiratory symptoms (dyspnea,

cough, and/or sputum production) that are beyond normal day-to-day variation necessitating an increase in medications above baseline[3],[77]. The average patient with COPD experiences two AECOPD events per year, with 10% of these events requiring hospitalization[78,79]. Symptoms of AECOPDs can last from 7 to 10 days per event[80]. The best predictor for exacerbation risk involves assessing the patient's history of exacerbations per year[81],[82],[83]. Individuals that have experienced one moderate AECOPD or less in the prior year are classified as 'low-risk for exacerbations', while those with two moderate or one severe AECOPD are classified as 'high-risk for exacerbations' phenotype[78]. AECOPD events are highly associated with a poor health-related quality of life, increased morbidity and mortality, and a substantial economic burden on the healthcare system as a result of multiple hospitalizations and readmission[81,83–86]. Thus, AECOPD should be quickly diagnosed and treated early.

1.5.1 Clinical evaluation of AECOPD

Evaluation of AECOPD is focused on medical history, physical examination and clinical symptoms. According to GOLD 2024: AECOPD severity may be classified as mild (requiring no additional treatment apart from short-acting bronchodilators (SABDs) only), moderate (requiring outpatient treatment with antibiotics and/or oral corticosteroids such as prednisone in addition to (SABDs)), or severe (requiring hospitalization or emergency department visits) (Table 2)[8]. It is important to assess and eliminate any other potential reasons for worsening of respiratory symptoms, especially since these conditions can often mimic exacerbations: asthma, pneumonia, pneumothorax, pleural effusion, pulmonary embolism, congestive heart failure or acute respiratory distress syndrome[87].

Table 2: *GOLD classification of the severity of acute exacerbations of COPD (based on required treatment strategy).*[8]

Classification	Criteria
Mild	*Out-patient treatment with short-acting bronchodilators (SABDs) only.*
Moderate	*Out-patient treatment with short-acting bronchodilators (SABDs) and oral corticosteroids +/- antibiotics* (if signs of bacterial infection or increased sputum purulence).
Severe	*Patient requires hospitalization or visits the emergency room.* In-patient treatment can involve bronchodilators, oral corticosteroids, antibiotics, supplemental oxygen therapy and/or non-invasive mechanical ventilation. May also be associated with acute respiratory failure.

1.5.2 Cost of AECOPD

AECOPD is associated with increased morbidity and mortality, and a substantial economic burden on the healthcare system[32,88,89]. In Canada, the average cost of treating a moderate or severe AECOPD event is $641 and $9,557, respectively[90]. Indeed, the annual health cost of patients with two or more exacerbations is 36% greater than the remaining COPD population[91,92]. Considerable costs savings and reduction in mortality risk may be realized by preventing and/or reducing AECOPD severity.

1.5.3 Management of AECOPD

The primary goal for the treatment of AECOPD is to minimize the negative impact of the current exacerbation[93]. As previously mentioned, depending on the severity of an exacerbation and the underlying disease, it can be managed in either an outpatient or inpatient setting (Table 2). Over 80% of exacerbation events are managed in an outpatient setting with pharmacological therapies such as short-acting inhaled beta2-agonist bronchodilators as the initial medication,

systemic corticosteroids like prednisone to improve lung function and oxygenation, and antibiotics (upon indication of purulent sputum) to reduce recovery time and treatment failure[94–99]. Hospitalization is only recommended upon severe indications such as high respiratory rate, decreased oxygen, confusion, acute respiratory failure, failure to respond to initial therapy, and/or presence of serious comorbidities[100]. Long-term prognosis after discharge is poor with a 5-year mortality rate of 50%, which can be worsened with older age, lower BMI, severity of previous exacerbations and comorbidities[101–104]. After an acute exacerbation event, appropriate measures need to be considered to identify patients at high risk of exacerbations and prevent future events.

1.6 COPD patients at high risk of exacerbations

The Canadian Thoracic Society's guidelines recommend stratifying patients based on the risk of future exacerbations to guide appropriate treatment measures and prevention of future exacerbations. As a reliable biomarker of AECOPD is not available, predicting the risk of future exacerbations currently hinges on having a history of exacerbations[82,105]. As aforementioned, individuals with ≤ 1 moderate AECOPD in the last year are low-risk, while those with ≥ 2 moderate or ≥ 1 severe AECOPD are at high-risk for future exacerbations[8],[106]. Despite maximal treatment with existing therapy, some COPD patients continue to experience frequent exacerbations; and they are associated with an accelerated decline in lung function and poor health status[91,92]. For example, patients treated with optimal pharmacotherapy continue to experience on average 0.91 moderate or severe AECOPD annually[107]. Recurrence of AECOPD may reflect that there are predisposing factors that are not yet identified and treated and/or

ineffective pharmacotherapies. Using AECOPD history as a predictor of future AECOPD risk may be insufficient in guiding treatment and identifying high-risk patients[108].

There is a strong interest in identifying biomarkers that facilitate clinical decision-making toward a personalized medicine approach. For example, national and international guidelines recommend using blood eosinophil counts to determine whether inhaled corticosteroids (ICS) could be added to maintenance inhaled therapy to prevent AECOPD[8]. The guidelines are based on studies showing that ICS has little to no benefit in patients with blood eosinophil counts <100 cells/μL[8,106]. Phenotyping AECOPD patients based on blood eosinophil levels has shown to be associated with exacerbation risk, however variability in the cut-off of blood eosinophil levels to predict high-risk of AECOPD has been suggested[109]. Furthermore, predictive biomarkers of exacerbations included elevated levels of CRP, leukocytes and fibrinogen. Unfortunately, these previously studied biomarkers correlated poorly with exacerbations and lacked specificity and predictive value[109]. Thus, the need for novel biomarkers to guide personalized therapies for preventing AECOPD in high-risk patients exists.

1.7 Causes of AECOPD

To better understand the risk of AECOPD, it is important to understand the underlying causes. Risk factors for exacerbations are not well understood, but likely multifactorial. Exacerbations are often triggered by bacterial or viral infections, environmental pollutants (cigarette smoke), or other unknown factors; these lead to hyper-inflated lungs, reduced airflow, and increased airway inflammation[110]. It has been well-established that respiratory infections trigger over 60% of AECOPD events in patients; with 40-60% cases of bacterial and 30% of viral infections[3,39,110]. Several AECOPD cases have been reported with antibiotic-resistant bacteria[3,39,110]. Frequent bacterial etiological agents of exacerbations include *Streptococcus*

pneumoniae, Staphylycoccus aureus, non-typeable *Haemophilus influenzae and Moraxella catarrhalis*[111]. *Pseudomonas aeruginosa* has been associated with accelerated FEV1 decline[112]. While the most common viral pathogens tend to be human rhinovirus, influenza viruses, and respiratory syncytial viruses[3,110]. Respiratory pathogens tend to target the airway epithelia, which serves as a barrier to inhaled pathogens, further increasing susceptibility to bacterial-viral co-infection and poor mucus clearance[113]. Past studies have also shown that viral and/or bacterial loads are higher after infection in COPD patient groups compared to healthy controls, suggesting that patients with frequent exacerbations have a higher susceptibility to infections and significant impairment in lung defence mechanisms[114–116]. One of the key immune responses that is impaired during an infection is the humoral immune response.

1.8 Role of humoral immune response in preventing respiratory infection

The immune system is composed of two responses: innate and adaptive. The adaptive immune response is made up of the humoral and cellular immune systems[117]. The HI response is complex and HI dysfunction can result from a spectrum of disorders ranging from disordered B-cell stimulation all the way downstream to disordered production of specific antibodies.

1.8.1 The Humoral immune (HI) response

The HI system is critical in preventing infections, particularly bacterial infections with polysaccharide capsules (e.g., *Streptococcus pneumoniae, Haemophilus influenzae*) [117]. In response to infection, naïve B cells proliferate and differentiate into effector B cells that produce antigen-specific antibodies, also known as immunoglobulins (Ig)[117].

Ig play a major role in protecting mucosal surfaces including the airways by binding to foreign antigens and neutralizing bacteria by disrupting their motility or their adherence to epithelial cells[117]. IgM is the first Ig isotype produced by activated naïve B cells prior to class

switching with gene rearrangement and somatic hyper-mutation processes[118]. Thus, IgM tends to be of low affinity, but it's early production and large structural molecule with 10 antigen-binding sites makes it essential in early immune defense[118]. IgA and IgG are Ig isotypes produced by B-cells that have undergone class switching[118]. IgA resists cleavage from proteases present in mucus, and thus is predominantly produced on the respiratory or gastrointestinal mucosa[118]. IgG is the most abundant Ig isotype in plasma, and is composed of four subclasses: IgG1, IgG2, IgG3, and IgG4[118],[119] . These IgG subclasses have similar functions with slight differences. For example, a reduction in IgG2 has been associated with increased susceptibility to infections with encapsulated organisms[18]. Different types of antigens induce B cell response differently, either with or without T cell help. Polysaccharide antigens, for example, induce a B cell response without T cell help (i.e., T cell-independent B cell response) [118]. Activated B cells undergo differentiation into memory B cells, which are long-lasting and can produce Ig rapidly in response to previously recognized antigens from repeated infections. Class-switched B-cells from the primary infection also progress to class-switched memory B cells[118].

1.8.2 Humoral immune dysfunction

Any abnormality in the HI system contributes to an ineffective immune response and recurrent infections. The term HI dysfunction may include inadequate antibody production and/or B cell dysfunction. HI dysfunction may be inherited (primary humoral immunodeficiencies) or acquired (secondary). Primary humoral immunodeficiency includes common variable immunodeficiency, while secondary humoral immunodeficiency results from hematological malignancies and immune-modulating medications (e.g., rituximab, which depletes B cells)[120]. Patients with HI dysfunction are frequently unable to mount sufficient B cell related immune responses to protect against harmful infections[121]. These individuals are at

increased susceptibility to recurrent bacterial sino-pulmonary tract infections, which can lead to chronic lung diseases such as bronchiectasis and COPD[122,123],[124]. Oksenhendler et al. (2008) showed that 91% of 252 common variable immunodeficiency patients developed viremia or infection symptoms [124]. Studies have also shown that over 60% percent of AECOPD cases are triggered by viral infections which target the airway epithelial cells, increasing susceptibility to bacterial co-infection and poor mucus clearance[113].Therefore, recurrent infections may result in AECOPD similar to data observed in humoral immunodeficiency patients.

Management of humoral immunodeficiencies involves preventative measures such as vaccinations and prophylactic antibiotic treatment to reduce recurrent infections. Unfortunately, these measures have limited efficacy, standard protocols are still lacking and there are undesired side effects such as antibiotic resistance[125]. Prompt recognition of HI dysfunction and early treatment with polyvalent IgG infusions (from healthy plasma donors) reduces infections, prevents complications, and improves quality of life[126]. Presently, Ig treatment is the cornerstone for the management of primary humoral immunodeficiencies[125]. It has shown to recover antibody levels, minimize complications, reduce infections and improve the health status of patients[125].

1.9 Evidence of possible humoral immune dysfunction in COPD

Although it is unknown whether COPD is definitively associated with HI dysfunction, there is growing evidence that recurrent respiratory tract infections (i.e. exacerbations) may be associated with reduced adaptive and mucosal immune function[127].

1.9.1 Animal studies

In C57BL/6 mouse models, Moghaddam et al. reported that chronic exposure to non-typeable *Haemophilus influenza* (NTHI), a causative pathogen for recurrent infections in COPD

patients, is associated with increased lung inflammation and compromised adaptive immunity[128],[129]. Immune cell infiltration was greatly present in the airways including CD8 T cells. NTHI-specific B cell responses involved in the adaptive immune system were impaired along with poor antibody production and systemic immune dysfunction[127],[129]. Immune responses were impaired across the mucosal surfaces, along with the spleen, bone marrow, and in serum samples[127],[130]. Motz et al. also showed expansion of T cells in the mucosal environment of mouse models of cigarette-induced COPD, indicative of a persistent adaptive T cell immune response[131]. Further, several studies identified low levels of markers such as CXCL12/1, and BAFF (B cell activating factor of tumor necrosis factor family); and upregulation of APRIL (A proliferation inducing ligand) involved in B cell survival and development; in COPD mouse model lungs[132].

1.9.2 Human studies

These findings from animal studies were also reflected in human studies. First, Pela et al. evaluated bronchial microbial flora to isolate predominant bacteria colonizing the airways of 56 COPD patients. Common infection agents, *H. influenzae, S. pneumoniae, and Moraxella catarrhalis*, in humoral immunodeficient patients, were frequently detected in COPD patients with high-risk of future exacerbations[133]. 54% AECOPD patients were also positive for non-typeable *Haemophilus influenzae* (NTHi)[134]. Furthermore, BAFF was significantly increased in the lungs of patients with COPD similar to mice, which may attenuate inflammation in the lung and emphysema[135]. COPD was associated with poor proportions of CD4+ T central memory cells, especially in patients with frequent exacerbations[136].

Provided that these AECOPD-associated pathogens and markers are mainly localized in the respiratory mucosa, it is expected that there is an impairment to elicit mucosal immune

responses. Studies have provided evidence that mucosal immunity is impaired in COPD patients including an abnormal mucosal IgA response, which is initiated upon the upregulation of CD8+ T cells in the narrow bronchioles and areas with emphysema[137]. Adaptive T helper and B cell infiltration was also observed in these areas[138]. Overall, this suggests that COPD may alter mucosal defense and promote dysregulated adaptive immune responses that underlie lung tissue destruction including the epithelial barrier against pathogens[138].

Additionally, prolonged and frequent use of systemic corticosteroids (e.g., prednisone in COPD) has been associated with low serum IgG levels, and inhaled corticosteroids are associated with increased risk of pneumonia[139,140]. Therefore, corticosteroids use in COPD patients with recurrent AECOPDs may suppress B cell function[141], and induce secondary humoral immunodeficiency, which may further increase risk of recurrent exacerbations.

Finally, Filho et al. investigated the relationship between circulating total IgG levels in COPD participants and the risk of exacerbations. The overall frequency of hypogammaglobulinemia (IgG<7 g/L) in cohorts of n=621 was 10.3% and n=262 was 11.5%. The findings demonstrated a linear relation between serum IgG levels and exacerbation frequency and hospitalization in two large COPD cohorts (n=976 and n=653) [142]. Hypogammaglobulinemia increased exacerbation risk by 70% and hospitalization by 60% in these large cohorts. Several studies have shown reduced concentrations of total IgA, IgG and IgG subclasses in serum and bronchoalveolar lavage; and blood gene expression profiles, indicative of poor lymphocyte function and impaired mucosal immunity [142–146]. Further work by McCullagh et al. demonstrated an association between IgG levels and pneumococcal IgG titers, indicating poor antibody levels in a subset of patients with COPD[147]. Although both of these studies indicate HI dysfunction in AECOPD patients, the primary data is limited by its

quantitative measure of immunity – serum IgG levels or pneumococcal antibody assessment in previously vaccinated individuals. A functional measure of humoral immunity is required to assess antibody responses and compare baseline and post-vaccination levels. This literature does not necessarily indicate whether COPD patients with frequent exacerbations tend to have defects in the ability of B-cells or their respective antibodies response to opsonize and eliminate pathogens[148].

1.9.3 Ig treatment reduces AECOPD

Studies have further demonstrated that polyvalent Ig treatment significantly reduces recurrent AECOPD events in patients with COPD[121],[85]. In a retrospective case series of 14 patients with COPD, Cowan et al. demonstrated a reduction in the frequency of annualized AECOPD from 4.7 ± 3.1 from the year before to 0.6 ± 1.0 in the year after Ig treatment (Figure 1)[149]. While these results were promising, a prospective observational study was needed to evaluate the effect of Ig treatment on AECOPD.

Cowan et al. then conducted a pilot single-center, randomized placebo-controlled study to determine the feasibility and safety of Ig prophylaxis to prevent AECOPD (IPRAC study)[150]. It was found that monthly intravenous Ig (IVIG) for 12 months in COPD patients with recurrent AECOPD was challenging due to poor adherence. Nevertheless, compared to placebo, there was a trend toward increased AECOPD-free days among participants who adhered to ≥80% of the treatments regardless of baseline serum IgG levels in the IVIG group. The median time free from AECOPD events was 161 days longer in the IVIG group[150].

These data suggest that Ig treatment may be a potential therapy to prevent AECOPD but several questions should be addressed before conducting a large efficacy trial[151]. One key question is “whether there is a subset of COPD patients who benefit from Ig therapy?” Perhaps

Ig therapy reduces AECOPD by preventing respiratory tract infections in a similar way to patients with congenital HI dysfunction[151]. Overall, this suggests that HI dysfunction may exist in some COPD patients and be a risk factor for frequent AECOPD.

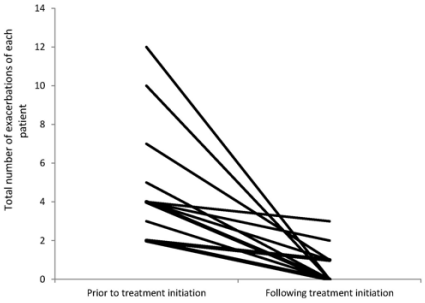

Figure 1: *Number of AECOPD events for one year before and after the initiation of Ig treatment in n=14.* 53 moderate and 12 severe AECOPD were observed in the year before Ig treatment, while 8 moderate and 1 severe AECOPD was observed in the year after Ig initiation. This figure illustrates a consistent reduction in the annual rate of AECOPDs in each individual. Adapted from Cowan et al. PLoS ONE. 10(11): e0142205[149].

1.10 Humoral immune function assessment methods

HI function can be assessed by measuring antibody response to a polysaccharide antigen vaccine and/or quantifying serum Ig isotypes.

1.10.1 Gold-standard detection of humoral immune dysfunction

The antibody response to the 23-valent pneumococcal vaccine (Pneumovax23), against *Streptococcus pneumoniae*, is the 'gold standard' for diagnosing HI dysfunction. Suspected HI dysfunction is traditionally detected by an immune response to generate antibodies against bacterial polysaccharide antigens without T-cell help, known as a T-cell-independent immune response[152]. Upon vaccination and activation of the adaptive immune response, B cells are activated which can be classified as T-cell dependent or independent based on the requirement for T cell help in producing Igs. T-cell-independent antigens are often polysaccharides with repeating epitopes and liposaccharides[153]. Polysaccharide vaccines often contain a polysaccharide antigen from the pathogen's surface, and when administered - the antigen

engages with the B cell receptor. This induces the B cells to perform antigen-specific responses which involve the synthesis of various immunoglobulin subtypes including high levels of IgG production[152]. This response is a blunted memory response in humans. The antigen-specific (i.e. anti-pneumococcal IgG) antibodies are released into circulation and the antibody response can be quantified by comparing the serum upon blood collection in pre- and post-vaccination samples. In adults, subnormal IgG concentrations represent a humoral immunodeficiency characterized by recurrent respiratory infections[153]. This method is a functional measure of humoral immunity.

Although the 23-valent pneumococcal vaccine response test is commonly used, its practicality may be challenging in COPD patients. Most individuals at high risk for pneumococcal infections (including COPD patients) are strongly recommended to receive vaccination as it is associated with a reduced hospitalization risk[154]. According to the Government of Canada, 20.3% of individuals with chronic illnesses and 41.6% of individuals over age 65 have received the pneumococcal vaccine[155]. Thus, this vaccine lacks a neo-antigen that can reliably differentiate between normal and abnormal IgG responses in a significant portion of our targeted population. This poses a barrier to reliably assessing HI responses using this testing modality.

1.10.2. Quantitative Ig isotypes

In clinical practice, access to the polysaccharide vaccine response may be limited. In these cases, a diagnosis of HI dysfunction can be made with serum IgG levels below 4 g/L with or without low levels of IgA (<0.7 g/L) and IgM (<0.4g/L). If IgG is mildly low (<7 g/L), then a polysaccharide vaccine response is recommended to confirm diagnosis because 2.5% of the general population can have mildly low IgG without HI dysfunction[156]. On the other hand, HI function in individuals with recurrent infections but normal serum IgG levels should also be

tested because HI dysfunction can still be present[157]. As such, functional analysis with vaccine response is superior to IgG quantification.

1.11 Novel Typhim Vi detection of humoral immune responses

Recent studies indicate that aforementioned boundaries can be overcome by measuring HI response with the *Salmonella* Typhi Vi polysaccharide vaccine, containing the VI capsular polysaccharide. This vaccine response test has been widely supported in literature and used by the Clinical Immunology community as an alternative to the pneumococcal vaccine. Bausch-Jurken et al. (2017) compared pre- and post-vaccination serum titers to the Typhim Vi vaccine in patients with diagnosed and suspected primary immunodeficiencies[158]. They found that a ≤2-fold increase in antibody titers post to pre-vaccination is both 100% sensitive and specific in detecting a known humoral immunodeficiency, with no false positives[159]. All healthy controls (100%) showed >2-fold specific antibody response to Typhim Vi vaccination across multiple studies[151, 156, 157]. Additionally, a study by Evans et al. showed a significant correlation between the detection of HI dysfunction using the 'gold-standard' pneumococcal and Typhim Vi vaccine[159]. Other studies have found consistent results and confirmed that IgG responses to Typhim Vi vaccination can be reliably used to assess HI dysfunction[160–162]. This vaccine is an optional Health Canada-approved vaccine recommended to individuals (i) travelling to areas with high risk of salmonellosis or typhoid exposure, (ii) likely to be in contact with a known typhoid carrier and/or (iii) working in a laboratory with *S. typhi*[159]. Thus, the baseline concentrations of Typhi Vi IgG antibodies in most Canadians should be low if not zero. This vaccine will be a suitable alternative tool to assess HI function in COPD patients in a simple manner.

1.12 Summary of rationale and hypothesis

There is a substantial gap in our understanding of the underlying factors contributing to AECOPD risk and different phenotypes of COPD. One notable issue is that some patients continue to experience frequent AECOPD despite receiving the maximum available therapies. Emerging data suggest a growing interest in studying the adaptive immune responses in COPD patients[110]. It is well-established that individuals with HI dysfunction are more susceptible to recurrent respiratory tract infections, a trait shared by some COPD patients who experience acute exacerbations. In our previous research, we observed that Ig treatment, commonly prescribed for patients with confirmed HI dysfunction, appeared to reduce the rate of AECOPD. **In this study, we hypothesize that HI dysfunction is present among patients with COPD, and those with HI dysfunction have a higher rate of AECOPD compared to those without HI dysfunction**. To identify HI dysfunction in COPD populations, we will use the Typhim Vi vaccine response test. We will quantify anti-Typhim Vi IgG antibody titers before and after immunization. Additionally, we will quantify total Ig isotypes to determine if these readily accessible clinical tests can be used as surrogate markers of the polysaccharide vaccine response in the COPD population as an exploratory exercise. If HI dysfunction is identified in a subset of COPD patients, the polysaccharide vaccine response and/or Ig isotypes may be used as clinical biomarkers to inform personalized therapies.

1.12.1 Primary objective

To assess the relationship between HI dysfunction, defined as a ≤2-fold increase in anti Typhi Vi IgG titers after Typhim Vi vaccination, and AECOPD frequency in COPD patients with at least moderate airflow obstruction.

1.12.2 Secondary objectives

To evaluate 1) Proportion of patients with different thresholds of post- vs pre- antibody titer fold change, 2) Association between HI dysfunction and serum measurements, COPD severity, medication use, and patient reported outcome measures.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Study design and site settings

The *Assessment of Humoral Immune Function in COPD Patients* (AiCOP) study is a single-center prospective observational (cohort) study at The Ottawa Hospital (TOH) approved by the Research Ethics Board. TOH serves 1.2 million people in Eastern Ontario and the respirology clinics at TOH care for approximately 500 patients with COPD. The study was conducted at the clinical investigation unit (CIU) of the Ottawa Hospital Research Institute (OHRI). All patients provided informed consent before study participation per the Declaration of Helsinki.

2.2 Study participants

Participants were recruited, since February 2022 until the present, from inpatient clinics, pulmonary rehabilitation programs and ambulatory clinics at TOH General Campus in Ottawa, Ontario. Patients whom provided permission to be contacted by research personnel were approached in the clinic to undergo screening and participate in the study. Eligibility criteria for the study are as follows:

Inclusion criteria included individuals that are over the age of 40 years with a confirmed diagnosis of COPD by post-bronchodilator spirometry FEV1/FVC <0.70, and at least moderate airflow obstruction FEV1, L (%) <80% predicted. The exclusion criteria excluded individuals who received previous Typhim Vi vaccination, immunosuppressants, prednisone >10mg in the past four weeks, Ig treatment within the last six months, with history of AECOPD or infection in the past four weeks, known immunodeficiency, history of organ or stem cell transplantation, and/or active malignancy within the past 12 months.

2.3 Study procedure

All eligible participants who provided informed consent were required to visit the study center twice. Figure 2 shows the study procedure, with details outlined in Table S1 (in Appendix). The baseline visit (Visit 1) included a review of the screening criteria, comorbidities (asthma, diabetes, heart disease, chronic liver/kidney disease, eczema, allergies, other), and medication use. A history of moderate and severe AECOPD in the past 12 months prior to enrollment was obtained from patients, and verified by reviewing electronic medical records, medications dispensed from the pharmacy and family physician/walk-in clinic documentation. Blood samples were collected to measure pre-vaccination anti-Typhi Vi IgG titers (U/mL), complete blood counts (white blood cells, 10^9/L), cellular differentials (neutrophils, eosinophils, lymphocytes, 10^9/L) and Ig levels (including IgA (g/L), IgM (g/L), IgE (ug/L), IgG (g/L), IgG1 (g/L), IgG2 (g/L), IgG3 (g/L), IgG4 (g/L)). This was followed up by administration of a single dose of the Typhim Vi vaccine (0.5mL) (from Sanofi Pasteur Ltd) intramuscularly. Respiratory questionnaires including the CAT (out of 40) and SGRQ (out of 100) scores were collected to assess quality of life and respiratory symptoms. Both English versions of the assessment are reliable and valid [74–76](Figure S1 and S2). At the follow-up visit (Visit 2), four weeks post-vaccination, blood samples were collected to measure post-vaccination anti-Typhi Vi IgG titers.

2.3.1 Prospective data collection

In addition, all enrolled participants were followed up via phone calls every 12, 24, 36, 48 weeks to document changes in their respiratory symptoms to prospectively capture AECOPD rates, medication changes and symptom burden (CAT) scores. AECOPD were defined as worsening in at least 2 of the following 3 characteristics: breathlessness, cough, and sputum production that is beyond normal day-to-day variation[8]. We captured mild, moderate and severe

AECOPD events during the follow-up period (Figure 2). Prospective AECOPD details were verified by referring to emergency department (ED) visits or hospital admissions through electronic medical records from patients' primary care physicians and pharmacies using the EPIC system at TOH. EPIC is an electronic medical record system used at TOH which allows the study team to receive notifications for every enrolled participant who presented to the emergency room or required hospitalization during the duration of the study. Analysis from prospective data is ongoing, and not reported in this thesis.

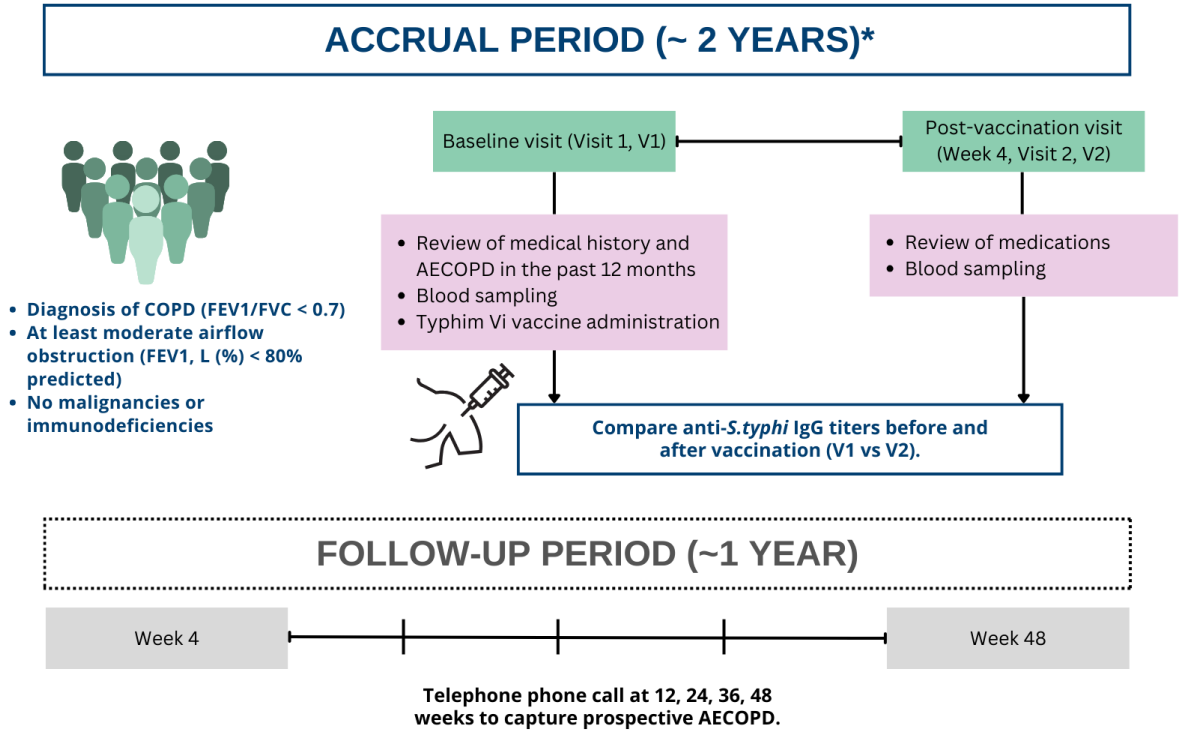

Figure 2: *AiCOP study work plan and timeline.* The accrual period (~2 years) involves the baseline and post-vaccination (V2) visit with a review of AECOPD history in the past 12 months. The follow-period (~1 year) involves prospective data collection over the next 12 months after enrolment. *This thesis is based on retrospective AECOPD data from the accrual period.

2.3.2 Vaccine safety

In consideration of vaccine safety, past immunogenicity and safety trials were conducted in the adult US population. Minor and transient adverse reactions were reported including local reactions such as injection site pain, elevated oral temperature (>38°C) and erythema; these reactions in duration were almost always resolved within 48 hours of vaccination. No serious or life-threatening systemic events were reported (Data from Sanofi Pasteur Ltd. Product Monograph - Typhim Vi. December 2018)[163].

2.3.3 Blood processing

Standard serum separation techniques were performed on the day of sample collection to obtain 1-2mL of sera from whole blood samples collected at Visits 1 and 2. The serum was centrifuged at 1600 rpm for 10 minutes before aliquoting and stored in a -80°C freezer until a sufficient sample number was available to be evaluated (i.e. batch analysis) using the VaccZyme™ *Salmonella* Typhi Vi IgG Enzyme-Linked Immunosorbent Assay Kit (The Binding Site Group, Ltd., UK).

2.3.4 VaccZyme™ *Salmonella* Typhi Vi IgG ELISA kit

This ELISA was carried out according to the manufacturer's instructions (The Binding Site) at room temperature. Samples were diluted 1:100 with sample diluent. 100uL of each diluent sample, five calibrators (ranging from 7.4, 22.2, 66.7, 200 and 600 U/mL), high and low positive control was dispensed into the appropriate wells and incubated for 30 minutes. Calibrators and controls were included in each run. Plates were washed three times. 100uL of conjugate was dispensed into each well in duplicate and incubated for 30 minutes. Plates were washed three times again. Then, 100uL of substrate (TMB) was dispensed into each well and incubated in the dark for 30 minutes. 100uL of stop solution was dispensed into each well (a

color from blue to yellow was observed) and the optical density (OD) of each well was read at 450nm on a microplate reader, within 30 minutes of stopping the reaction. The mean optical densities for each calibrator, control and sample were calculated. A calibration curve was plotted between anti-Typhi Vi IgG antibody concentration on the log scale and the OD on the linear scale for each calibrator. The curve was used to read the anti-Typhi Vi IgG concentration (U/mL) of the controls and diluted samples.

2.4 Outcome measures

2.4.1 Objective 1

Overall, the primary objective is studying an association between humoral immune response (presence or absence of HI dysfunction) and frequency of AECOPD. HI dysfunction or impaired antibody was defined by a ≤2-fold increase in anti-Typhi Vi IgG titers after Typhim Vi vaccination as measured by the using the VaccZyme™ *Salmonella* Typhi Vi IgG ELISA kit (The Binding Site Group, Ltd., UK). A 2-fold increase of post-vaccination Ab titer compared to the pre-vaccination titer was shown to have 100% [95% CI 88.43% – 100%] sensitivity, and 100% [95% CI 84.56% – 100%] specificity to diagnose HI dysfunction in a known immunodeficiency population. This ratio was referred to as 'antibody fold change' interchangeably throughout this thesis.

As previously defined, AECOPD involve periods of worsening in at least 2 of the following 3 characteristics: breathlessness, cough, or sputum production that are beyond normal day-to-day variation necessitating an increase in medications above baseline[164]. AECOPD frequency was determined by capturing moderate (requiring physician visits, antibiotics, and/or systemic corticosteroids) and severe AECOPD (requiring hospitalization) history which are less

subjective than mild AECOPD (requiring short-acting bronchodilators only). During the follow-up period; mild, moderate and severe AECOPD events were captured every 12 weeks.

To meet Objective 1, the proportion of HI dysfunction in AECOPD and no AECOPD patients was determined. Then mean annual rates of moderate and severe AECOPD were compared based on the presence of HI dysfunction in our COPD cohort. Total post-immunization anti-Typhi Vi IgG titers (U/mL) and post: pre-immunization antibody fold change (AU) were also compared with AECOPD rates. Secondary analysis involved assessing HI dysfunction in association with i) only moderate AECOPD, ii) only severe AECOPD, iii) low-risk AECOPD patients (defined by GOLD), and IV) high-risk.

2.4.2 Objective 2

The secondary outcomes are:

I) Proportion of COPD patients with HI dysfunction using different thresholds of post- versus pre- antibody titer fold change.

Other thresholds to define HI dysfunction were deemed through previous literature; a ≤3-fold, ≤4-fold and ≤6-fold increase in anti-Typhi Vi IgG titers after immunization were considered[159,165,166]. The proportion of patients with HI dysfunction using these various cut-offs was compared between patients with and without AECOPD.

II) Sociodemographic and clinical characteristics

Demographic information such as sex (female and male), age (years), body mass index ($kg \cdot m^{-2}$), smoking history (pack-years) was recorded. The proportion of patients with HI dysfunction was compared between different age ranges (0-49, 50-59, 60-69, 70-79, 80-89 and 90-99 years), females and males, BMI categories (underweight, normal, over-weight and obese) and smoking history (pack years, active vs quit smoking). All variables were

compared between patients with and without dysfunction and also with degree of antibody fold change.

III) Serum measurements

Blood test results (white blood cells, neutrophils, eosinophils, lymphocytes, IgG, IgM, IgA, IgE, IgG1, IgG2, IgG3, IgG4, CRP) were reported. Pre-immunization serum IgG (g/L), IgG subclasses (g/L), IgA (g/L), IgM (g/L), IgE (ug/L) and CRP (mg/L) were measured at the TOH laboratory core with a nephelometry technique (Siemens Vista 1500). Deficiency of quantitative Ig measures was defined as IgG, IgG1, IgG2, IgG3, IgG4, IgA, or IgM levels below the lower limits of the reference range established per assay (refer to Table S2 in Appendix). All serum measurements were compared between patients with and without HI dysfunction. Additionally, IgG levels were compared with degree of antibody fold change.

IV) Measures of quality of life and respiratory symptoms

Both disease specific health-related quality of life and symptom burden was measured by the St. George's Respiratory Questionnaire (SGRQ) and COPD Assessment Test (CAT), respectively. SGRQ scores were measured out of 100 ranging from Quartile 1-4, while to CAT scores were measured out of 40 from low to very high (refer to Table S3 in Appendix). Higher scores indicate worse quality of life and health status or respiratory symptoms, respectively. The proportion of patients with HI dysfunction and different score ranges on the CAT and SGRQ were determined. Further CAT and SGRQ scores were compared between patients with and without HI dysfunction.

V) Pulmonary function and COPD severity

Spirometry results, indicating pulmonary function, were collected including forced expiratory volume in the first second (FEV1, L (%)), forced vital capacity (FVC, L (%))

values and an FEV1/FVC ratio were obtained at the baseline visit (V1). The FEV1, L (%) value and FEV1/FVC ratio were compared between patients with and without HI dysfunction to assess possible associations with pulmonary function. Further, the spirometry results were used to classify COPD patients according to the GOLD criteria (Table 1) to assess a possible association between COPD severity and HI dysfunction. The proportion of patients with HI dysfunction in each GOLD category was identified, along with a comparison to antibody fold change.

VI) Therapeutic interventions

Clinical information such as medications, vaccination history and pulmonary rehabilitation was reported. The usage of these medications was recorded and assessed for an association with HI responses: cumulative systemic corticosteroid usage equivalent to dosage of prednisone in the 12 months prior to study enrollment, current use of chronic azithromycin 250-500mg thrice weekly or 250mg daily for at least 3 months prior to study enrolment; and current use of beta-2-agonists, muscarinic antagonists, inhaled corticosteroids, or supplemental oxygen. The completion of pulmonary rehabilitation in the past five years and initiation of Ig treatment during the follow-up period was also recorded. Finally, previous pneumococcal and influenza vaccination in the past 5 years (including details regarding type and date of vaccinations for secondary analysis) was collected. The proportion of HI dysfunction between patients on and off these interventions was determined, and antibody fold change was also assessed.

All of these other measures were compared between patients with and without HI dysfunction.

2.5 Sample size calculation (for the AiCOP study)

The sample size was calculated using Mann-Whitney t-tests on the difference between AECOPD rates across patients with and without HI dysfunction from the pilot data (n=40). Thus, the calculation was based on the estimate that a third or more of the COPD cohort will have HI dysfunction, and annual exacerbation rates were roughly 0.8 and 1.5 events in the non-dysfunction and dysfunction groups, respectively. Given the observed standard deviations of 1.1 and 1.3, it was estimated that this is a conservative pooled standard deviation of 1.2. A two-sample t-test would require 107 patients to detect this difference with 80% power and with 25% participant loss to follow-up and/or withdrawal, a total recruitment goal of 140 participants was determined (for the AiCOP study, recruitment is ongoing). Since the negative binomial model gains power from the fact that patients can experience more than one AECOPD, 140 patients will provide ample power to detect a difference.

2.6 Statistical and descriptive analyses

GraphPad – Prism (Version 10.0.3, La Jolla, CA, USA) was used for all statistical analysis and graph plotting. Patient clinical and demographic characteristics at baseline were described using means and standard deviations for continuous variables and proportions or percentages for categorical variables. After testing for normality, statistical comparisons between HI dysfunction and AECOPD frequency and other variables was performed using a Spearman correlation, Mann-Whitney t-test ((nonparametric unpaired analysis, 2 groups) and/or Kruskal-Walis test (nonparametric, unpaired analysis, >2 groups). Proportions were analysed by Fischer's exact test. Antibody fold change was presented with mean +/- standard deviation and 95% confidence intervals. P-values <0.05 were considered significant unless stated otherwise.

CHAPTER THREE: RESULTS

3.1 Recruitment outcomes

Figure 3 illustrates a consort diagram of the enrolled study population. 371 participants were screened for eligibility into the AiCOP study using the aforementioned inclusion and exclusion criteria between March 2022 and March 2024 from TOH, Ottawa, Canada. Among these participants, 137 are being followed up via email or phone call for interest in participation. 182 patients were excluded from study enrolment due to failed exclusion criteria (n=80), refused participation in research (n=82), or long commuting distance (n=20). Please refer to Figure 3 for details on the participants (n=80) whom did not meet the exclusion criteria.

Fifty-two patients have passed screening and been enrolled. A total of 46 patients have completed their baseline visits: 44 patients have completed the Week 4 in-person visit including the post-immunization blood draw and 15 patients have completed the entire study. 44 patients have had both pre- and post- anti-Typhi Vi IgG (U/mL) measurements.

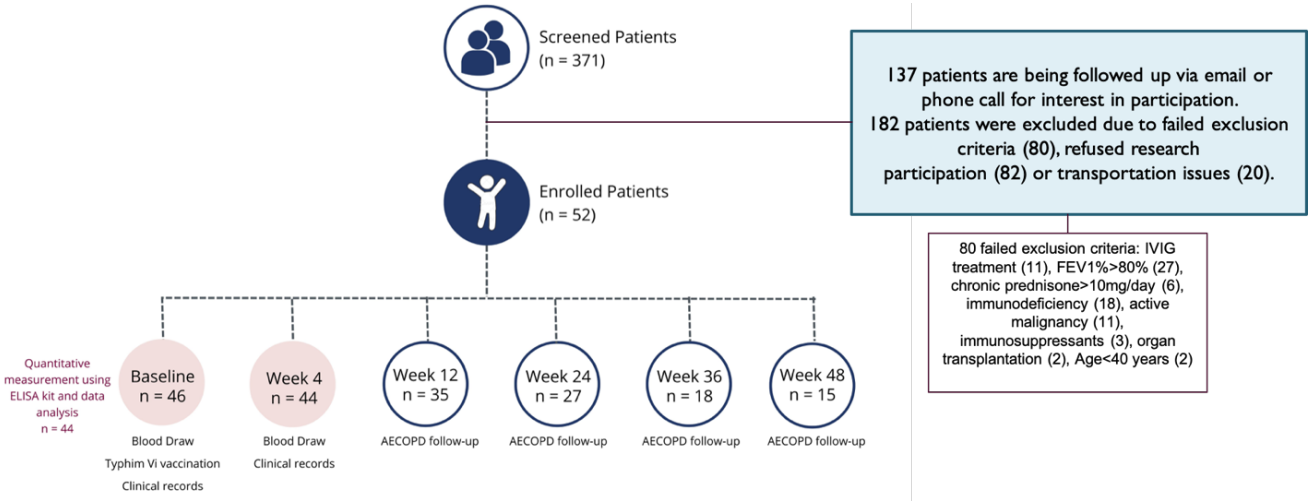

Figure 3: *Consort diagram of the study population.* Eligible patients were recruited from out-patient settings. 371 patients were screened with the defined inclusion and exclusion criteria. From which, 137 participants are being followed up and 182 were excluded due to the provided rationale. 52 patients were enrolled into the AiCOP study with and without AECOPD. The number of participants whom have completed each visit of the study are displayed.

Abbreviations: AECOPD – acute exacerbations of COPD, ELISA – enzyme-linked immunosorbent assay. FEV1 – forced expiratory volume in the first second

3.2 Clinical and demographic characteristics

Baseline clinical characteristics and history of AECOPD frequency for all participants (n=46) are presented in Table 3, and further divided into only AECOPD (n=26) and no AECOPD (n=20) participants. Demographics, cellular components, immunoglobulin levels, pulmonary function, comorbidities, medical interventions, and health status were recorded. Mean age of the total COPD cohort (25 females, F) was 69.6 +/- 9.82 years; with a mean smoking history of 43.0 pack-years (6 active smokers), mean FEV1, L of 49.1 +/- 17.1% and mean annual rate of 1.13 +/- 1.38 AECOPD per year. For subgroup of participants with history of AECOPD in the past year, mean age was 67.4 +/- 10.2 years (12F); with a mean smoking history of 41.0 pack years, mean FEV1, L of 52.6% and mean annual rate of 2.00 +/- 1.26 AECOPD per year. For subgroup of participants with no AECOPD, mean age was 72.1 +/- 8.98 years (13F) with a mean smoking history of 50.6 pack years and mean FEV1, L of 45.6%.

There were no important differences between age, sex, smoking history, pulmonary function, and SGRQ scores for AECOPD and no AECOPD patients. BMI (kg/m^2) was significantly lower for participants with AECOPD compared to those without AECOPD (Mann Whitney t-test, 0.01 ($P<0.05$)). CAT scores, indicating respiratory symptom burden, were significantly higher (Mann Whitney t-test, 0.02 ($P<0.05$)). Cellular components such as white blood cells, neutrophils, eosinophils and lymphocytes; and immunoglobulin subtypes (IgG, IgA, IgM) and IgG subclasses (IgG1, IgG2, IgG3, IgG4) in circulation were within normal limits for the COPD cohort. IgE was slightly higher in circulation.

Table 3: *Baseline demographics and clinical characteristics for COPD patients (n=46): All COPD; subgroups of AECOPD vs no AECOPD.*

Parameter	COPD (n=46)	AECOPD (n=26)	No AECOPD (n=20)	P-value
Female: male, n	25:21	12:14	13:7	--
Age, years	69.6 +/- 9.82	67.4 +/- 10.2	72.1 +/- 8.97	0.54, ns
Body mass index, kg・m^{-2}	28.2 +/- 9.58	25.7 +/- 7.60	31.2 +/- 10.9	0.01*
Smoking history, pack years	43.0 +/- 32.3	41.0 +/- 26.9	50.6 +/- 34.8	0.54, ns
Active smoking status, n (%)	6 (13%)	3 (12%)	3 (15%)	--
GOLD classification: II, III, IV	23:17:6	16:8:2	7:9:4	--
Cellular component levels				
White blood cells, 10^9・L^{-1}	7.08 +/- 1.64	7.07 +/- 1.76	7.08 +/- 1.54	0.93, ns
Neutrophil, 10^9・L^{-1}	4.70 +/- 1.46	4.67 +/- 1.51	4.74 +/- 1.44	0.80, ns
Lymphocyte, 10^9・L^{-1}	1.44 +/- 0.51	1.45 +/- 0.52	1.42 +/- 0.51	0.93, ns
Eosinophil, 10^9・L^{-1}	0.24 +/- 0.29	0.27 +/- 0.34	0.22 +/- 0.24	0.65, ns
CRP, mg・L^{-1}	5.36 +/- 7.45	4.61+/- 4.15	6.18+/- 9.95	0.55, ns
Immunoglobulin levels				
IgG total, g・L^{-1}	9.65 +/- 4.79	8.97 +/- 3.88	10.3 +/- 5.57	0.27, ns
IgA, g・L^{-1}	2.14 +/- 1.47	2.15 +/- 1.57	2.12 +/- 1.40	0.71, ns
IgM, g・L^{-1}	1.15 +/- 1.16	1.22 +/- 0.85	1.09 +/- 1.42	0.20, ns
IgE, ug・L^{-1}	310 +/- 569	337 +/- 661	283 +/- 473	0.97, ns
IgG1, g・L^{-1}	5.10 +/- 3.42	5.65 +/- 4.55	4.57 +/- 1.72	0.92, ns
IgG2, g・L^{-1}	2.45 +/- 1.35	2.46 +/- 1.34	2.44 +/- 1.39	0.85, ns
IgG3, g・L^{-1}	0.51 +/- 0.37	0.50 +/- 0.42	0.51 +/- 0.44	0.81, ns
IgG4, g・L^{-1}	0.52 +/- 0.55	0.43 +/- 0.55	0.61+/- 0.65	0.20, ns
Post-bronchodilator spirometry				
FEV1, L (%)	49.1 +/- 17.1	52.6 +/- 16.4	45.6 +/- 17.4	0.31, ns
FEV1/FVC (%)	49.5 +/- 16.7	53.0 +/- 17.9	45.9 +/- 15.1	0.38, ns
Chest radiography[a]				
Normal	15 (33%)	9 (35%)	6 (30%)	--
Emphysema	26 (57%)	15 (58%)	11 (55%)	--
Bronchitis	5 (11%)	2 (8%)	3 (15%)	--
Bronchiectasis	2 (4%)	1 (4%)	1 (5%)	--
AECOPD rate per year				
Total # of AECOPD per year	1.13 +/- 1.38	2.00 +/- 1.26	0	--
Moderate AECOPD, n	0.72 +/- 0.98	1.27 +/- 1.00	0	--
Severe AECOPD, n	0.33 +/- 0.87	0.58 +/- 1.10	0	--
Comorbidities				
Asthma, n (%)	16 (35%)	9 (35%)	7 (35%)	--
Diabetes mellitus, n (%)	7 (15%)	3 (12%)	4 (20%)	--
Hypertension, n (%)	15 (33%)	7 (27%)	8 (40%)	--
Coronary Artery Disease, n (%)	4 (9%)	3 (12%)	1 (5%)	--

Congestive Heart Failure, n (%)	3 (7%)	2 (8%)	1 (5%)	--
Chronic Kidney Disease, n (%)	1 (2%)	1 (4%)	0 (0%)	--
Eczema, n (%)	3 (7%)	2 (8%)	1 (5%)	--
Environmental allergies, n (%)	12 (26%)	7 (35%)	5 (19%)	--
Other, n (%)	16 (35%)	10 (38%)	6 (30%)	--
Pharmacological intervention, n (%)				
Current home oxygen use	17 (37%)	9 (35%)	8 (40%)	--
Oral azithromycin	10 (22%)	7 (27%)	3 (15%)	--
Prednisone > 10mg/day	4 (9%)	3 (12%)	1 (5%)	--
Inhaled beta-agonist	44 (96%)	25 (96%)	19 (95%)	--
Inhaled muscarinic antagonist	32 (70%)	18 (69%)	14 (70%)	--
Inhaled corticosteroid	19 (41%)	10 (38%)	9 (45%)	--
Non-Pharmacological Intervention				
Pulmonary rehabilitation, n (%)	25 (54%)	16 (62%)	9 (45%)	--
Vaccination history (past 60 months)				
Prevnar 13, n (%)	41 (89%)	22 (85%)	19 (95%)	--
Pneumovax 23, n (%)	41 (89%)	23 (88%)	18 (90%)	--
Influenza, n (%)	34 (74%)	18 (69%)	16 (80%)	--
Health status and quality of life				
SGRQ score, out of 100	46.4 +/- 21.5	50.0 +/- 22.4	41.9 +/- 20.0	0.43, ns
CAT score, out of 40	18.3 +/- 8.80	20.9 +/- 9.30	15.1 +/- 7.16	0.02 *

Note: All measures indicate n (%) and mean +/- standard deviation for dichotomous and continuous variables, respectively.
*Values indicate statistical significance (P<0.05).
[a] Not all results available for every patient.

[b]Other comorbidities include obesity, sleep apnea, spinal injury, migraines, depression, anxiety, diverticulitis, hypothyroidism, multiple sclerosis, arthritis, osteoporosis, disease of thyroid, and gastroesophageal reflux disease.

Abbreviations: AECOPD – acute exacerbations of COPD, CAT – COPD assessment test, COPD – chronic obstructive pulmonary disease, FEV1 – forced expiratory volume in the first second, FVC – forced vital capacity, GOLD – Global Initiative for Chronic Obstructive Lung Disease, ns = not significant, Prevnar 13 - Pneumococcal conjugate vaccine (13-valent), Pneumovax - Pneumococcal polysaccharide vaccine (23-valent), SGRQ – St. George Respiratory Questionnaire.

3.3 Typhim Vi vaccine response in COPD patients (n=44)

Humoral immune response, measured by anti-Typhi Vi IgG post: pre-immunization fold change, was quantified in n=44. Of 44 COPD participants, 15 (34.1%) had HI dysfunction or impaired antibody response (≤2-fold post: pre-anti-Typhi Vi IgG titers) referred to as 'non-

responders'. 12 out of 15 (80%) COPD patients with HI dysfunction had ≥1 moderate or severe AECOPD events in the 12 months before enrollment.

The proportion of COPD participants with or without HI dysfunction in the AECOPD and no AECOPD groups was assessed. The proportion of patients with HI dysfunction was significantly higher for participants with AECOPD (Figure 4A) compared to those without AECOPD (Fischer's exact test, P=0.025). 12 out of 24 (50%) participants with ≥1 moderate or severe AECOPD (n=24) in the past year, presented with HI dysfunction (Figure 4B).

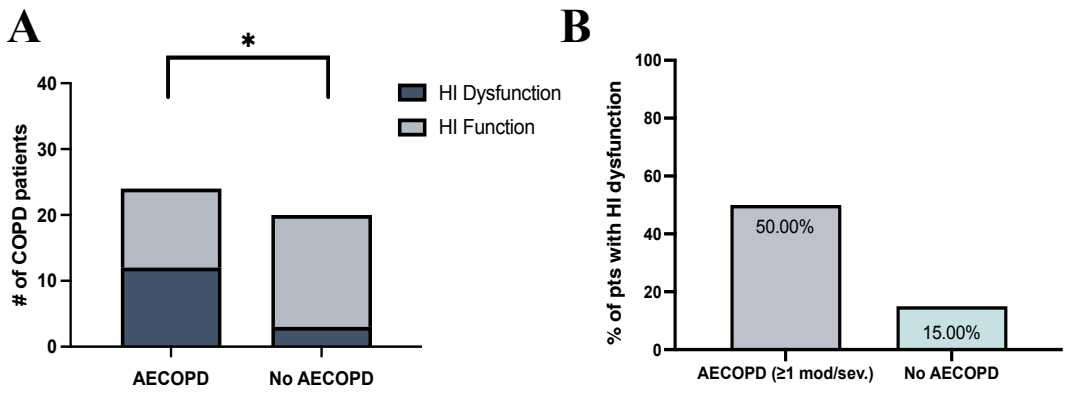

Figure 4: *Proportion of COPD patients with Humoral Immune Dysfunction (measured by Typhim Vi vaccine response* of anti-*Typhi Vi IgG ≤2-fold change).* A) Fisher's exact test of the number of COPD patients with and without HI dysfunction in AECOPD and no AECOPD subgroups. Statistical significance was shown with P=0.025. Navy blue indicates HI dysfunction, and gray indicates HI function. B) Bar graph comparing percentage of patients with HI dysfunction in AECOPD (12/24, 50%) and no AECOPD (3/20, 15%) subgroups.
Abbreviations: IgG – Immunoglobulin G, HI dysfunction – humoral immune dysfunction, pts – patients.

3.4 Total anti-Typhi Vi IgG levels

Mean anti-Typhi Vi IgG concentrations were low (10.91 +/- 4.85 U/mL) and comparable (Mann Whitney t-test, P = 0.599, ns) among all COPD participants at the baseline visit before immunization (Figure 5A). All pre-immunization samples were within the low range (< 25.2 U/mL) of the ELISA kit except one patient at 38.3 U/mL. Mean post-immunization anti-Typhi

Vi IgG concentrations were 145.6 +/- 244.2 U/mL. Antibody concentrations were significantly reduced (Mann Whitney t-test, P = 0.011) among participants with ≥1 moderate/severe AECOPD in the past year (mean anti-Typhi Vi IgG of 130.3+/- 281.0 U/mL) compared to those without AECOPD (mean anti-Typhi Vi IgG of 164.0 +/- 196.7 U/mL) (Figure 5B).

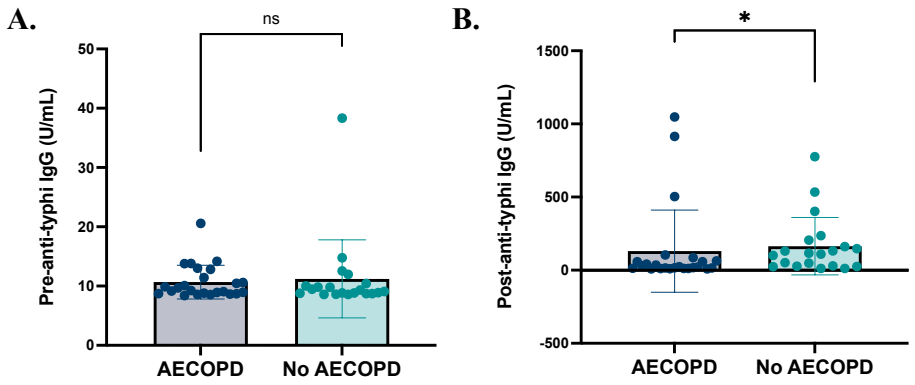

Figure 5: *Total anti-Typhi Vi IgG titers before and after vaccination in subgroups of AECOPD and no AECOPD patients.* A) Mann-Whitney t-test reported a non-significant P=0.599 for anti-Typhi Vi IgG before immunization in AECOPD versus no AECOPD subgroups. B) Mann-Whitney t-test reported a significant P=0.011 after immunization in AECOPD versus no AECOPD subgroups.
Abbreviations: IgG = immunoglobulin G, ns = not significant.

3.5 Pre: Post-immunization anti-Typhi Vi IgG fold change and AECOPD frequency

A ratio of post- to pre-immunization anti-Typhi Vi IgG, referred to as anti-Typhi Vi IgG fold change or 'antibody fold change' was compared with AECOPD frequency in the past 12 months before enrolment. The mean anti-Typhi Vi IgG fold change was 12.75 +/- 26.99 AU for participants with AECOPD and 16.29 +/- 21.88 AU for those without AECOPD. Anti-Typhi Vi IgG fold change was significantly lower among participants with ≥1 moderate/severe AECOPD (Mann-Whitney t-test, P = 0.0052) (Figure 6A). When comparing antibody fold change between participants with varying number of acute exacerbations (0, 1, ≥2 events), a decrease in antibody fold change was associated with increased AECOPD frequency. A significant association

between lower antibody fold change and having had ≥2 moderate/severe AECOPD as compared to those without exacerbations in the previous year was observed (Kruskal-Wallis test, P = 0.028) (Figure 6B). The mean anti-Typhi Vi IgG fold change across participants with 0, 1, ≥2 events are shown in Table 4.

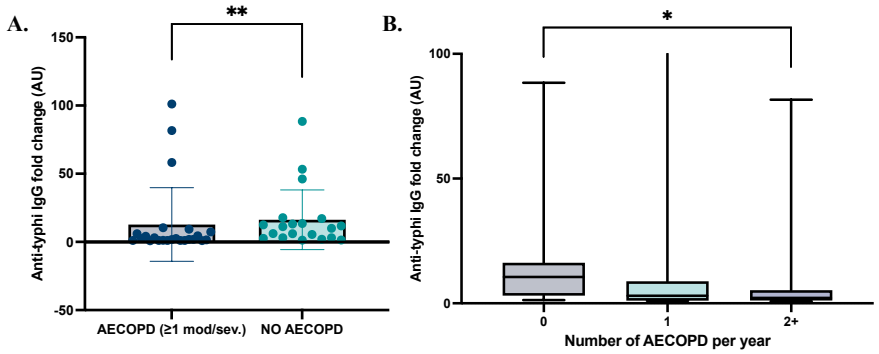

Figure 6: *Total Moderate and Severe AECOPD Frequency and* anti-*Typhi* Vi IgG *fold change.* A) Mann-Whitney t-test reported a significant P=0.0052 for anti-Typhi Vi IgG fold change in AECOPD versus no AECOPD subgroups. B) Kruskal-Wallis test reported a significant P=0.028 for anti-Typhi Vi IgG fold between patients with 0 exacerbations versus +2 exacerbations. Non-significant P=0.0145 and >0.999 were observed between patients with 0 vs 1 AECOPD and 1 vs +2 AECOPD, respectively.
Abbreviations: IgG = immunoglobulin G, mod. = moderate, sev. = severe.

Table 4: *Mean Anti-Typhi Vi IgG fold change across participants with 0, 1, ≥2 AECOPD events.*

	# of past AECOPD	Anti-Typhi Vi IgG fold change, AU, mean +/- SD (95% CI)
No AECOPD	0	16.29 +/- 21.88 (6.053-26.53)
AECOPD	1	16.04 +/- 31.22 (-3.795-35.88)
	≥2	9.457 +/- 22.90 (-5.091-24.01)

3.5.1 Humoral immune response and AECOPD frequency

With the objective of analyzing anti-Typhi Vi IgG produced in response to immunization in COPD patients, AECOPD frequency was compared between patients with and without HI dysfunction, referred to as non-responders (n=15, ≤2-fold post: pre-anti-Typhi Vi IgG titers) and responders (n=29), respectively. Mean annual exacerbations were significantly higher for patients with HI dysfunction (Mann-Whitney t-test, 1.600 ± 1.404 AECOPD, P value = 0.030) than those with adequate function (0.862 ± 1.329 AECOPD) (Figure 7A). AECOPD rates between responders and non-responders were reported in Table 5. A significant weak-moderate correlation was observed with anti-Typhi Vi IgG fold change and number of past AECOPDs (Spearman correlation, ρ= -0.403, P=0.007) (Figure 7B).

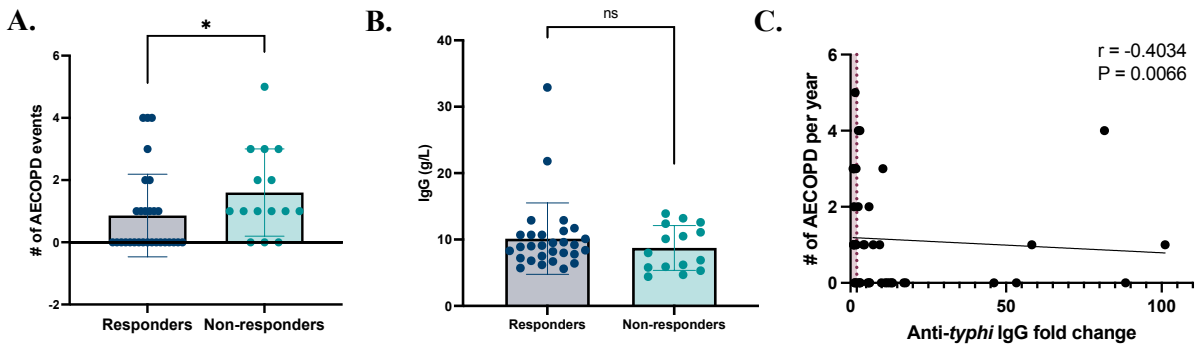

Figure 7: *Humoral Immune Response and Total Moderate and Severe AECOPD Frequency.* A) Number of AECOPD events in patients with and without humoral immune dysfunction (non-responders and responders, respectively). A Mann Whitney t-test reported a significant P=0.030. B) Serum IgG titers at baseline in responders versus non-responders. A Mann Whitney t-test reported a non-significant P=0.481. C) Spearman correlation between anti-Typhi Vi IgG fold change and number of AECOPD in the past 12 months from enrolment reported a significant ρ value of -0.4034 (P=0.0066).

Table 5: *Mean annual rates of moderate and/or severe AECOPD in the past 12 months by anti-*Typhi *Vi IgG antibody response (no or yes).*

Typhim Vi Antibody Response	**AECOPD History**	**AECOPD rate per year, mean +/- SD (95% CI)**
No, HI dysfunction	All moderate and/or severe AECOPD	1.600 ± 1.404 (0.822-2.378)

(≤2-fold) (n=15)	events	
	Only moderate AECOPD events requiring prednisone/antibiotics + bronchodilators	1.133 +/- 1.125 (0.510-1.757)
	Only severe AECOPD events requiring ED visit or hospitalization	0.267 +/- 0.799 (-0.176-0.709)
Yes, HI function (> 2-fold) (n=29)	All moderate and/or severe AECOPD events	0.862 ± 1.329 (0.357-1.368)
	Only moderate AECOPD events requiring prednisone/antibiotics + bronchodilators	0.483 +/- 0.871 (0.152-0.814)
	Only severe AECOPD events requiring ED visit or hospitalization	0.379 +/- 0.942 (0.021-0.738)

Abbreviations: CI = confidence intervals, ED = emergency department, SD = standard deviation.

3.5.2 Comparison of only moderate versus severe AECOPD frequency

Across 44 COPD participants, there was a total of 31 moderate and 15 severe AECOPD events in the past 12 months. When comparing HI response and moderate AECOPD frequency, non-responders had a significantly higher moderate AECOPD frequency in the past year when compared to responders (Mann-Whitney t-test, $P = 0.024$) (Figure 8A). Anti-Typhi Vi IgG fold change had a significant weak-moderate correlation with moderate AECOPD rates in the past year (Spearman correlation, $\rho = -0.4483$, $P = 0.0023$) (Figure 8B). Severe AECOPD rates between responders and non-responders were comparable (Mann-Whitney t-test, $P = 0.615$, ns)

(Figure 8C). A comparison between HI response and all, only moderate and only severe AECOPD frequency was reported in Table 5 above.

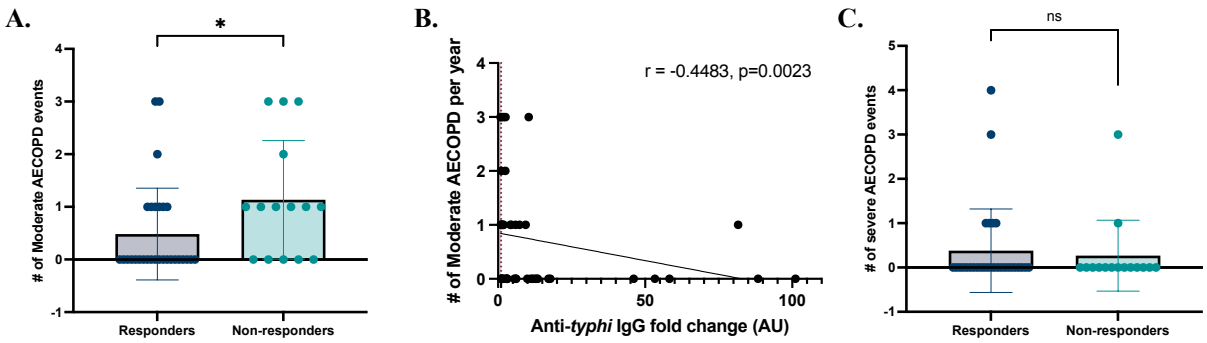

Figure 8: *Humoral Immune Response and Moderate versus Severe Only AECOPD Frequency.* A) Number of moderate AECOPD events in patients with and without HI dysfunction (non-responders and responders, respectively). A Mann Whitney t-test reported a significant P=0.024. B) Spearman correlation between anti-Typhi Vi IgG fold change and number of moderate AECOPD in the past 12 months from enrolment reported a significant ρ value of -0.4483 (P=0.0023). C) Number of severe AECOPD events in patients with and without HI dysfunction (non-responders and responders, respectively). A Mann Whitney t-test reported a non-significant P=0.615.

3.5.3 Humoral immune dysfunction in low-risk versus high-risk AECOPD patients

From our COPD cohort, a total of 32 participants have been identified as low-risk (≤1 moderate AECOPD in the past year) for future AECOPD and 12 are high-risk (≥2 moderate or >1 severe AECOPD). Proportional analysis showed that the high-risk COPD group had a higher number of patients of HI dysfunction than low-risk patients (Fishers exact test, P = 0.7222 ns) (Figure 9A). 42% of high-risk AECOPD patients presented with HI dysfunction (Figure 9B). Anti-Typhi Vi IgG fold change was comparable between low-risk and high-risk AECOPD patients (Mann Whitney t-test, P = 0.267) (Figure 9C).

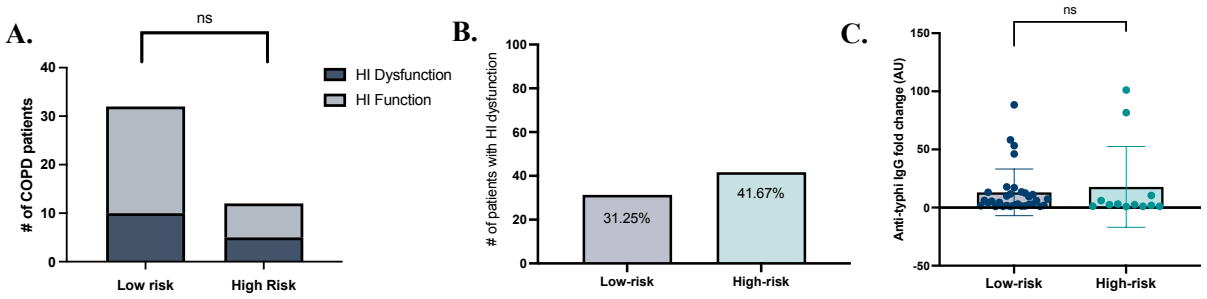

Figure 9: *Humoral immune response in patients at low-risk vs high-risk for frequent AECOPD.* A) Fisher's exact test of the proportion of COPD patients with and without HI dysfunction in low-risk and high-risk subgroups showed a non-significant P=0.7222. Navy blue indicates HI dysfunction, and gray indicates HI function. B) Bar graph comparing percentage of patients with HI dysfunction in low-risk (31%) versus high risk (42%) subgroups. C) Anti-Typhi Vi IgG fold change in patients at low-risk vs high-risk. A Mann Whitney t-test reported a non-significant P=0.267.

Low-risk non-responders had a significantly higher AECOPD frequency in the past year when compared to responders (Mann-Whitney t-test, P = 0.013) (Figure 10A). While findings were comparable between high-risk responders and non-responders (Mann-Whitney t-test, P = 0.905, ns) (Figure 10B). Mean annual AECOPD rates between participants with and without HI dysfunction in the low-risk and high-risk groups was reported in Table 6.

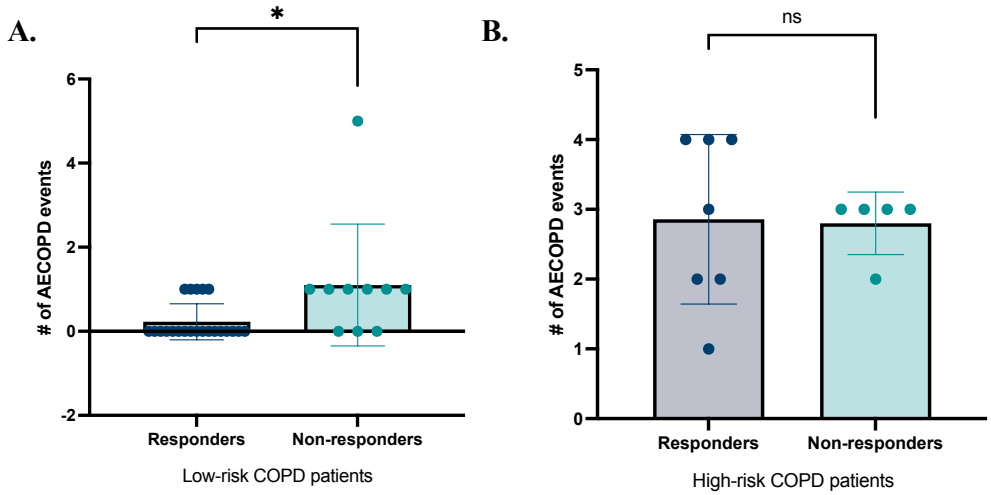

Figure 10: *Number of AECOPD events in low-risk vs high-risk responders and non-responders.* A) Mann-Whitney t-test of the number of AECOPD between responders and non-

responders in the low-risk subgroup reported a significant P=0.013. B) Mann-Whitney t-test in the high-risk subgroup reported a non-significant P=0.905.

Table 6: *Mean annual rate of AECOPD by Typhim Vi antibody response in patients at high-risk vs low-risk of AECOPD (defined by GOLD criteria).*

Typhim Vi Antibody Response	**Risk of Future AECOPD**	**AECOPD rate per year, mean +/- SD (95% CI)**	**P-value**
No, HI dysfunction (≤2-fold) (n = 15)	High-risk (n = 5)	2.800 +/- 0.447 (2.245-3.355)	0.905
	Low-risk (n = 10)	1.100 +/- 1.449 (0.063-2.137)	0.013
Yes, HI function (> 2-fold) (n = 29)	High-risk (n = 7)	2.857 +/- 1.215 (1.733-3.981)	0.905
	Low-risk (n = 22)	0.227 +/- 0.429 (0.037-0.418)	0.013

Note: High risk = patients with ≥2 AECOPD; Low risk = patients with ≤1 moderate or 0 severe AECOPD.
Abbreviations: CI = confidence intervals, SD = standard deviation.

3.6. Proportion of study patients with different thresholds of post- vs pre- antibody titer fold change

To explore the proportion of COPD patients with different thresholds of post- vs pre-antibody titer fold change, a ≤3-fold, ≤4-fold and ≤6-fold cut-off for HI dysfunction was considered. The proportion of patients with HI dysfunction was significantly higher for participants with AECOPD compared to those without AECOPD using the ≤3-fold, ≤4-fold and ≤6-fold cut-offs: Fischer's exact test, P = 0.0064, 0.0329, 0.0128, respectively (Figure 11A, B, C, top). In comparison to the standard 2-fold cut-off (n=15, 34% HI dysfunction, Fischer's exact test, P = 0.025), a 3-fold cut-off identified 19 (43%) participants with HI dysfunction. With a 4-fold cut-off and 6-fold cut-off, 22 (50%) and 27 (61%) presented with HI dysfunction, respectively. Similar to a 2-fold cut-off, mean annual exacerbations were significantly higher for

patients with HI dysfunction than those with adequate function when using a 3-fold ((Mann-Whitney t-test, P = 0.009), 4-fold (Mann-Whitney t-test, P = 0.0147) and 6-fold cutoff (Mann-Whitney t-test, P = 0.0179) (Figure 11A, B, C bottom, respectively). Both the proportions of HI dysfunction patients using each threshold cut-off and mean AECOPD rates between responders and non-responders are highlighted in Table 7.

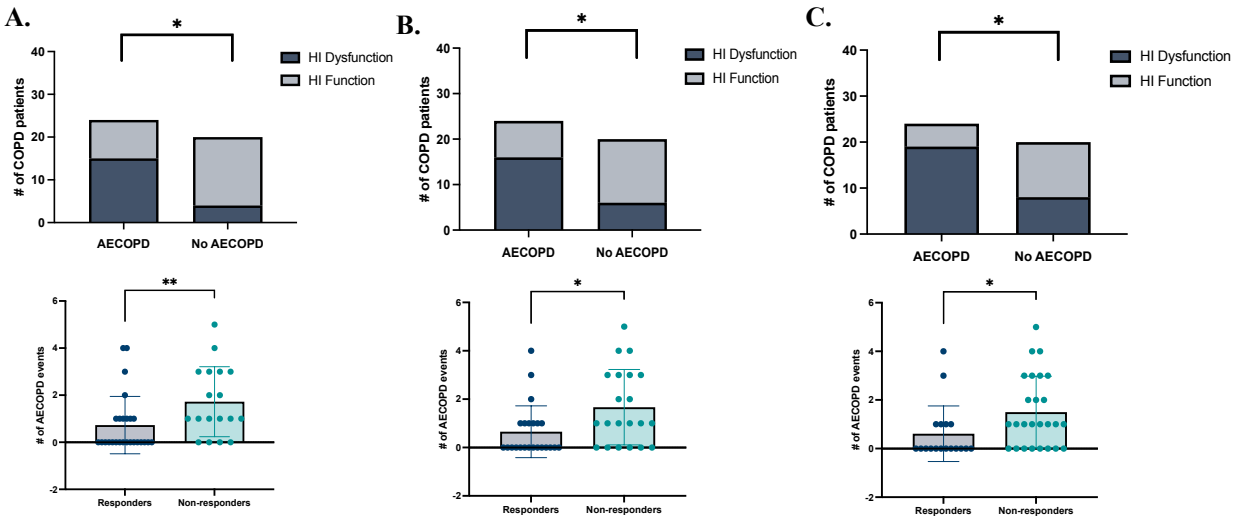

Figure 11: *Humoral immune responses based on varying thresholds for HI dysfunction.* Top figures show Fischer's exact test identifying proportions of patients with HI dysfunction across AECOPD and no AECOPD subgroups. Bottom figures show Mann-Whitney t-tests of the number of AECOPD events between responders and non-responders. A) Fischer's exact test for HI dysfunction based on a ≤3-fold cutoff reported a significant P=0.0064 (top) and Mann-Whitney t-test reported a significant P=0.009 (bottom). B) Fischer's exact test for HI dysfunction based on a ≤4-fold cutoff reported a significant P=0.0329 (top) and Mann-Whitney t-test reported a significant P=0.0147 (bottom). C) Fischer exact test for HI dysfunction based on a ≤6-fold cutoff reported a significant P=0.0128 (top) and Mann-Whitney t-test reported a significant P=0.0179 (bottom).

Table 7: *Mean annual rate of AECOPD in patients with HI dysfunction defined by varying thresholds (≤2-fold, 3-fold, 4-fold, 6-fold cut-offs).*

Threshold for HI dysfunction	**Proportion of patients with HI dysfunction**	**AECOPD rate, mean+/- SD (95% CI)**

	AECOPD (n=24)	No AECOPD (n=20)	Responders	Non-responders
≤2-fold	12 (50%)	3 (15%)	0.862 ± 1.329 (0.357-1.368)	1.600 ± 1.404 (0.822-2.378)
≤3-fold	15 (63%)	4 (20%)	0.731 +/- 1.218 (0.239-1.223)	1.722 +/- 1.487 (0.983-2.462)
≤4-fold	16 (67%)	6 (30%)	0.652 +/- 1.071 (0.189-1.115)	1.667 +/- 1.560 (0.957-2.377)
≤6-fold	19 (79%)	8 (40%)	0.611 +/- 1.145 (0.042-1.180)	1.500 +/- 1.476 (0.904-2.096)

Abbreviations: CI = confidence intervals, SD = standard deviation.

3.7 Humoral immune response and demographic characteristics

3.7.1 Age

All enrolled COPD patients are over the age of 40 years, with a majority of participants within the age range of 60-79 years. The HI response has been observed to vary in elderly populations compared to youth. When comparing the proportion of patients with HI dysfunction across various age ranges, a greater number of participants (n=9) between 70-79 years presented with HI dysfunction (Figure 12A). The number of patients with HI dysfunction within each age group was reported in Table 8. To evaluate possible effects of age on HI responses in our COPD cohort, the age of responders and non-responders was compared. Age was similar between responders and non-responders (Mann-Whitney t-test, $p = 0.1115$, ns) (Figure 12B). Also,

antibody fold change was comparable across different age groups (Kruskal-Wallis test, P value = 0.4599) (Figure 12C).

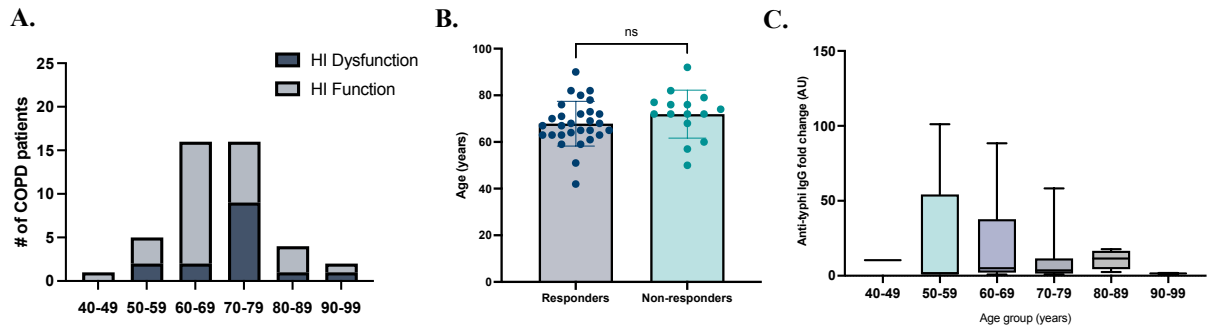

Figure 12: *Age and Humoral Immune Response in COPD.* A) Proportion of patients with HI dysfunction across varying age ranges. B) Mann-Whitney t-test of age (years) between responders and non-responders reported a non-significant P = 0.1115. C) Anti-Typhi Vi IgG fold change across varying age ranges was non-significant on a Kruskal-Wallis test with a P value = 0.4599.

Table 8: *Proportion of COPD patients with HI dysfunction across age ranges (years).*

Age (years)	# of COPD Patients with HI dysfunction (%)
40 - 49	0 (0%)
50 - 59	2 (40%)
60 - 69	2 (13%)
70 - 79	9 (56%)
80 - 89	1 (25%)
90 - 99	1 (50%)

3.7.2 Sex (female and male)

Out of the 44 COPD patients assessed for HI dysfunction, there are 24 (55%) females and 20 (45%) males. The proportion of HI dysfunction in males (n = 9, 60%) was slightly increased compared to females (n = 6, 40%) (Fischer's exact test, P value = 0.2097, ns). (Figure 13A-B).

When comparing AECOPD frequency in male COPD participants only, non-responders had a significantly higher number of AECOPD (2.222 +/- 1.641 events) than responders (0.546 +/- 1.214 events) (Mann-Whitney t-test, P value = 0.018) (Figure 13C). No statistical differences

were seen between responders and non-responders in female COPD participants (Mann-Whitney t-test, P value = 0.216); non-responders had a slightly increased AECOPD rate than responders.

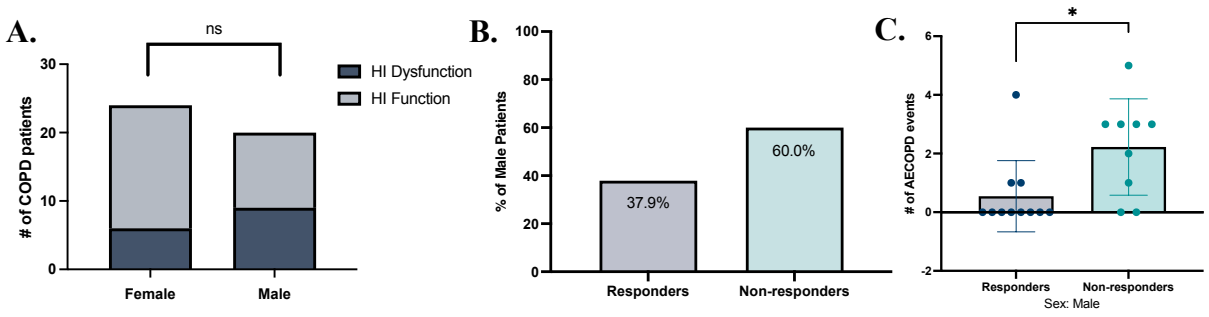

Figure 13: *Sex (female/male) and humoral immune response in COPD.* A) Fischer's exact test for proportion of patients with and without HI dysfunction in males and females reported a non-significant P=0.2097. B) Percentage of male COPD patients with and without HI dysfunction. C) Mann-Whitney t-test of the number of AECOPD events between male responders and non-responders reported a significant p-value of 0.018.

3.7.3 Body mass index and nutritional status

Table 9 shows the number of COPD patients among the four BMI categories: underweight, normal, over-weight and obese. A majority of COPD patients were in the normal, overweight and obese categories. Proportional analysis showed a higher proportion of HI dysfunction (50%) in patients with an underweight BMI (no statistical analysis performed) compared to proportions (43%, 38%, 15%) in normal, overweight and obese patients, respectively (Figure 14A, Table 9). COPD participants with an underweight and normal BMI presented with reduced anti-Typhi Vi IgG fold change (Kruskal-Wallis test, P value = 0.1355) (Figure 14B). Mean anti-Typhi Vi IgG fold change for each BMI category was reported in Table 9. There was no statistical difference in BMI (kg/m^2) between responders and non-responders (Mann-Whitney t-test, P value = 0.118) (Figure 14C).

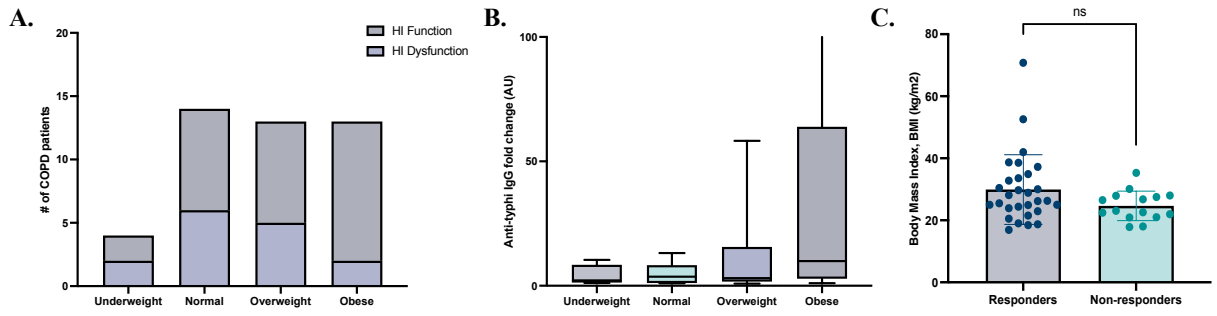

Figure 14*: Nutritional status per body mass index (BMI) and humoral immune response in COPD.* A) Number of patients with HI dysfunction across BMI categories (underweight, normal, overweight and obese). B) Anti-Typhi Vi IgG fold change across BMI categories was non-significant on a Kruskal-Wallis test with a P value = 0.1355. C) Mann-Whitney t-test of BMI (kg/m^2) between responders and non-responders reported a non-significant P = 0.118.

Table 9: *Proportion of COPD patients with HI dysfunction and respective mean anti-*Typhi *Vi IgG fold change across body mass index (BMI) categories.*

BMI category, kg/m^2	**# of patients with HI dysfunction (%)**	**Anti-Typhi Vi IgG fold change, AU, mean +/- SD (95% CI)**
Underweight <18.5 (n=4)	2 (50%)	3.943 +/- 4.335 (-2.956- 10.84)
Normal 18.5-24.9 (n=14)	6 (43%)	4.855 +/- 4.377 (2.327- 7.382)
Overweight 25.0 – 29.9 (n=13)	5 (38%)	13.16 +/- 19.66 (1.280- 25.04)
Obese	2 (15%)	29.00 +/- 37.12 (6.574- 51.44)

>30 **(n=13)**		

3.7.4 Smoking history and status

Forty-three (98%) participants had previous smoking history; 37 (86%) individuals had quit smoking and 6 (14%) were current smokers. A higher proportion (4/6, 67%) of current smokers (active smoking) had HI dysfunction than individuals whom had quit smoking (12/37, 32%) (Fischer's exact test, P-value = 0.159, ns) (Figure 15A, 15B). No association was observed between antibody fold change and smoking status (active or quit smoking) (Mann-Whitney t-test, P value = 0.6523) (Figure 15C). Also, there were no significant differences observed in the smoking history (pack-years) of responders and non-responders (Mann-Whitney t-test, P-value = 0.935) (Figure 15D).

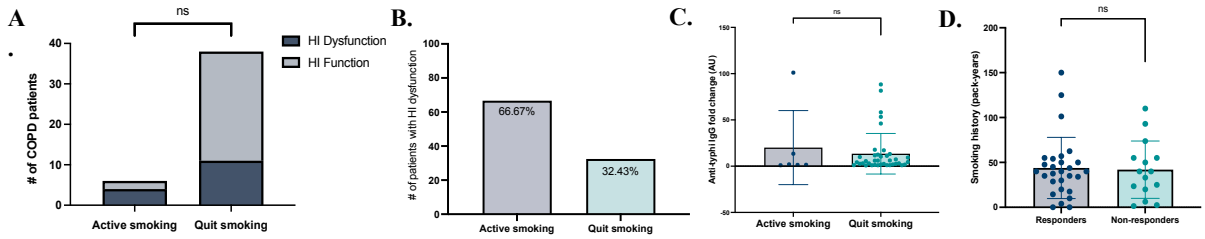

Figure 15: *Smoking status and Humoral Immune Response in COPD.* A) Fischer's exact test for proportion of patients with and without HI dysfunction by smoking status (active or quit) reported a non-significant P=0.159. B) The percentage of patients with HI dysfunction in individuals whom are actively smoking (66.67%) versus quit smoking (32.43%). C) Anti-Typhi Vi IgG fold change in patients whom are actively smoking versus quit smoking. A Mann Whitney t-test reported a non-significant P=0.6523. D) Mann-Whitney t-test of the number of pack-years (smoking history) between responders and non-responders reported a significant P=0.935.

3.8 Humoral immune response and serum measurements

3.8.1 Cellular component levels

A complete blood count including white blood cells, and cellular differentials including neutrophils, eosinophils, lymphocytes and CRP were measured and compared between responders and non-responders. There were no statistical differences ($p>0.05$) regarding all cellular measures (white blood cells, neutrophils, lymphocytes and CRP) between responders and non-responders (Mann Whitney t-test, P-value = 0.481, P-value = 0.504, P-value = 0.347, P-value = 0.541, respectively) (Figure 16A, B, D, E). Blood eosinophils were significantly higher (0.255 +/- 0.244 x 10^9 g/L) in responders than non-responders (0.223 +/- 0.372 x 10^9 g/L) (Mann-Whitney t-test, P value = 0.048) (Figure 16C).

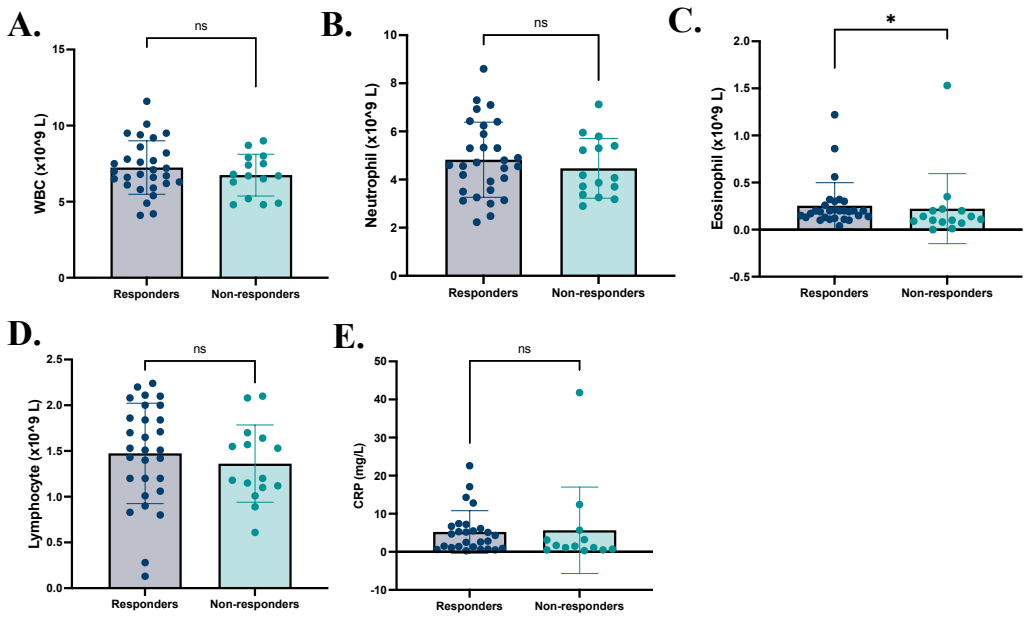

Figure 16: *Cellular measurements in circulation and humoral immune response in COPD.* A) Mann-Whitney t-test of white blood cell (WBC) levels between responders and non-responders reported a non-significant P=0.481. B) Mann-Whitney t-test of neutrophil levels between responders and non-responders reported a non-significant P=0.504. C) Mann-Whitney t-test of eosinophil levels between responders and non-responders reported a significant P=0.048. D) Mann-Whitney t-test of lymphocyte levels between responders and non-responders reported a non-significant P=0.347. E) Mann-Whitney t-test of C-reactive protein (CRP) levels between responders and non-responders reported a non-significant P=0.541.

3.8.2 Immunoglobulin levels

Among 44 COPD patients recruited with and without AECOPD, 3 (7%) had low IgA, 6 (14%) IgM and 13 (30%) IgG; 18 (41%) patients had at least one immunoglobulin below the normal thresholds. 14 (32%) patients had high IgE above the normal threshold. All measured Ig levels (IgA, IgM, IgE and IgG) were comparable between responders and non-responders (Mann Whitney t-test, P-value = 0.709, P-value = 0.435, P-value = 0.524, P-value = 0.481, respectively) (Figure 17A, B, C, D).

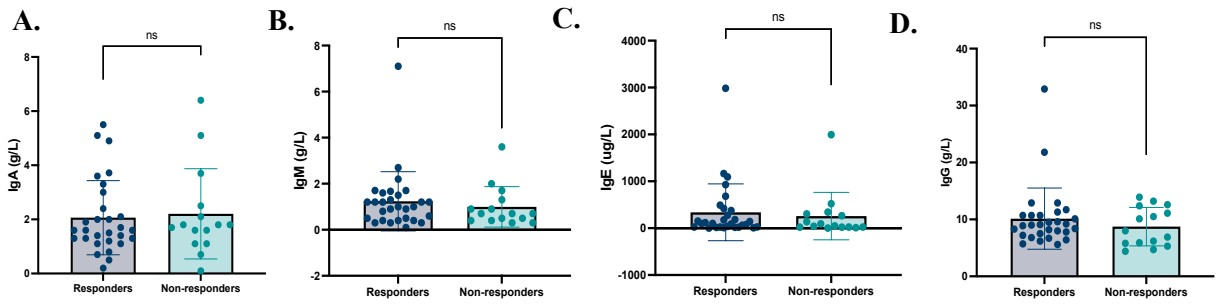

Figure 17: *Immunoglobulin levels in circulation and humoral immune response in COPD.* A) Mann-Whitney t-test of IgA levels between responders and non-responders reported a non-significant P=0.709. B) Mann-Whitney t-test of IgM levels between responders and non-responders reported a non-significant P=0.435. C) Mann-Whitney t-test of IgE levels between responders and non-responders reported a non-significant P=0.524. D) Mann-Whitney t-test of IgG levels between responders and non-responders reported a non-significant P=0.481.

3.8.3 IgG and subclasses levels

Furthermore, the proportion of patients with HI dysfunction was compared among those with normal and low IgG levels (Figure 18A). A greater proportion of patients with low IgG levels (54%) had HI dysfunction compared to those with normal IgG levels (35%) (Fischer's exact test, P-value = 0.0924, ns) (Figure 18A). 7 of 15 (47%) patients with HI dysfunction had low baseline IgG levels in circulation. Also, patients with lower IgG levels presented with

reduced anti-Typhi Vi IgG fold change of 4.829 +/- 5.321 AU compared to those with normal IgG at 18.36 +/- 28.26 AU (Mann-Whitney t-test, P value = 0.1115) (Figure 18B).

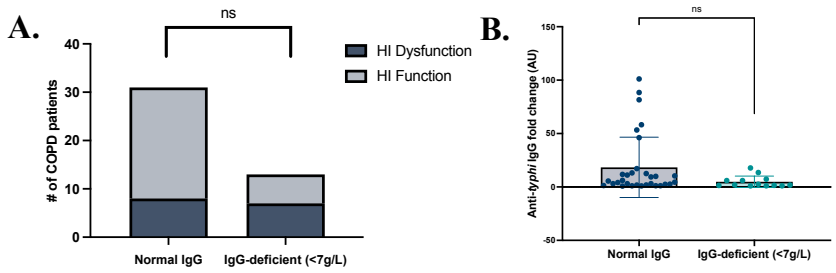

Figure 18: *Serum IgG levels (in circulation) and humoral immune response in COPD.* A) Fischer's exact test for proportion of patients with and without HI dysfunction by normal IgG levels versus IgG deficiency reported a non-significant P=0.0924. B) Anti-Typhi Vi IgG fold change in patients with and without IgG levels within normal range. A Mann Whitney t-test reported a non-significant P=0.1115.

IgG subclasses including IgG1, IgG2, IgG3 and IgG4 were also measured and compared between responders and non-responders. There were no statistical differences ($p>0.05$) regarding IgG1, 2, 3, and 4 between responders and non-responders (Mann Whitney t-test, P-value = 0.917, P-value = 0.855, P-value = 0.263, P-value = 0.394, respectively) (Figure 19A, B, D, E).

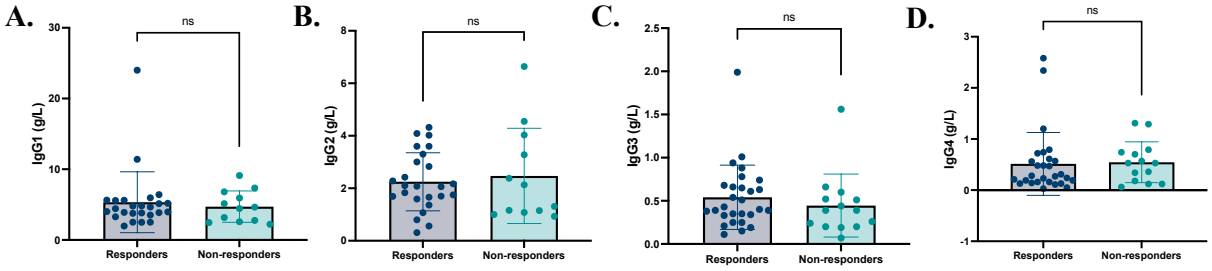

Figure 19: *Immunoglobulin G (IgG) subtype levels in circulation and humoral immune response in COPD.* A) Mann-Whitney t-test of IgG1 levels between responders and non-responders reported a non-significant P=0.917. B) Mann-Whitney t-test of IgG2 levels between responders and non-responders reported a non-significant P=0.855. C) Mann-Whitney t-test of IgG3 levels between responders and non-responders reported a non-significant P=0.263. D) Mann-Whitney t-test of IgG4 levels between responders and non-responders reported a non-significant P=0.394.

3.9 Disease-specific health-related quality of life and symptom burden

All patients completed the CAT with scoring from low to very high and SGRQ ranging from Quartile 1-4; higher scores indicating worse respiratory symptoms or health status and quality of life, respectively. 6 (14%) patients scored low on the CAT questionnaire, 21 (48%) had medium scores, 11 (25%) had high scores and 4 (%) had very high scores. While 9 (20%) patients scored within Quartile 1 on the SGRQ, 11 (25%) within Quartile 2, 9 (20%) within Quartile 3, and 15 (34%) within Quartile 4. COPD participants with HI dysfunction varied between low to very high scores on both the CAT and SGRQ (Figure 20A and Figure 20B). There were no statistical differences ($p>0.05$) regarding both CAT scores (Figure 20B; Mann-Whitney t-test P-value = 0.373) and SGRQ scores (Figure 20D; Mann-Whitney t-test, P-value = 0.422) between responders and non-responders at baseline.

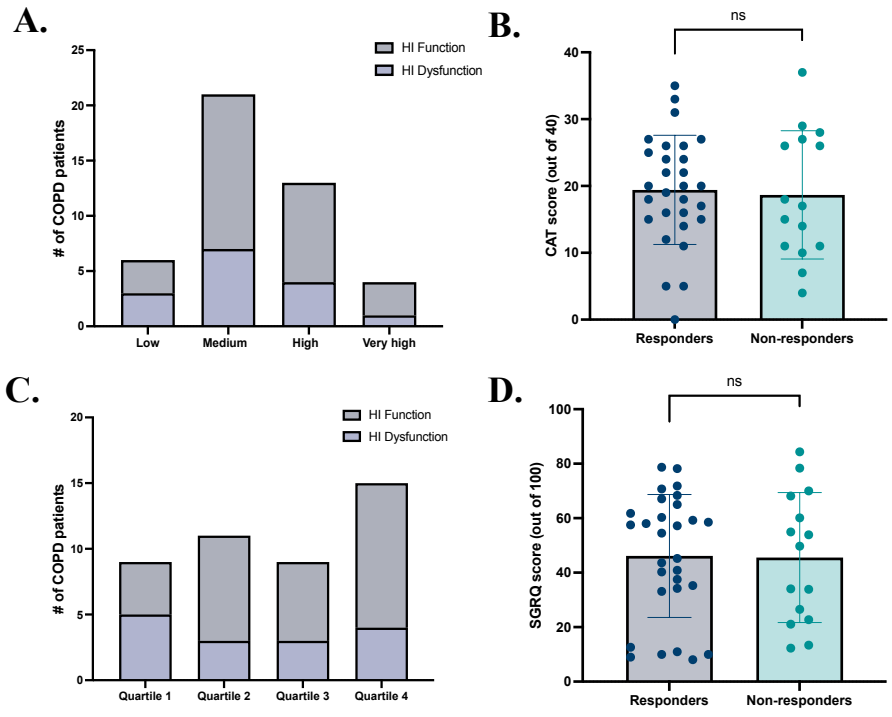

Figure 20: *Quality of life and humoral immune response in COPD.* A) Number of patients with HI dysfunction across CAT score ranges (low, medium, high, very high). B) Mann-Whitney t-test of CAT scores (out of 40) between responders and non-responders reported a non-significant P = 0.373. C) Number of patients with HI dysfunction across SGRQ score ranges (quartile 1, 2,3, 4). D) Mann-Whitney t-test of SGRQ scores (out of 100) between responders and non-responders reported a non-significant P = 0.422.

3.10 Humoral immune response and COPD severity (based on spirometry)

Assessment of spirometry findings showed no significant differences for FEV1, L (%) and FEV1/FVC (%) between responders and non-responders (Figure 21A, B). Based on these spirometry results, 21 COPD patients were grouped into GOLD stage II, 17 in GOLD III, and 6 under GOLD IV. A higher number of patients with HI dysfunction were classified as GOLD II (53%) and III (47%) (moderate and severe), with none under GOLD IV (very severe) (Figure 22A). Antibody fold change was not significantly different across different GOLD categories, indicating COPD severity, however patients in the GOLD II and III categories had lower antibody fold change than GOLD IV (Kruskal-Wallis test, P = 0.3147) (Figure 22B).

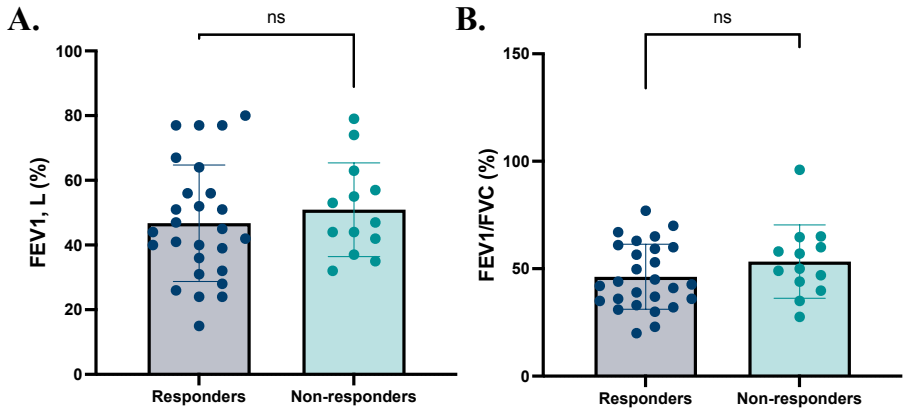

Figure 21: *Pulmonary function results and humoral immune response in COPD.* A) Mann-Whitney t-test of FEV1, L (%) predicted between responders and non-responders reported a non-significant P=0.395. B) Mann-Whitney t-test of FEV1/FVC (%) between responders and non-responders reported a non-significant P=0.261.

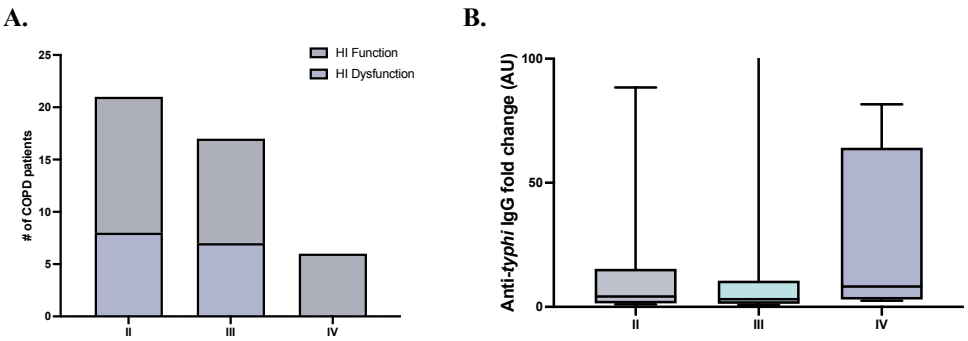

Figure 22: *COPD severity based on GOLD classification and Humoral Immune Response.* A) Number of patients with HI dysfunction across categories of COPD severity (II, III, IV). B) Anti-Typhi Vi IgG fold change across GOLD II, III, IV was non-significant on a Kruskal-Wallis test with a P value = 0.3147.

3.11 Humoral immune response and therapeutic interventions

Pharmacological and non-pharmacological interventions administered for COPD management are summarized in Table 3. There were no significant differences between prednisone dosage over the year and chronic azithromycin dosage per week between responders and non-responders (Mann Whitney t-test, P = 0.101, P = 0.812) (Figure 23A, B). However, non-responders had higher usage of prednisone (mg/year) in the 12 months prior to study enrolment and lower usage of oral azithromycin (mg) per week for at least 3 months prior. A comparison of mean prednisone or azithromycin dosage taken by patients with and without HI dysfunction can be observed in Table 10.

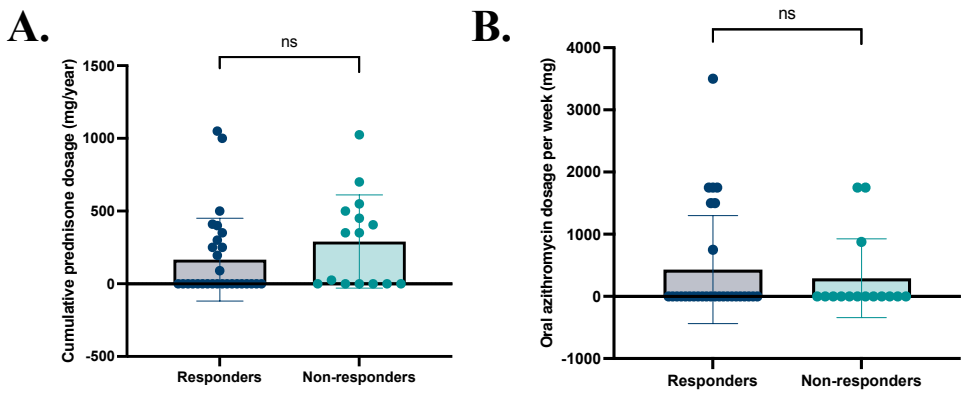

Figure 23: *Cumulative prednisone dosage and chronic azithromycin usage and humoral immune response in COPD.* A) Mann-Whitney t-test of cumulative prednisone dosage (mg/year) between responders and non-responders reported a non-significant P=0.101. B) Mann-Whitney t-test of oral azithromycin dosage (mg/week) between responders and non-responders reported a non-significant P=0.812.

Table 10: *Cumulative prednisone dosage and chronic azithromycin usage in all COPD patients; sub-grouped into patients with and without HI dysfunction.*

Parameter	**All COPD, (n=44)**	**HI Function (n=29)**	**HI Dysfunction (n=15)**	**P-value**
Cumulative prednisone dosage in the past 12 months (mg/year)	208.0 +/- 299.9	165.3 +/- 284.8	290.3 +/- 320.8	ns
Chronic azithromycin usage (mg/week)	384 +/- 791	431 +/- 868	292 +/- 633	ns

Note: All data was presented as mean +/- SD.

The proportion of patients currently using B-agonists, muscarinic antagonists, inhaled corticosteroids, and/or supplemental oxygen was reported in Table 3. 15 (100%) patients with HI dysfunction used B-agonists (Figure 24A; Fischer's exact test, P value = 0.540, ns), 10 (67%) used muscarinic antagonists (Figure 24B; P >0.9999, ns), 8 (53%) used ICS (Figure 24C; P = 0.357), 7 (47%) used supplementary home oxygen (Figure 24D; P = 0.521). 6 (40) non-

responders had completed pulmonary rehabilitation in the past 5 years (Figure 24E; P = 0.342) and 1 (6.7%) non-responder had started Ig treatment during the follow-up period of the study (Figure 24F; P = 0.647). No significant differences were observed between patients on and off these therapeutic interventions (Mann Whitney t-test, P = 0.6110, P = 0.1489, P = 0.7250, P = 0.9430, P = 0.6358, P = 0.6250) (Figure 24A, B, C, D, E, F). These trends were noticeable: patients on B-agonists, muscarinic antagonists, and Ig treatment during follow up had slightly higher anti-Typhi Vi IgG fold change, while those on supplemental oxygen had reduced antibody fold change.

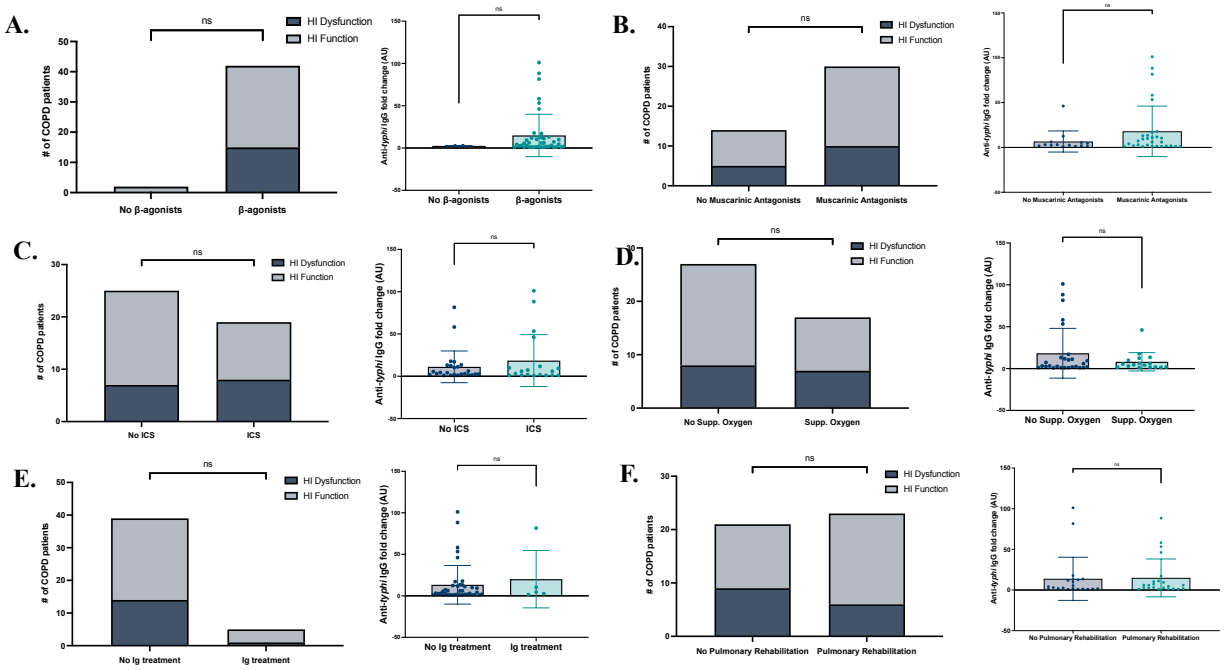

Figure 24: *Common medical interventions and humoral immune response in COPD.* A) Fischer's exact test for proportion of patients with and without HI dysfunction by current treatment with and without B-agonists reported a non-significant P=0.540 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without B-agonist treatment reported a non-significant P=0.6110. B) Fischer's exact test for proportion of patients with and without HI dysfunction by current treatment with and without muscarinic antagonists reported a non-significant P>0.9999 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without muscarinic antagonist treatment reported a non-significant P=0.1489. C) Fischer's exact test for proportion of patients with and without HI dysfunction by current treatment with and without inhaled corticosteroids (ICS) reported a non-significant

P=0.7250 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without inhaled corticosteroid treatment reported a non-significant P=0.149. D) Fischer's exact test for proportion of patients with and without HI dysfunction by current treatment with and without supplementary oxygen reported a non-significant P=0.521 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without supplementary oxygen reported a non-significant P=0.9430. E) Fischer's exact test for proportion of patients with and without HI dysfunction by treatment with and without Ig treatment (during the follow-up period) reported a non-significant P=0.647 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without Ig treatment reported a non-significant P=0.6358. F) Fischer's exact test for proportion of patients with and without HI dysfunction by past/current treatment with and without pulmonary rehabilitation reported a non-significant P=0.342 (left figure). A Mann Whitney t-test of anti-Typhi Vi IgG fold change in patients with and without pulmonary rehabilitation reported a non-significant P=0.6250.

Finally, previous pneumococcal and influenza vaccination in the past 5 years was assessed. 13 (87%) non-responders had received the Prevnar 13 pneumococcal vaccine, 13 (87%) Pneumovax 23 and 12 (80%) influenza vaccine (Fischer's exact test, P = >0.9999, P = >0.9999, P=0.500, ns) (Figure 25A, B, C top). Vaccinated individuals had reduced antibody fold change; no statistical significance was observed with Prevnar 13 (Mann Whitney t-test, P = 0.4274), Pneumovax (Mann Whitney t-test, P = 0.7198, ns) and influenza vaccination (Mann Whitney t-test, P = 0.4116, ns) (Figure 25A, B, C, bottom).

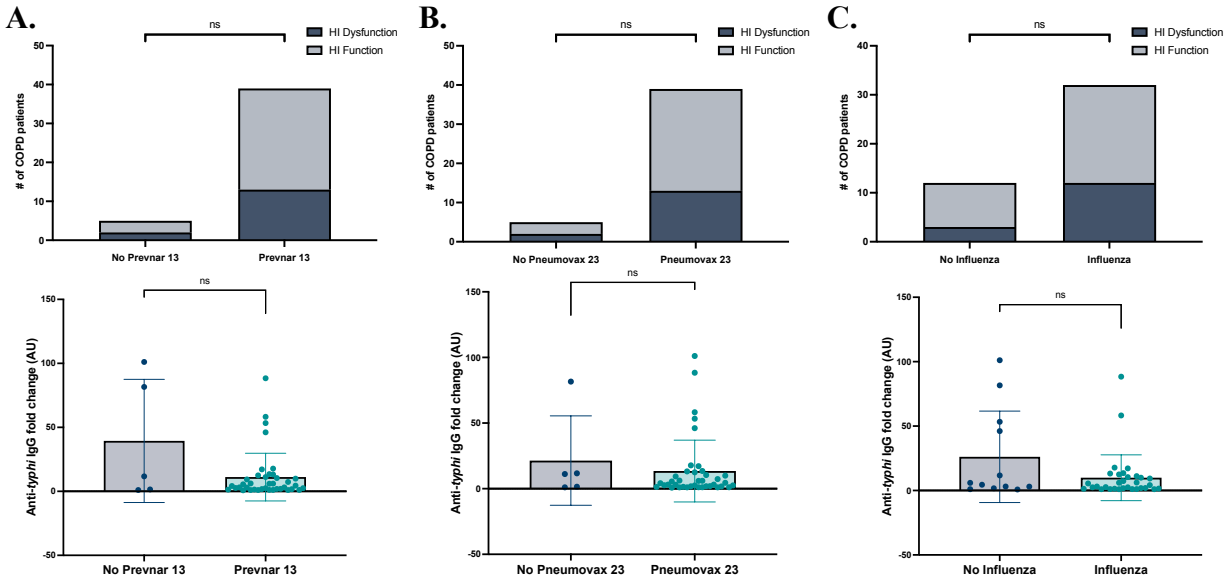

Figure 25: *Vaccination status in the past five years and humoral immune response in COPD.* Top figures represent Fischer's exact test identifying proportions of patients with HI dysfunction across unvaccinated and vaccinated individuals. Bottom figures show Mann-Whitney t-tests of anti-Typhi Vi IgG fold change between unvaccinated and vaccinated individuals. A) Fischer's exact test for HI dysfunction in individuals whom have received the Prevnar 13 vaccine reported a non-significant p>0.9999 (top) and Mann-Whitney t-test reported a non-significant P=0.4274 (bottom). B) Fischer's exact test for HI dysfunction in individuals whom have received Pneumovax reported a non-significant P>0.9999 (top) and Mann-Whitney t-test reported a non-significant P=0.7198 (bottom). C) Fischer's exact test for HI dysfunction in individuals whom have received the influenza vaccine reported a non-significant P=0.500 (top) and Mann-Whitney t-test reported a non-significant P=0.4112 (bottom).

CHAPTER FOUR: DISCUSSION

4.1 Summary of main findings

In this thesis, we sought to identify humoral immune dysfunction in COPD patients with and without acute exacerbations. The failure to produce an adequate HI response may leave an individual at risk of recurrent infections; response to a polysaccharide vaccine is a reflection of HI dysfunction[167]. Unfortunately, the IgG response to the pneumococcal vaccine may have limitations. To our knowledge, this marks the first study to assess HI responses in COPD patients using an innovative and reliable Typhim Vi polysaccharide vaccine test and compare with AECOPD frequency. A Typhim Vi polysaccharide vaccine test is appropriate because i) it is a reliable test to measure HI dysfunction that is well-accepted in the field of Immunology ii) it overcomes barriers with using a pneumococcal vaccine response test as mentioned in Chapter One. This thesis contributes to COPD literature by introducing a novel biomarker, typhoid vaccine response, to identify individuals with risk of frequent AECOPD.

To summarize our main findings, antibody fold change was quantified in 44 patients. From which a subset of COPD patients (34%) were identified to have HI dysfunction measured by ≤2-fold post: pre-anti-Typhi Vi IgG ratio, of whom 80% had history of AECOPD in the year before enrolment. Mean annual exacerbation rates were significantly higher for patients with HI dysfunction (1.4±1.6 events) than without HI dysfunction (0.95±1.12 events). These findings were primarily seen in male COPD patients. Further, HI dysfunction had a significant correlation with frequent moderate only AECOPD in low-risk patients. We did not find differences between other variables and COPD patients with and without HI dysfunction. This thesis work suggests a signal that HI dysfunction in COPD patients may be associated with higher frequency of AECOPD.

4.2 Interpretation of the main findings and comparison to previous literature

4.2.1 HI dysfunction and AECOPD frequency

Chronic lung disease has been identified as a frequent complication or clinical manifestation of humoral immune dysfunction or humoral immunodeficiencies, most commonly atelectasis and bronchiectasis (27%), which is often a key feature of COPD[168]. Studies have also shown that asthmatic patients (24-33%) are likely to be diagnosed with common variable immunodeficiency than non-asthmatics and controls[169]. Systemic corticosteroid use was associated with a 21.7-fold increased risk of humoral immunodeficiencies[170]. Deficiencies in IgA were observed in asthma patients and associated with increased mucosal antigen exposure; causing elevated levels of IgE against allergens and worsening airway inflammation[137][171]. Additional investigation of pulmonary findings in those with common variable immunodeficiency showed these proportions: 30% had allergic rhinitis, 25% develop non-necrotizing granuloma and 15% had interstitial lung disease[172,173]. Further, clinical and biological data showed that 11.5-18.5%% of COPD patients had hypogammaglobulinemia, increasing with severity. These patients were associated with lower BMI and more frequent hospital admissions. These studies suggested that hypogammaglobulinemia and humoral immune dysfunction may be involved in poor outcomes of COPD patients[174].

Our study's primary outcome was to assess the relationship between HI dysfunction and AECOPD frequency using Typhim Vi vaccine response. In our case, we classified our patients into non-responders and responders based on a 2-fold increase in titers. We demonstrated that the measurement of anti-Typhi Vi IgG in response to Typhim Vi vaccination was able to identify non-responders in our COPD cohort. We further found that non-responders presented with

greater mean annual exacerbations than responders. When associating degree of antibody fold change with exacerbations, we saw a significant negative correlation, indicating that a lower antibody fold increase was moderately associated with frequent AECOPD. When comparing antibody fold change based on the number of AECOPD events experienced by patients, we observed a decreasing trend in antibody fold change, with a significantly lower fold-change for patients with more than 2 exacerbations in the past year than patients with no AECOPD.

Furthermore, the comparison of only moderate AECOPD events with antibody fold change reflected a similar significant correlation. However, this significance was not observed when considering only severe AECOPD events. This was similarly observed when comparing patients at low risk (≤1 moderate AECOPD in the past year) vs high risk (≥2 moderate or >1 severe AECOPD) for future exacerbations. Low-risk non-responders had frequent AECOPD, while no statistical differences were seen between high-risk non-responders and responders. It must be taken into consideration that there were significantly fewer severe AECOPD (15 events) than moderate (31 events); and also, few participants identified as high-risk (n=12) for future AECOPD than low-risk (n=32). Overall, these findings suggest that the measurement of antibodies in response to Typhim Vi antibody response may be able to identify COPD patients with possible humoral immunodeficiency and/or a higher risk of AECOPD. The severity of AECOPD events may be unrelated to HI dysfunction in COPD or the lack of significance may be due to reduced statistical power from the small sample size of high-risk COPD patients and severe events.

While, there have been no previous studies examining HI response using a functional measure for antibody response such as the Typhim Vi (polysaccharide) vaccine response test in COPD patients. A few studies have provided evidence for possible HI dysfunction in AECOPD

patients. As mentioned in the introduction, lab groups such as Palikhe et al. showed that at least one immunoglobulin (IgA, IgG and IgM) was below the normal range in serum samples of AECOPD patients[175]. Although serum Ig is a quantitative measure and cannot provide insight into HI function, these findings suggest that the immune system is likely unable to induce an immediate response against the infectious agent during an exacerbation; leading to increased respiratory tract infections[142,176]. These findings were supported by Filho et al. whom investigated the relationship between circulating total IgG and risk of acute exacerbations and hospitalization in COPD patient samples from 2 large COPD trials (MACRO, STATCOPE). Patients with reduced IgG levels had 50-100% increased risk for exacerbations and hospitalization[143]. Our study goes a step further by showing an association between a functional measure of humoral immunity and AECOPD frequency.

Furthermore, several non-steroidal immunomodulatory agents have been administered to AECOPD patients, however they have failed to effectively reduce AECOPD frequency[177–181]. Considering that patients with AECOPD have low IgG, suppressed mucosal and systemic immunity, and novel immunomodulatory therapy are required to prevent AECOPD; Cowan et al studied Ig treatment as a preventative measure for frequent AECOPD[149,150]. RCT findings showed a significant reduction in AECOPD rates[150]. Ig treatment may reduce AECOPD by promoting mucosal immunity to protect against infectious agents, reduce autoantibodies or inhibit inflammatory pathways triggered by viral or bacterial infections[149,182–184]. However, these two studies were limited by a small, heterogeneous sample size, low adherence and poor feasibility[150]. Our study's findings can help identify patients who may be most responsive to Ig treatment to further conduct an adequately powered RCT to assess efficacy of IVIG in AECOPD.

4.2.2 Proportion of patients with different thresholds of post- vs pre- antibody titer fold change

A pre- to post- immunization titer ratio of ≤2.0 (AU) has been shown to be 100% sensitive and specific to identify a defect in the ability to produce a sufficient HI response in previous literature and according to the ELISA kit protocol[158]. While, a 4-fold cutoff is often used to define a recent infection in the field of infectious disease, and 3-fold to 5-fold cutoffs have been used to assess HI dysfunction in common variable disease patients using Pneumovax II[159,167].

Provided that several studies have identified varying fold increase cut-offs to define an adequate response to polysaccharide vaccines; we considered identifying HI dysfunction based on different fold increases that are predominantly considered in immunodeficiency populations[185]. Our findings demonstrated significant findings with a cutoff of 3-fold, 4-fold, and 6-fold which was comparable to a 2-fold cut-off. An increase in the antibody fold cutoff facilitated an increase in the proportion of patients with HI dysfunction. Greater differences between the proportion of patients with and without HI dysfunction signals the possibility of introducing more false positive results and reducing specificity of the typhoid vaccine response test when using a higher cut-off such as 4-fold and 6-fold. A 2-fold and 3-fold cutoff produces similar findings with likely good specificity. We plan to perform further analysis to consider both a 2-fold and 3-fold threshold and establish an optimal cut-off for HI dysfunction in COPD patients[120]. A future study with receiver-operating curve analysis comparing IgG responses to Typhim Vi and 'gold-standard' Pneumovax 23 vaccine in a group of COPD patients may be helpful to validate a cut-off for HI dysfunction in this population.

4.2.3 Association between HI dysfunction and other variables

Our next step was to evaluate the relationship between HI response in COPD and other measurements including demographics, serum measurements, patient-reported outcomes, COPD severity and medication use that are often associated with exacerbation risk.

Among 44 patients, a majority of the patients were of older age (60-79 years), within normal to obese BMI categories, and with a previous smoking history of approximately 43 pack-years. Although not statistically significant, HI dysfunction was more prevalent in patients between 70-79 years, underweight BMI and current smokers. Miravitlles et al. reported that AECOPD risk increases by 20% with every 10 years of age; possibly due to multiple comorbidities, poor adherence to medications or other consequences of impaired cognitive functions[186,187]. An association between HI dysfunction and lower BMI may suggest that poor nutritional status or malnutrition of the patient may be linked to their antibody response, in addition to previous literature which has shown that exacerbation risk increases with lower BMI[188]. Underweight individuals with COPD were shown to have a higher risk of severe AECOPD than overweight individuals[25]. Malnutrition has been associated with significantly diminished proportions of B cell subsets, primarily including memory B cells and plasma cells; it has been suggested that low nutrient availability may impact B cell survival and activation[189]. It is important to also note that a greater proportion of underweight individuals (44%) were identified to be current smokers than overweight individuals[190].

Cigarette smoking has been well-studied as a critical risk factor for AECOPD, as a significant proportion of patients with COPD have smoking history[191,192]. A nationwide study of 48, 836 COPD patients by Nielsen et al. showed that active smokers had the highest risk of exacerbations compared to former and never smokers[191,192]. Active smoking has shown to

interfere with effective antiviral CD8+ T-cell immunity, as severe COPD patients whom are actively smoking were shown to have reduced T cell proportions[193,194]. A relationship between active smoking and adaptive immunity suggests possible interference with B cell antibody responses in AECOPD patients[193]. Since these demographics have an association with exacerbation risk, a slightly higher proportion of individuals with HI dysfunction and old age, poor nutritional status, or active smoking status is somewhat expected. Although there were trends observed with proportional analysis, no statically significant differences in age, BMI and smoking history or number of cigarettes smoked was observed between typhoid vaccine responders and non-responders. Although these variables are particularly relevant to exacerbation risk, our t-tests suggest that they may be unrelated to the association between HI dysfunction and AECOPD frequency.

Furthermore, males and females have shown to differ in their adaptive responses, with elevated humoral immunity in females[195]. These sex-based differences across anti-Typhi Vi IgG response was also investigated in our study. A higher percentage of male patients had HI dysfunction than females. Although this was not statistically significant, it may be speculated that female COPD patients may naturally have a lower prevalence of HI dysfunction. Fink and Klein discuss that females have higher antibody responses than males to numerous vaccines including influenza, hepatitis B, yellow fever, rabies, herpes, and smallpox viruses[195]. Higher IgA titers, memory B cells, and plasma cells involved in the humoral response were also observed in females; while males experienced a drastic decrease in B cell numbers and CD8+ T memory cells with age[196–199]. A higher proportion of males are also known to be active smokers and experience undernutrition than females, which may lead to a diminished immune status including impaired regulatory B cells, memory B cells and antibody production. These previous

findings may explain why only male non-responders had significantly higher AECOPD events than female non-responders whom only had a slightly increased AECOPD rate (not statistically significant). Overall, these findings regarding sex-based differences may suggest that the prevalence of HI dysfunction is similar regardless of sex, however it may be associated with increased AECOPD events in males and does not hold true for females. In contrast, a slight increase in AECOPD rate among female participants may indicate that other factors are influential in exacerbation risk in females with COPD aside from or in addition to HI dysfunction[200].

The complete blood counts and cellular differentials including white blood cells, neutrophils, lymphocytes and CRP revealed no significant differences between responders and non-responders, indicating that there were no notable variations in baseline serum measurements between these two groups. Although the presence of elevated blood eosinophils in our COPD cohort was low (4/44 patients'), blood eosinophil levels were significantly higher in responders than non-responders. Recent research has shown that lower blood eosinophil levels have an association with increased risk of bacterial infections, which is observed in non-responders whom have a higher AECOPD frequency than responders[201]. This finding may also be influenced by the confounding effect of comorbidities on the distinct immunological profile of responders compared to non-responders. Further insight into the clinical characteristics showed that three responders and one non-responder had elevated eosinophils, above the reference range ($\geq 0.50 \times 10^9$/L). 2/3 of these responders had clinical history of asthma, which has been highly associated with eosinophilic inflammation[202]. Singh has also shown that higher blood eosinophils are associated with an increased response to ICS treatment; our data showed that individuals on ICS had higher antibody fold change (indicative of a greater proportion of

responders on ICS)[201]. These speculations may be a possible rationale for why non-responders have lower blood eosinophils compared to responders.

As previous literature has associated lower total IgG, IgG1 and IgG2 with an increased risk of AECOPD and hospitalizations, we were interested to analyze their association with HI dysfunction[143]. We found that a majority of patients had at least one immunoglobulin (IgA, IgM, IgE, IgG) outside the normal limits, and there was a greater prevalence of HI dysfunction (54%) and lower antibody fold change in patients with IgG deficiency compared to those with normal IgG levels. Despite these trends, no statistical differences were observed in serum immunoglobulin levels and IgG subclasses between responders and non-responders. These findings suggest that while there may be a prevalence of COPD patients with IgG deficiencies, IgG levels do not directly correlate with AECOPD frequency. Additionally, it highlights the indication that not all COPD patients with HI dysfunction exhibit IgG deficiency. Typhoid vaccine response, as a functional biomarker of humoral response, may be able to better identify HI dysfunction in COPD patients with or without IgG deficiency (hypogammaglobulinemia) than quantitative measures such as serum IgG levels. Furthermore, IgE levels were slightly higher in the overall COPD cohort; with slightly higher levels in AECOPD patients. IgE has been established as a key role in exacerbations of allergic asthma. Previous studies have shown elevated IgE levels in men with recurrent AECOPD (WISDOM trial)[203]. There are a larger proportion of males (n=14) and individuals with history of asthma/allergy (n=16) in the AECOPD subgroup, which may result in elevated IgE levels compared to the no AECOPD subgroup. This likely contributes to elevated IgE levels for the overall cohort. However, no significant differences were observed between responders and non-responders, likely indicating no confounding effect on the primary association.

CAT and SGRQ scores, indicative of respiratory symptom burden and quality of life, were not associated with HI dysfunction in COPD patients. These questionnaires are likely to reflect change in clinical status and worsening of respiratory symptoms as observed during AECOPDs[204]. However, the data suggests that CAT and SGRQ scores are likely unable to reliably predict immune dysfunction. Further, pulmonary function results and COPD severity had no association with HI response. It is worth to note that a majority of patients were in the moderate and severe categories of airflow obstruction, thus it may be difficult to observe differences in pulmonary function between responders and non-responders in this COPD cohort.

We understand that many of our participants will be receiving interventions to improve COPD symptoms or prevent recurrent AECOPD (e.g. chronic azithromycin, prednisone, vaccinations, pulmonary rehab, etc.). Thus, it was important to assess the association of interventions and HI response. In our study, cumulative prednisone dosage in the past year was comparable between non-responders than responders, with a slightly higher usage in non-responders; this was expected with an association between HI dysfunction and frequent moderate/severe AECOPD which are often treated with prednisone. Cuevas et al provided evidence that chronic azithromycin usage, at doses of 250mg daily and 500mg three times a week, significantly reduced the number of exacerbations and severity of AECOPD compared to a control group[205]. No significant association was observed regarding chronic azithromycin usage in responders versus non-responders; however, a slightly higher usage of azithromycin in responders may be expected as these patients have reduced AECOPD rates. Thus, prednisone nor chronic azithromycin usage likely do not influence the association between HI dysfunction and recurrent AECOPD.

In addition, our findings show that a majority of COPD patients are on standard medications such as B-agonists and ICS, however, several of them continue to experience recurrent AECOPD in the year. While ICS are commonly used to improve lung function and oxygenation during exacerbations, they have been cited by Singanayagam et al. to have limited effectiveness in reducing AECOPD events, increase pneumonia risk and impair innate and acquired antiviral immune responses[206]. Over-use of B-agonists has also shown to be associated with increased risk of exacerbation in chronic lung diseases like asthma and COPD[207,208]. Our data reiterates the importance of identifying better biomarkers for AECOPD frequency to improve personalized treatment strategies such as Ig treatment, which is commonly prescribed for humoral immunodeficiencies. Current muscarinic antagonist treatment and Ig treatment during the follow-up year was associated with slightly higher anti-Typhi Vi IgG fold change in our COPD cohort. Previous literature supports this finding as a reduction in exacerbation rates of 12% using muscarinic antagonistic treatment and from mean 4.7 to 0.6 events per year using Ig treatment in COPD was observed[151,209]. We observed no significant differences in antibody fold change between patients on and off these treatments to indicate an influence on HI dysfunction. However, since these interventions had associations with AECOPD rates in previous literature, the possible effects of these medications will continue to be monitored between responders and non-responders.

Finally, COPD patients are recommended to receive these aforementioned vaccines as a preventative strategy against respiratory infections[210]. Vaccination status in our COPD cohort showed that a large percentage of individuals including those with HI dysfunction had received Prevnar 13, Pneumovax and influenza vaccines in the past five years. All three vaccinations were associated with lower anti-Typhi Vi IgG fold change. Unfortunately, this indicates that there are

many vaccinated non-responders whom are unable to induce adequate antibody responses as a result of HI dysfunction and protect themselves from infectious agents (confirmed via proportional analysis). While the mechanisms are not fully understood, using Typhoid vaccine response to identify these patients with poor antibody response regardless of vaccination may facilitate clinicians to seek alternative therapies or adapt vaccination strategies such as booster doses or frequent vaccination schedules[211].

4.3 Strengths

As previously mentioned, this was the first cohort study to establish HI dysfunction in COPD patients and to evaluate an association between HI dysfunction and AECOPD frequency using a functional measure of humoral immunity. Our study approach is innovative in that we used a well-accepted Typhoid vaccine to measure HI function in COPD patients, which overcomes the barrier to reliably assess HI responses using the 'gold-standard' pneumococcal vaccine response test. The Typhim Vi vaccine response test has been widely supported by the Clinical Immunology community as a reliable and simpler alternative to the pneumococcal vaccine with a pre-vaccination titer of zero for most individuals living outside of countries where typhoid fever is endemic. Previous studies have determined associations between quantitative measures such as IgG in circulation, however a typhoid (polysaccharide) vaccine response test allows for the comparison of baseline antibody levels with post-vaccination levels, providing insight into an individual's ability to adequately produce an antibody (HI) response; as such this testing modality is likely superior to IgG quantification[185]. If this testing modality is introduced to the clinical community, it will be a relatively simple test to identify individuals with COPD whom are likely to develop a frequent exacerbator phenotype.

Furthermore, the AiCOP study is a prospective cohort study. This thesis presented findings on HI dysfunction and frequent AECOPD in the year before enrolment. Through the AiCOP study, all enrolled patients are also being contacted every 12 weeks for 48 weeks to document changes in their respiratory symptoms and prospectively capture AECOPD rates. Upon completion, this will be the first study to potentially demonstrate an association between HI dysfunction and frequent AECOPD prospectively. We have calculated a sufficient sample size goal of 140 participants to adequately power differences between HI dysfunction and 1) AECOPD history, 2) prospective AECOPD rates, and 3) other variables.

Also, our study consists of a well-defined patient cohort with few missing data. We are recruiting COPD patients (>40 years) with moderate airflow obstruction (FEV1, L<0.80) to capture subjects with more severe disease to demonstrate utility of the typhoid vaccine response strategy using a total sample size goal of 140 participants. Also, we included both participants with and without AECOPD to enable comparison of HI dysfunction between the groups and confidently report our findings that poor humoral immunity may be associated with AECOPD. For our study, we are comparing HI dysfunction with only moderate and severe AECOPD events. By excluding mild exacerbation events, we are reducing potential bias as mild AECOPD heavily rely on participant self-reporting. Finally, our study outcome measures were well-defined, relevant and patient-oriented, with significant clinical implications (see Section 4.6.1).

4.4 Limitations

4.4.1 Sample size and recruitment

Despite these advantages, the interpretation of the current results was limited in that the sample size of n=44 for the primary outcomes was relatively small because of its dependence on

recruitment in the ongoing AiCOP study, which was significantly affected by the COVID-19 pandemic. Other recruitment barriers have involved commuting distances, hesitancy to enter hospitals, vaccine hesitancy or travel costs. Since the findings in n=44 are likely underpowered to adequately detect differences in AECOPD frequency between responders and non-responders, the reported findings should be interpreted solely as exploratory rather than conclusive until further data is collected. To overcome the recruitment rate, we plan to i) ensure feasibility of our total sample size and ii) introduce mitigation strategies to overcome recruitment barriers. Based on the mean annual exacerbations rates in the dysfunction and non-dysfunction groups of n=40, we had re-calculated our sample size to feasibly recruit n=140 for ample power. To overcome recruitment barriers, we have been employing various strategies including option of at-home visits to minimize transportation barriers, compensation for parking, and increasing social media presence across respiratory organizations. By further expanding our recruitment to two additional centers in Hamilton (Ontario) and Montreal (Quebec), we believe that the recruitment will be feasible and our study will be adequately powered.

The event rate for severe AECOPD events and number of patients in the high-risk (>1 severe or >2 moderate AECOPD) category were also relatively low to compare HI dysfunction. This may affect the subgroup analysis which compared HI dysfunction between moderate only and severe only AECOPD; and low-risk versus high risk patients. Neither analysis in severe only and high-risk patients demonstrated a significant difference in mean annual exacerbation rates between responders and non-responders. This limitation may be overcome with a larger COPD cohort which may introduce more patients classified as high-risk and more severe AECOPD events to the study population to report powered differences with these analyses.

4.4.2 Confounding factors

Since HI dysfunction has not been well-studied in this patient population, an additional limitation of our study includes the influence of potential confounding variables on our primary finding between HI dysfunction and frequent AECOPD. Our primary and secondary data analysis suggested possible confounding factors that may influence the association between our primary variables. BMI and CAT scores were significantly different between AECOPD and no AECOPD subgroups. Blood eosinophil counts were significantly different between responders and non-responders. Thus, in addition to confounding factors like age, sex and FEV1, L % predictive value; we will be adjusting for BMI, CAT scores and blood eosinophil counts. A future direction is to perform negative binomial modelling to adjust for these potential confounding factors.

4.4.3 Variability between clinical cutoffs

Despite widespread use of polysaccharide vaccine response tests for diagnosis of HI dysfunction, previous studies and different laboratories have shown variability in the clinical cutoffs such as the typhoid and 'gold-standard' pneumococcal vaccine response test. While some studies use a 2-fold cutoff, others have performed analysis with a 3-fold, 5-fold and 10-fold cut-off in patients with humoral immunodeficiency. This raises a possible limitation for variability in the clinical cut-off of our testing modality, typhoid vaccine response test, to identify HI dysfunction in COPD (which may possibly be a milder form of immunodeficiency). Another future direction involves more investigation to confirm a cut-off or threshold for HI dysfunction in COPD; as mentioned above.

4.5 Future Directions

4.5.1 Next steps

The AiCOP study is ongoing and our next course of action beyond this thesis is:

I) Ongoing recruitment and analysis of HI dysfunction and AECOPD frequency in a larger COPD cohort (n=140).

II) Adjusted analyses for confounding variables that may affect the association between HI dysfunction and frequent AECOPD. Factors were identified based on previous literature, statistical significance testing of baselines differences between participants with and without AECOPD (Table 3), and participants with and without HI dysfunction. The selected variables were age, sex, baseline FEV1% predicted value, BMI and CAT QoL scores.

III) Prospective data collection and analysis of AECOPD frequency over the study follow-up period (of 48 weeks) using negative binomial modelling while adjusting for the same covariates as our primary analysis. By doing so, we would be able to establish typhoid vaccine response test as a predictive biomarker for risk of frequent AECOPD in COPD patients with HI dysfunction.

4.5.2 Clinical implications

This study is novel, clinically relevant, and will potentially have a significant impact on clinical management of COPD. The association between HI dysfunction and recurrent AECOPD will facilitate a promising new therapeutic paradigm for COPD care. If HI dysfunction is associated with recurrent AECOPD, this will have implications on the characterization of the frequent exacerbation phenotype by including identification of HI dysfunction. By identifying COPD patients who are likely to develop a frequent exacerbation phenotype, this will allow for

valuable allocation of more intense interventions and identification of individuals with severe disease for inclusion in future clinical trials.

Furthermore, patients with HI dysfunction often benefit from alternative therapies such as additional immunization with conjugate vaccines (in addition to polysaccharide pneumococcal vaccination), IgG replacement therapy, intensive antibiotic action plans and/or antimicrobial prophylaxis[212]. Current therapeutic approaches to prevent and treat AECOPD are often administered without consideration of the patient's immune status or infectious and non-infectious etiology. The incorporation of a typhoid vaccine response test to characterize HI defects and augment antibody levels and/or function would broaden eligibility criteria for these alternative therapies. This could facilitate clinicians to apply targeted treatment strategies by reducing use of oral or inhaled corticosteroids and instead offering Ig treatment in patients with frequent AECOPD. Additionally, anti-Typhi IgG fold change after vaccination may be assessed to monitor and follow-up with patients in urgent need of medical management (high risk for severe AECOPD) or possible discontinuation of Ig-replacement therapy after a certain time period. We hope to use typhoid vaccine response as a simpler and clinically pragmatic biomarker of poor humoral immunity in COPD that should be tested for predictive value of AECOPD or vice versa in combination with clinical judgment and patient preference.

4.5.3 Research implications

There are a limited number of publications reporting on original research studies that validate large clinical trials on Ig treatment in COPD. The knowledge from this proposed study will facilitate the design of an adequately powered randomized control trial with well-defined inclusion criteria and sample size to better evaluate the efficacy of Ig treatment for recurrent AECOPD. We also hope that a better understanding of typhoid vaccine response, as a functional

measure of HI in COPD, will pave the path to pursue mechanistic explanations. A recent study examined the presence of memory and switched memory B cells between Typhim Vi responders and non-responders with primary immunodeficiency; they found a significant difference (P=0.006) in the proportion of total B cells ($CD19^+$) and switched memory B cell subset ($CD19^+$ $CD27^+$ IgD^-)[158],[158,159]. Similar studies that involve B-cell immunophenotying may provide insight into the novel mechanism of HI dysfunction in AECOPD. Figure 26 summarizes possible mechanism of humoral immune dysfunction in COPD patients leading to frequent exacerbations. Overall, the study has potential to develop new knowledge around the mechanisms for frequent COPD exacerbations, which can inform effective treatment strategies, and enable clinicians and scientists to use immune function as an innovative biomarker for COPD phenotyping.

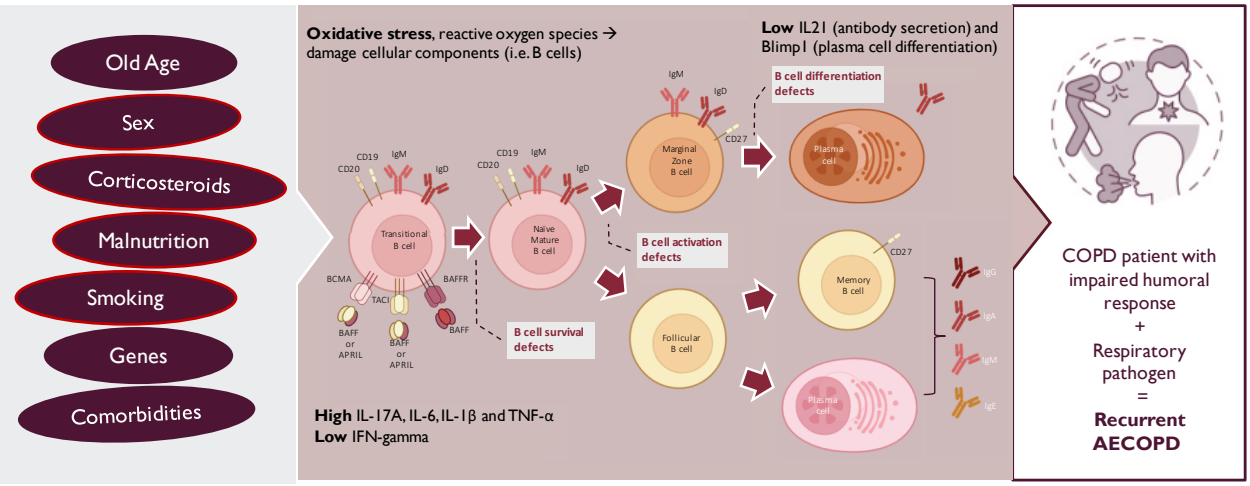

Figure 26: *Proposed summary of impaired humoral immune response in COPD patients, contributing to recurrent AECOPD.* Factors associated with increased inflammation and exacerbations such as ageing, corticosteroid suppression, malnutrition, sex (males), active smoking, specific genetic signatures and comorbidities contribute to i) oxidative stress by production of reactive oxygen species via inflammatory and structural cells in the lungs which are known to damage cellular components such as B cells, and ii) release of inflammatory mediators including abnormal cytokine/protein levels (IL-17A, IL-6, IL-1B, TNF-alpha, IFN-gamma, Blimp1). These abnormalities further complicate the development process and cause B cell survival, activation and/or differentiation defects. Specific markers can be studied such as BAFF (a fundamental survival factor for transitional B cells to mature B cell development) to

assess B cell proportions. Overall, B cell defects may cause low antibody production. An impaired antibody response enables certain respiratory pathogens to thrive and surpass the epithelial barrier; thus, a subset of patients tend to be more susceptible to recurrent infections and AECOPD.

4.6 Conclusion

This thesis examined HI dysfunction in COPD patients and whether it is more prevalent in those with frequent AECOPD in the past 12 months through a prospective cohort study called the AiCOP study. The key findings of this study indicate that HI dysfunction is present in a subset of COPD patients, specifically in male participants with higher frequency of AECOPD. However, this conclusion is not definitive, provided the limitations of the current data. Further work is required to assess this association in a larger COPD cohort, adjust for confounding variables that may influence the primary and secondary analysis, determine the efficacy of Ig treatment in AECOPD, and uncover mechanisms underlying HI dysfunction and its relationship with exacerbation risk. Overall, the study has the potential to improve health outcomes in COPD patients by providing an innovative tool to guideline leaders, clinicians and scientists to better phenotype their COPD patients for important clinical decisions such as diagnostics, phenotyping and management.

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APPENDIX

Table S1: *All study visits and participant timeline from enrolment to Week 48.*

	Enrolment/Week 0	**Week 4**	**Week 12**	**Week 24**	**Week 36**	**Week 48**
Written informed consent	X					
Demography	X					
Eligibility verification	X					
Vital signs including O2%	X					
Post-bronchodilator spirometry	X					
CT Chest[2]	X					
Typhim Vi vaccination	X					
Blood Collection	X	X				
Blood Test (CBC, Ig subtypes)	X					
Medical and AECOPD history (moderate/severe)	X	X	X	X	X	X
Adverse event assessment	X	X	X	X	X	X

Concomitant medications	X	X	X	X	X	X
SGRQ questionnaire	X					
CAT Questionnaire	X		X	X	X	X

All laboratory tests were performed at the hospital.[1]Most recent results of post bronchodilator spirometry were used for FEV1, L (%) and FEV1/FVC (%). [2]CT of chest data and findings (including emphysema, bronchitis and bronchiectasis) will be collected and recorded if available.

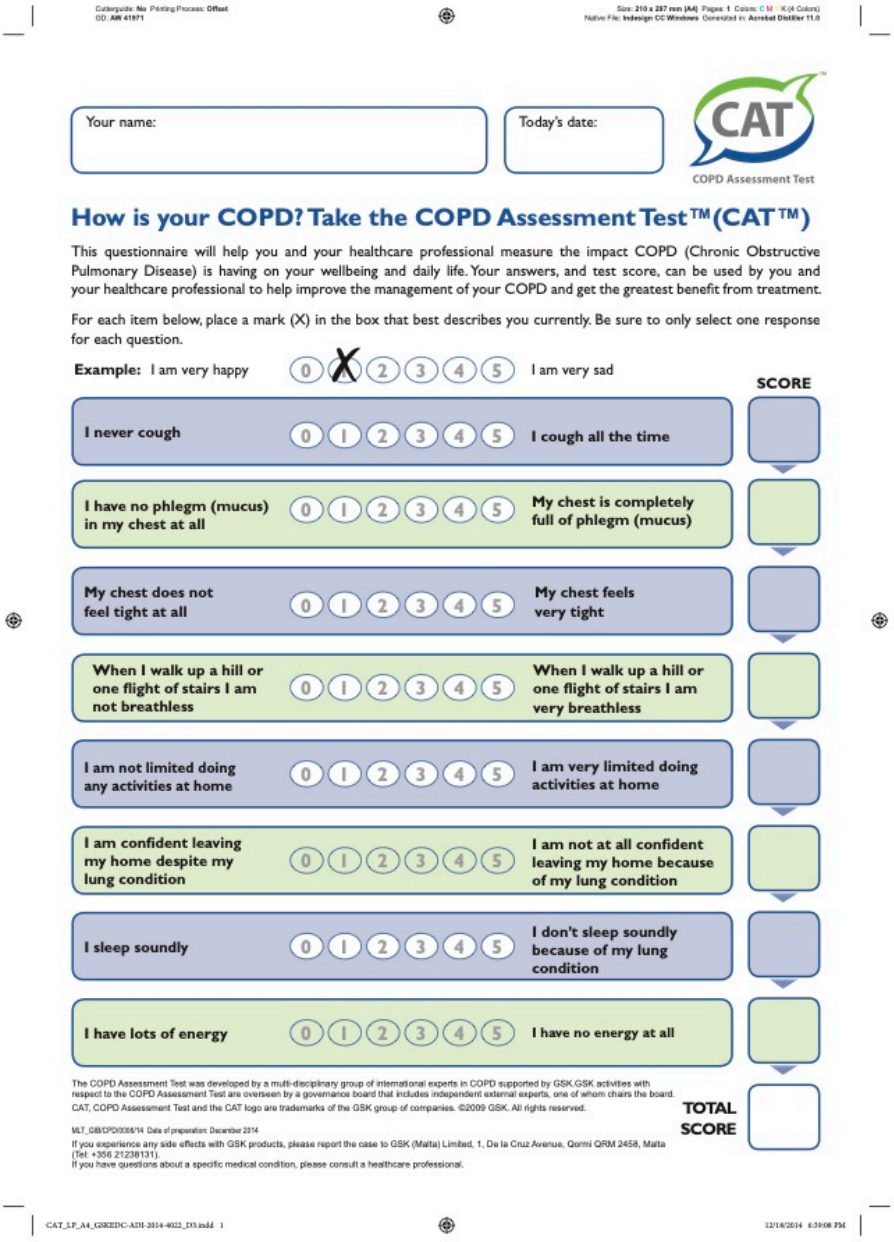
Abbreviations: CBC – complete blood count; SGRQ - St. George's Respiratory Questionnaire; CAT - COPD Assessment test.

Table S2: *Reference range of serum immunoglobulin concentrations.*

Ig type or subclass	Reference range
IgG (g/L)	7.0-16.0
IgG1 (g/L)	3.82-9.29
IgG2 (g/L)	2.42-7.00
IgG3 (g/L)	0.22-1.76
IgG4 (g/L)	0.04-0.86
IgA (g/L)	0.7-4.0
IgM (g/L)	0.4-2.3
IgE (ug/L)	<=240

Table S3: *Scoring and categorization of COPD Assessment Test and St. George Respiratory Questionnaire scores.*

COPD Assessment Test scores (out of 40)	
Low	<10
Medium	10-20
High	>20 - 29
Very High	>30
St. George Respiratory Questionnaire scores (out of 100)	
Quartile 1	<32
Quartile 2	≥32 to <46
Quartile 3	≥46 to <60
Quartile 4	>60



Your name:

Today's date:

CAT
COPD Assessment Test

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

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

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

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

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

The COPD Assessment Test was developed by a multi-disciplinary group of international experts in COPD supported by GSK.GSK activities with respect to the COPD Assessment Test are overseen by a governance board that includes independent external experts, one of whom chairs the board.

MLT_GB/CPD/0006/14 Date of preparation: December 2014

If you experience any side effects with GSK products, please report the case to GSK (Malta) Limited, 1, De la Cruz Avenue, Qormi QRM 2458, Malta (Tel: +356 21238131).
If you have questions about a specific medical condition, please consult a healthcare professional.

Supplementary Figure S1: *CAT Questionnaire*

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest problem you have had over the past 4 weeks.

Please checkmark (✓) *one box for each question*:

	Most days a week	Several days a week	A few days a month	Only with chest infections	Not at all
1. Over the past 4 weeks, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 4 weeks, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3. Over the past 4 weeks, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 4 weeks, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 4 weeks, how many severe or very unpleasant attacks of chest problem have you had?

Please checkmark (✓) *one box only*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest problem last:
(Go to question 7 if you had no severe attacks)

Please checkmark (✓) *one box only*:

- a week or more ☐
- 3 days or more ☐
- 1 or 2 days ☐
- Less than a day ☐

7. Over the past 4 weeks, in an average week, how many good days (with little chest problem) have you had:

Please checkmark (✓) *one box only*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- Nearly every day was good ☐
- Every day was good ☐

8. If you have a wheeze, is it worse in the morning:

Please checkmark (✓) *one box only*:

- No ☐
- Yes ☐

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please checkmark (✓) *one box only*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes me no problem ☐

If you have ever had paid employment.

Please checkmark (✓) *one box only*:

- My chest problem made me stop work altogether ☐
- My chest problem interferes with my work or made me change my work ☐
- My chest problem does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

For ***each item***, please checkmark (✓) the box as it applies to you ***these days***:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around at home	☐	☐
Walking outside on the level	☐	☐
Climbing up a flight of stairs	☐	☐
Climbing hills	☐	☐
Playing sports or games	☐	☐

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest problem may have on you <u>these days</u>.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest problem is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication. If you are taking no medication go straight to Section 6.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

For ***each item***, please checkmark (✔) the box as it applies to you ***because of your breathing***:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as climbing up hills, carrying things up stairs, light gardening such as weeding, dancing, playing bowls or golf	☐	☐
My breathing makes it difficult to do things such as carrying heavy loads, digging the garden or shovelling snow, jogging or walking at 8 kilometres per hour, playing tennis or swimming	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, running, cycling, swimming fast or playing competitive sports	☐	☐

Section 7

We would like to know how your chest problem <u>usually</u> affects your daily life.

For ***each item***, please checkmark (✔) the box as it applies to you ***because of your chest problem***:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the groceries	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest problem may prevent you doing (you do not have to checkmark these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest problem may stop you doing:

.....

.....

.....

.....

Now, would you checkmark the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish, would you check to see that you have answered all the questions.

Supplementary Figure S2: *SGRQ questionnaire*