University of Alberta

Chronic Obstructive Pulmonary

Disease And Systemic Inflammation

by ©

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List of Abbreviations

BMI	Body mass index
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COT	Serum cotinine
CRP	C-reactive protein
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
IL-6	Interleukin-6
IL-8	Interleukin-8
NHANES III	Third National Health and Nutrition Examination Survey
NR	Not reported or not calculable.
OR	Odds ratio
Pred	Predicted
SD	Standard deviation
TNF-α	Tumor necrosis factor-α

Chapter 1

Introduction

1.1 INTRODUCTION

Systemic complications such as weight loss,[1-3] cachexia,[4, 5] osteoporosis,[5, 6] and cardiovascular disease[7-9] are commonly observed among patients with stable chronic obstructive pulmonary disease (COPD), especially those with moderate (forced expiratory volume in 1 second, FEV_1, 50 to 80% predicted value) or severe COPD (FEV_1< 50% predicted value).[5, 10] Several prospective epidemiologic studies have demonstrated that impaired lung function is a strong predictor of future cardiovascular death, which is the leading cause of death for individuals with impaired lung function.[7-11] One study elucidated that a 10% decrease of FEV_1 among COPD patients is associated with about 30% increase in the risk of cardiovascular-related deaths. [12]

The mechanistic pathways to explain the strong association between impaired lung function and cardiovascular diseases are largely unknown. However, there is convincing epidemiologic and experimental evidence linking systemic inflammation with the occurrence of cardiovascular diseases, including atherosclerosis, ischemic heart disease, strokes, and coronary deaths.[10, 13-17] Furthermore, systemic inflammation has also been implicated in the pathogenesis of weight loss,[18] cachexia,[18] anorexia,[18, 19] and osteoporosis.[20] Interestingly, inflammation is a prominent feature of airways of individuals with impaired lung function.[21, 22] If this inflammation were to "spill over" into the systemic circulation, this may contribute to atherosclerosis and cardiovascular morbidity in these patients.[23-25] However, to date, there is little consensus on whether impaired lung function is indeed associated with persistent systemic inflammation. The main

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purpose of this project was to evaluate the relationship between impaired lung function (and in particular impaired lung function associated with COPD, which is the most common cause of impaired lung function in the community) and systemic inflammation and to determine whether inhaled corticosteroids, which are commonly used non-specific anti-inflammatory agents for the management of COPD, can down-regulate airway inflammation (which in turn might repress systemic inflammation) in those with severe lung function impairment.

In the first study, I conducted a systematic review and a meta-analysis to explore the relationship between COPD and systemic inflammation. In the second study, I extended the findings of study number one by evaluating whether the relationship between COPD and systemic inflammation was severity-dependent such that those with the most profound lung function impairment would have the largest burden of systemic inflammation and those with the least lung function impairment would have the smallest inflammatory burden. This study was conducted using a large population-based database (Third National Health and Nutrition Examination Survey, NHANES III). In the third study, I conducted a systematic review and meta-analysis of randomized controlled clinical trials to examine the effects of inhaled corticosteroids on the airway inflammation in stable COPD. If they are indeed effective in down-regulating airway inflammation, they may also be effective in repressing systemic inflammation since it may be presumed that the source of systemic inflammation in such patients is the airways.

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1.2 OBJECTIVES

The major objectives of the project were to determine:

1. Whether systemic inflammation is present in patients with stable COPD.

2. Whether the levels of systemic inflammatory markers are associated with impaired lung function, independent of cigarette smoking and other main confounders.

3. Whether inhaled corticosteroids, which are potent but non-specific anti-inflammatory agents, could effectively suppress airway inflammation in patients with stable COPD.

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Chapter 2

The Association Between Chronic Obstructive

Pulmonary Disease And Systemic Inflammation:

A Systematic Review And A Meta-Analysis

2.1   INTRODUCTION

Increasingly, systemic inflammation is being recognized as a risk factor for variety of different complications including atherosclerosis,[1] cachexia,[2] anorexia,[2, 3] and osteoporosis.[4] Notably, all of these complications are commonly observed in patients with chronic obstructive pulmonary disease (COPD).[5-10] Whether systemic inflammation is present in stable COPD and whether it is wholly or partially responsible for these associations is controversial. Although several studies have been done to evaluate this potential relationship, most of the studies have been small in size and scope, and, as such, may have, on their own, lacked sufficient statistical power to adequately address this issue. To overcome these and other limitations and to better understand the relationship between COPD and systemic inflammation, we conducted a systematic review and a meta-analysis, with the specific aim of examining the associations between stable COPD and serum levels of C-reactive protein, fibrinogen, leukocytes, and other pro-inflammatory cytokines. We chose these markers of systemic inflammation because they have been well-studied and have been intimately linked with the development of ischemic heart disease and stroke,[11-13] which, interestingly, are also the leading causes of mortality among COPD patients.[14]

2.2   METHODS

2.2.1   Search for Relevant Studies

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Using MEDLINE (1966-2003), EMBASE, CINAHL (1982-2003), and the Cochrane Databases, we conducted a systematic literature search to identify relevant studies published before November 1, 2003, which evaluated the potential relationship between stable COPD and various markers of systemic inflammation. We combined disease-specific search terms (COPD, bronchitis, emphysema, forced expiratory volume, or vital capacity), and inflammatory marker-specific search terms (systemic inflammation, biological markers, C-reactive protein, fibrinogen, leukocyte, interleukin, interleukin-8, interleukin-6, or tumor necrosis factor-alpha) in all our searches. We supplemented the electronic searches by scanning the reference lists from retrieved articles to identify additional articles that may have been missed during the initial search. We also contacted the primary authors for additional data and/or clarification of data, when necessary, to ensure that all relevant articles were represented in the meta-analysis. We decided a priori to include only those studies wherein stable patients (or individuals) were studied. All acute exacerbation studies were, therefore, discarded. We also discarded studies that did not have a suitable control (comparator) group.

2.2.2 Study Selection and Data Abstraction

The primary outcome of this systematic review was to compare serum C-reactive protein (CRP), fibrinogen, leukocyte, tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) levels between study participants with and without stable chronic obstructive pulmonary disease (COPD). From each

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relevant article, two investigators abstracted the following information: the source of data, the study design, the baseline characteristics of study participants including their age, predicted forced expiratory volume in one second (FEV_1), and smoking status. We also evaluated the laboratory methods that were employed to determine the levels of systemic inflammatory markers. Any questions or discrepancies regarding these data were resolved through iteration and consensus. We used the definition of COPD provided by each individual study, however they were defined. Although there was some heterogeneity in the way in which COPD was defined across the studies, most defined COPD using spirometric criterion of FEV_1/forced vital capacity (FVC) <0.70 or <0.60. For population-based studies wherein COPD was not explicitly defined, we assumed that participants in the lowest quartile group of predicted FEV_1 had COPD, while those participants in the highest quartile group of predicted FEV_1 did not (and therefore served as controls). We assumed that most individuals in the former category had COPD, since, from a population perspective, COPD is the most common cause for chronic airflow limitation in the adult population. For these studies, we included data only from those participants who had a history of smoking; data from life-time non-smokers were censored from the main analyses. We excluded any studies, which did not provide spirometry data on the study participants to ensure comparability of the COPD definition across the studies.

2.2.3 Statistical Methods

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To accommodate differences in the way in which inflammatory markers were measured and reported across various laboratories, we converted the absolute levels of above inflammatory markers into a common unit by calculating standardized effect sizes. Standardized effect sizes were derived by dividing the mean difference in CRP levels between COPD and control subjects of each study by the pooled standard deviation of the CRP levels across the two groups.[15] We employed the same technique in calculating standardized mean differences for leukocytes, fibrinogen, and other inflammatory cytokines. For each outcome, we tested the heterogeneity of results across the studies, using a Cochran Q test. If significant heterogeneity was observed (p <0.10), then a random effects model, which assigns a weight to each study based on individual study variance as well as between-study variance, was employed to pool the results together. In the absence of significant heterogeneity, a fixed effect model was used.[16] As a sensitivity analysis, we also pooled the data together using a weighted mean difference technique. All analyses were conducted using Review Manager version 4.2 (Revman; The Cochrane Collaboration, Oxford, England).

2.3   RESULTS

A summary of the search strategy is shown in **Figure 2.1**. The original search yielded 911, 666, 279, and 16 citations in MEDLINE, EMBASE, CINAHL, and the Cochrane Databases, respectively. The abstracts of these articles were selected and reviewed. Of these, 19 articles were retrieved for a detailed review: seven for C-reactive protein,[17-23] six for fibrinogen,[17, 20, 24-27] six for leukocyte,[17, 18,]

[20, 28-30] six for TNF-α,[22, 31-35] two for IL-6,[22, 23] and two for IL-8,[23, 35] respectively. Five studies were excluded for the following reasons: two studies were publications of the same cohort;[17, 34] and two studies provided data on leukocytes based only on a linear regression model, which made it impossible to ascertain the relationship between COPD and leukocytes;[28, 30] and one study diagnosed chronic bronchitis based only on symptoms (without spirometry).[27] This process left 14 original studies meeting the inclusion and exclusion criteria, which were then used for the analyses: five for C-reactive protein,[18-22] four for fibrinogen,[20, 24-26] four for TNF-α,[22, 31-33] three for leukocyte,[18, 20, 29] two for IL-8,[23, 35] and one for IL-6,[22] respectively. The relevant baseline data from each of the selected studies are summarized in **Table 2.1**.

Patients with COPD had higher levels of CRP than control subjects in all studies. Overall, the standardized mean difference in the CRP level between COPD and control subjects was 0.53 units (95% confidence interval, CI, 0.34 to 0.72) (**Table 2.2, Figure 2.2**) or 1.86 mg/L (95% CI, 0.75 to 2.97 mg/L) using a weighted mean difference technique. The heterogeneity in results across the studies (test for heterogeneity, p=0.006) likely resulted from the differences in the severity of underlying COPD population and way in which control subjects were selected for each of the study. Mannino et al [20] and Mendall et al,[21] for instance, used data from a population-based study; whereas, Eid et al [19] and Yasuda et al [22] recruited their patients from respiratory clinics. Not surprisingly, the standardized mean difference values in CRP were larger in the latter studies than the former. Importantly, however, even in population-based studies, which are less

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susceptible to selection bias, a strong relationship between CRP and COPD was observed, suggesting that COPD is, indeed, a risk factor for elevated CRP in the community.

Similarly, COPD patients had higher fibrinogen levels relative to control subjects. Overall, the standardized mean difference in the fibrinogen level was 0.47 units (95% CI, 0.29 to 0.65) (**Table 2.3, Figure 2.3**) or 0.37 g/L (95% CI, 0.18 to 0.56 g/L) using a weighted mean difference technique. As with the CRP results, there was some heterogeneity in the results between the studies (test for heterogeneity, p<0.0001). However, all studies (both large and small) demonstrated that fibrinogen levels were higher in COPD than in control subjects. For population based studies,[20, 25, 26] the standardized mean difference between the lowest quartile group and the highest quartile group of predicted FEV_1 among smokers was 0.43 units (95% CI, 0.24 to 0.61).[25, 26]

Overall, serum leukocytes were higher in COPD than in control subjects. The standardized mean difference was 0.44 units; 95% CI, 0.20 to 0.67 (test for heterogeneity, p=0.003) (**Table 2.4, Figure 2.4**) or 0.88 x10⁹ cells/L (95% CI, 0.36 to 1.40 x10⁹ cells/L) using a weighted mean difference technique. Likewise, serum TNF-α levels were higher in COPD than in control subjects. The standardized mean difference was 0.59 units; 95% CI, 0.29 to 0.89 (**Table 2.5, Figure 2.5**) (test for heterogeneity, p=0.87) or 2.64 pg/mL (95% CI, -0.44 to 5.72 pg/mL) using a weighted mean difference technique.

There was only one study with analyzable data for IL-6.[22] Compared with healthy controls (N=22), COPD patients (N=39) had significantly elevated serum

levels of IL-6 (mean difference 13.10 pg/mL; 95% CI, 7.23 to 18.97 pg/mL).

There were two studies on IL-8.[23, 35] One study showed that 17 out of 30 COPD

patients had detectable IL-8 level, whereas none of the 26 healthy controls had

detectable serum IL-8 (using an assay with a detectable limit of 20 pg/mL).[23]

Another study reported that 4 out of 18 COPD patients had detectable IL-8 levels

in their serum, while none of the 17 healthy controls had detectable serum IL-8

(using an assay with detectable limit of 8 pg/mL).[35]

2.4   DISCUSSION

In this systematic review, we found that compared with healthy controls,

individuals with chronic airflow limitation had significantly elevated levels of CRP,

fibrinogen, leukocyte, and TNF-α, indicating that persistent systemic inflammation

is present in COPD. Even among non-current smokers, there was evidence for

low-grade systemic inflammation in those with chronic airflow limitation.

How and why individuals with COPD develop systemic inflammation is

uncertain and unknown. COPD is characterized by an intense inflammatory

process in the airways, parenchyma, and pulmonary vasculature.[36] It is possible in

some cases that the inflammatory process may "spill" over into the systemic

circulation, promoting a generalized inflammatory reaction.[37-40] It is also possible

that there may be common genetic or constitutional factors that may predispose

individuals with COPD to both systemic and pulmonary inflammation.[36, 41] Finally,

while we believe that COPD is responsible for the systemic inflammation, there

exists the possibility of reverse causation. The possibility that systemic

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inflammation causes injuries to the airways, leading to COPD changes, cannot be fully discounted.[25]

Whatever the mechanism, the presence of systemic inflammation in COPD has been linked with a variety of complications including weight loss,[19, 31, 32] cachexia,[8, 10] osteoporosis,[9, 10] and cardiovascular diseases.[5-7] Moreover, data from Dahl et al suggest that individuals with elevated systemic inflammatory markers such as fibrinogen experience an accelerated decline in lung function and are at increased risk of COPD hospitalizations in the future.[25] The relationship of COPD, systemic inflammation and cardiovascular diseases may be especially germane, as over half of patients with COPD die from cardiovascular causes.[42, 43] Indeed, airflow limitation increases the risk of cardiovascular mortality by two-fold, independent of smoking.[5-7, 17, 26] Moreover, during periods of exacerbation, plasma levels of fibrinogen and serum levels of interleukin-6 increase significantly, which may further contribute to increased cardiovascular morbidity and mortality in COPD patients.[44]

Several limitations of this study should be emphasized. First, all relevant studies regarding the association between impaired lung function and systemic inflammation were cross-sectional in nature, thus the temporal relationships between these two factors were unclear. Second, there was some variation in the way in which study participants were sampled and inflammatory markers were analyzed. There was also heterogeneity as to the mean differences of inflammatory markers across separate studies. Even within the COPD group, some were selected on the basis of weight loss or poor nutritional status and, as such,

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selected study sample may not represent the general pool of COPD patients. However, despite these variations, it was reassuring that in nearly all studies (regardless of the sample size, the baseline FEV_1, and composition of the study and control groups), those with airflow limitation, on average, had higher levels of systemic inflammatory markers compared with healthy controls. This suggests that selection and sampling biases were unlikely to be responsible for the observed associations. Third, there was a marked paucity of studies that evaluated the relationship between COPD and IL-6, IL-8. IL-6 has been implicated in the pathogenesis of atherosclerosis,[45-47] while IL-8 may be an important signaling molecule for neutrophils chemotaxis, which may have significance in COPD.[48-49] In view of their potential relevance in COPD, more studies are needed in the future to determine whether the systemic expression of these cytokines is increased in COPD.

In summary, there is now a large body of evidence to indicate that systemic inflammation is present in patients with stable COPD. This finding may explain, at least in part, the high prevalence of systemic complications such as cachexia, osteoporosis, and cardiovascular disease among patients with COPD. Future studies are needed to determine whether attenuation of the systemic inflammatory level can modify the risk of these complications in COPD.

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2.5   TABLES

Table 2.1. Baseline Information on Original Studies Included in the Meta-Analysis

Source	Study Design and Original Purposes	COPD Patients	Controls	Laboratory Measurement
Alessandri [24] 1994	Conducted in Italy. To test whether a hypercoagulability state is present in patients with COPD.	1.FEV_1/ FVC <0.7; 2. A hematocrit value <50%; 3. Without comorbid diseases.	Healthy volunteers without any disease.	Fibrinogen, clauss method employing the KoaguLab 32-S coagulometer.
Dahl [25] 2001	Population-based study conducted in Denmark. To test whether increased fibrinogen concentrations correlate with lung function and COPD hospitalization rates among adults.	The lowest quartile group of FEV_1 % pred.	The highest quartile group of FEV_1 % pred.	Fibrinogen, standard colorimetric assay.
de Godoy [31] 1996	Conducted in the US. Age-mached healthy volunteers were chosen as controls. To examine whether TNF-α and IL-1β produced by peripheral blood monocytes are increased in weight-losing COPD patients.	1. FEV_1/ FVC < 0.6; 2. At least 6 wk stability; 3. Exclusion of patients receiving oral corticosteroids or with comorbid diseases.	Age-matched healthy volunteers.	TNF-α, enzyme-linked assay (R&D System).
Dentener [18] 2001	Conducted in Netherlands. To test the hypothesis that the chronic inflammatory process present in COPD is due to a defective endogenous anti-inflammatory mechanism.	1. FEV_1 <80% predicted; 2. β₂ agonist reversibility of <15% or 200 ml; 3. Ratio of FEV_1 to FVC of < 70%; 4.Stable clinical condition; 5.Exclusion of patients with comorbid diseases.	Healthy subjects with no evidence of COPD.	CRP, polyclonal ELISA. Leukocyte, COBAS Micro.
Di Francia [32] 1994	Conducted in France. 30 patients met the criteria were consecutively admitted. To test whether serum levels of TNF-α is related to weight loss in patients with COPD.	1.FEV_1/ FVC <0.6; 2.Irreversibility of airflow obstruction; 3.Creatine clearance in the normal range; 4. Stable clinical condition; 5.Exclusion of patients with comorbid diseases.	Healthy laboratory staff members.	TNF-α , immunoradio-metric method.
Eid [19] 2001	Conducted in the UK. Community-based patients were recruited from a hospital respiratory clinic. To test whether skeletal muscle loss is associated with inflammatory and catabolic responses in COPD.	1. Smoking history; 2. Respiratory symptoms; 3. Reversibility of <10% after bronchodilator; 4.Further confirmation during a 1-yr period of follow-up; 5. Stable clinical condition; 6. Without comorbid diseases.	Healthy age- and sex-related subjects free of lung disease.	CRP, enzyme-linked immunosorbent assay.

Engstrom [26] 2002	Population-based study conducted in Sweden. To explored whether plasma levels of fibrinogen and other inflammatory mediators are related to FVC and whether these proteins contribute to the increased incidence of MI and death among men with reduced FVC.	Participants in the lowest quartile group of FVC % pred (< 85%) without comorbid diseases. Men with reported long-term cough associated with increased mucus production were excluded.	Participants in the highest quartile group of FVC % pred (>105%).	Fibrinogen, Electroimmuno-assay method.
James [29] 1999	Cross-sectional survey of adults aged 25-79 yrs in Busselton, Western Australia. To investigate whether lung function and respiratory illness were related to leukocytes.	Participants in the lowest quartile group of FEV_1 % pred and with the ratio of $FEV_1/FVC < 0.7$.	Highest quartile group of FEV_1 %pred and with the ratio of $FEV_1/FVC >0.7$.	Leukocyte, NR.
Mendall [21] 2000	Caerphilly Prospective Heart Disease Study conducted in South Wales. To examine whether the low grade inflammation indicated by C-reactive protein may be the mechanism whereby non-circulating risk factors may influence pathogenesis of ischaemic heart disease.	Participants in the lowest 25th percentile of FEV_1.	Participants in highest 25th percentile of FEV_1.	CRP, in-house ELISA method.
Schols [23] 1996	Conducted in Netherlands. To investigate whether the increased resting energy expenditure seen in some COPD patients is related to systemic inflammatory response.	1. With moderate to severe COPD (FEV_1% pred of 37%); 2. β_2 agonist bronchodilator (400ug salbutamol) reversibility of <10%; 3. Stable clinical condition; 4. Resting energy expenditure <105% or >120% of predicted.	Randomly selected from a population sample in the same area as the patients and aged >50.	IL-6, ELISA assay with a detectable limit of 10 pg/mL IL-8, ELISA assay with a detectable limit of 20 pg/mL.
Mannino [20] 2003	A cross-sectional, multistage probability representative sample of the civilian non-institutionalized U.S. population. To assess the relation of impaired lung function to circulating levels of CRP and fibrinogen among adults.	$FEV_1/FVC < 0.70$.	$FEV_1/FVC \geq 0.7$, FVC % $\geq$ 80, and free of lung disease.	Fibrinogen, immunochemical method. CRP, latex-enhanced nephelometry. Leukocyte, standard method.

Takabatake[33] 2000	Conducted in Japan. To test whether systemic hypoxemia observed in male patients with COPD might contribute to activation of TNF-α system and therefore cause weight loss.	According to the criteria of the American Thoracic Society. 1.Irreversible chronic airflow obstruction; 2. Stable for at least 3 mo. 3. Exclusion of patients with conditions known to affect serum TNF-α level.	Age-matched health male volunteers.	TNF-α, enzyme-linked immunosorbent assay (ELISA) kits.
Vernooy[35] 2002	Conducted in Netherlands. To elucidate the relationship between local and systemic inflammation in smoking-induced COPD.	Diagnosed according to the criteria of the American Thoracic Society. 1. Stable clinical condition; 2. Predicted $FEV_1 < 70\%$; 3. β_2 agonist bronchodilator reversibility of <11% or 200ml; 4. Previous history of at least 20 pack-years of smoking; 5. Exclusion of patients receiving inhaled steroids or with comorbid diseases.	17 subjects with a normal FEV_1 and no medical history of lung disease. A smoking history of ≥15 pack-years.	IL-8, specific sandwich ELISA with a detectable limit of 8 pg/mL.
Yasuda[22] 1998	Conducted in Japan. To test whether the concentrations of sFas-L and sFas are related to CRP, TNF-α, or IL-6.	Diagnosed by history, physical examination, roentgenographic examination and lung function tests. Conditions: 1. Stable clinical condition; 2. No recent change of drugs; 3. Normal left ventricular ejection fraction; 4. Normal plasma creatinine concentration; 5. Absence of other pathological conditions.	Healthy age- and sex-matched volunteers without any disease.	CRP, latex nephelometric immunoassy with a detection limit of 0.3 mg/L. TNF-α, a sandwich ELISA kit.

Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; MI, myocardial infarction; NR, not reported; Pred, predicted; sFas, soluble Fas/Apo-1 receptor; sFas-L, soluble Fas/Apo-1 receptor ligand; TNF-α, tumor necrosis factor-α.

Table 2.2. C-Reactive Protein Levels and Demographic Features in the Patients with Stable COPD and Healthy Controls

Author	Group	N	Age (yr)	FEV$_1$ (% pred)	Current Smoker(%)	Men (%)	CRP (mg/L)
Dentener [18]	COPD	55	69 (4)	36 (14)	NR	100	20.4 (21.1)
	Control	23	64 (3)	110 (17)	NR	70	9.0 (16.0)
Eid [19]	COPD	68	68 (7)	31 (8)	100	57	3.5 (3.4)
	Control	45	NR	NR	33	NR	1.3 (1.9)
Mannino [20]	COPD	2366	64 (16)	78 (20)	32	60	3.3 (2.0)
	Control	8446	40 (17)	103 (13)	21	48	2.7 (1.0)
Mendall [21]	COPD	349*	45-59	NR	NR	100	0.8 (2.8)*
	Control	349*	45-59	NR	NR	100	0 (0)
Yasuda [22]	COPD	39	66 (3)	35 (1)	41	69	18.7 (17.8)
	Control	22	66 (1)	82 (0)	32	68	0.9 (2.8)

* Imputed from the regression coefficient between mean FEV$_1$ (25th to 75th percentile) and CRP.

Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; Pred, predicted; NR, not reported/not calculable.

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Table 2.3. Fibrinogen Levels and Demographic Features in the Patients with Stable COPD and Healthy Controls

Author	Group	N	Age (yr)	FEV$_1$ (% pred)	Current Smoker(%)	Men (%)	Fibrinogen (g/L)
Alessandri [24]	COPD	37	68 (8)	1.1(0.5)*	19	73	3.0 (0.9)
	Control	30	58 (10)	NR	20	70	2.3 (0.5)
Dahl [25] (smokers)	COPD	1427	62 (12)	66 (15)	100	51	3.5 (1.0)
	Control	785	55 (15)	117 (9)	100	43	3.0 (0.8)
Engstrom [26] (smokers)	COPD	720	47 (4)‡	<85†	100	NR	3.8 (0.9)
	Control	449	47 (4)‡	>105†	100	NR	3.6 (0.8)
Mannino [20]	COPD	2065	67 (12)	77 (21)	31	60	3.1 (0.6)
	Control	3488	57 (13)	104 (14)	17	47	2.8 (0.5)

* FEV1, L.

† Based on forced vital capacity.

‡ Estimated.

Abbreviations: COPD, chronic obstructive pulmonary disease; Pred, predicted; NR, not reported/not calculable.

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Table 2.4. Leukocyte Levels and Demographic Features in the Patients with Stable COPD and Healthy Controls

Author	Group	N	Age (yr)	FEV$_1$ (% pred)	Current Smoker(%)	Men (%)	WBC (10^9/L)
Dentener [18]	COPD	55	69 (4)	36 (14)	NR	100	8.0 (2.7)
	Control	23	64 (3)	110 (17)	NR	70	5.1 (1.2)
Mannino [20]	COPD	2366	64 (16)	78 (20)	32	60	7.1 (1.3)
	Control	8446	40 (17)	103 (13)	21	48	6.7 (1.3)
James [29]	COPD	294	56 (12)	66 (12)	100	74	7.7 (1.3)
(smokers)	Control	326	44 (13)	114 (9)	100	58	7.3 (1.3)

Abbreviations: COPD, chronic obstructive pulmonary disease; Pred, predicted; WBC, white blood cell; NR, not reported.

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Table 2.5. Tumor Necrosis Factor-α Levels and Demographic Features in the Patients with Stable COPD and Healthy Controls

Authors	Group	N	Age (yr)	FEV$_1$ (% pred)	Current Smoke(%)	Men (%)	TNF-α (pg/mL)
de Godoy [31]	COPD	20	69 (9)	67 (13)	NR	70	7.0 (2.9)†
	Control	13	64 (6)	106 (17)	NR	85	5.8 (1.5)†
Di Francia [32]	COPD	30	65 (9)	0.4 (0.1)*	NR	100	40.6 (73.1)
	Control	21	47 (13)	NR	NR	100	7.8 (3.9)
Takabatake [33]	COPD	27	73 (7)	52 (20)	NR	100	6.2 (1.1)
	Control	15	70 (6)	74 (7)	NR	100	5.4 (1.6)
Yasuda [22]	COPD	39	66 (3)	35 (1)	41	69	21.8 (33.6)
	Control	22	66 (1)	82 (0)	30	68	3.9 (2.4)

* FEV$_1$/FVC.

† Deduced from medians and ranges.

Abbreviations: COPD, chronic obstructive pulmonary disease; Pred, predicted; NR, not reported; TNF-α, Tumor necrosis factor-α.

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2.6 FIGURES

Figure 2.1. Study Selection Process

Search results: N=1872
  MEDLINE: n=911
  EMBASE: n=666
  CINAHL: n=279
  Cochrane Library: n=16

Did not meet criteria:
n=1853

Studies retrieved:
n=19

Did not have sufficient data: n=3
Different publications but the
same cohort: n=2

Studies included in analyses: n=14
  C-reactive protein: n= 5
  Fibrinogen: n= 4
  Tumor necrosis factor-a: n= 4
  Leukocyte: n= 3
  Interleukin-8: n= 2
  Interleukin-6: n= 1

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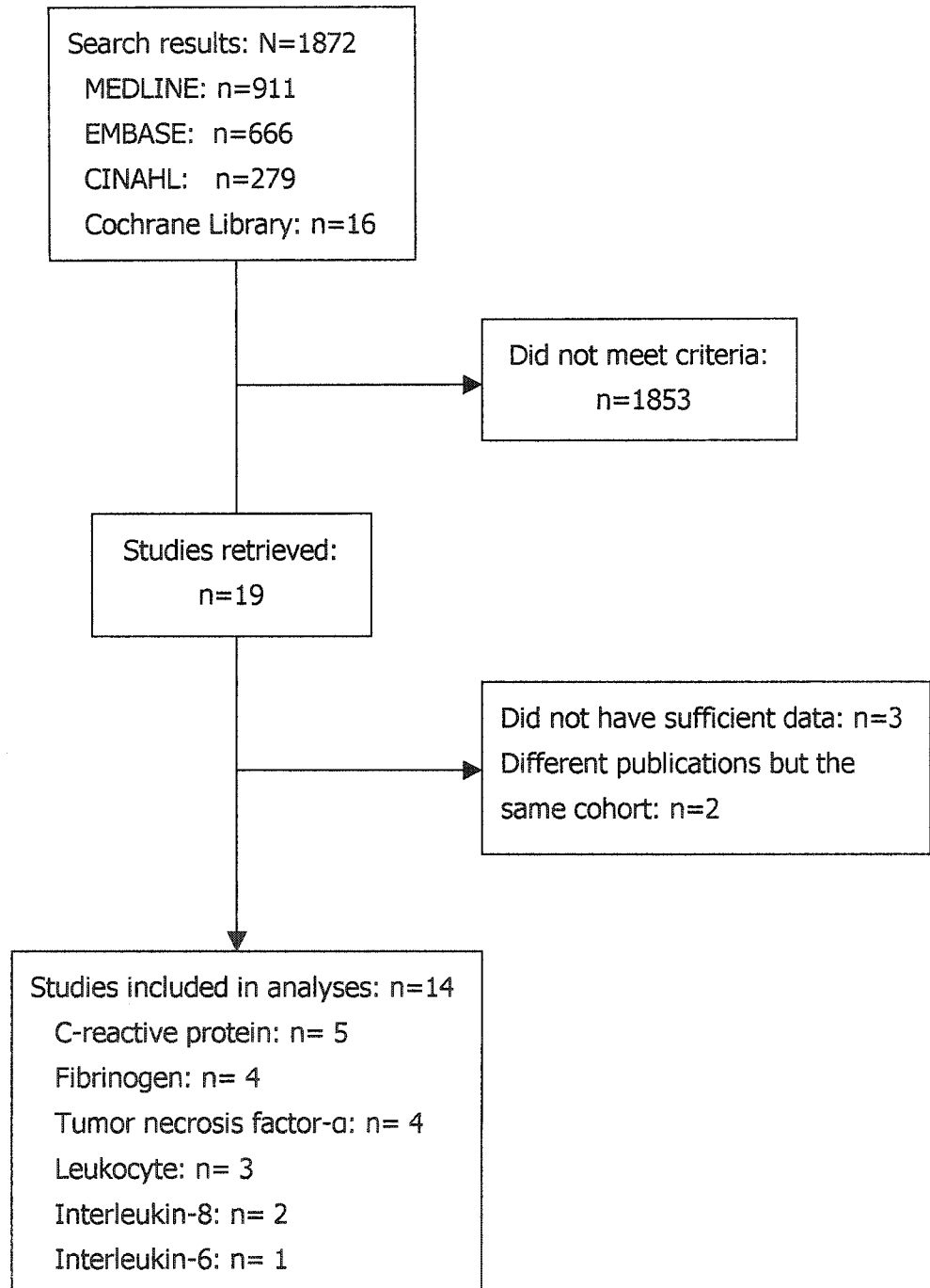
Figure 2.2. The Relationship of C-Reactive Protein and COPD
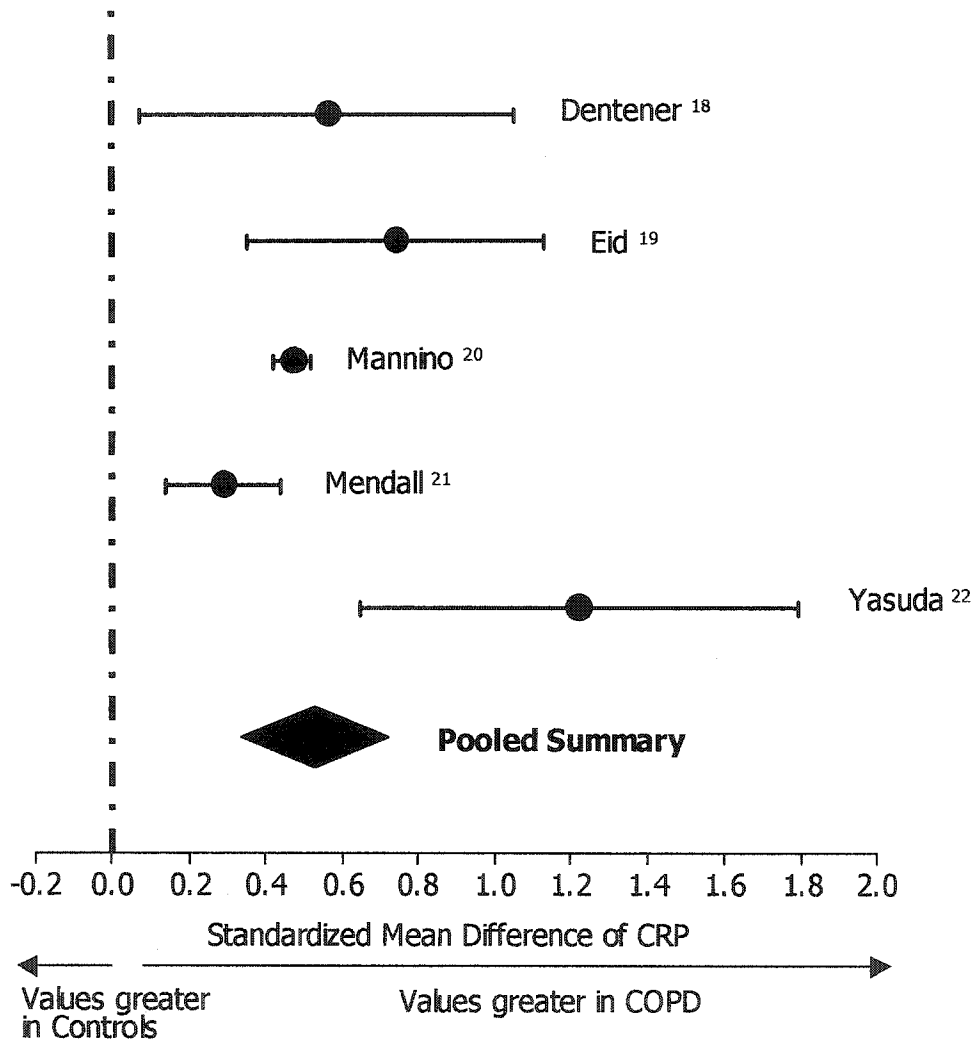
Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein.

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Figure 2.3. The Relationship of Fibrinogen and COPD

Abbreviations: COPD, chronic obstructive pulmonary disease.

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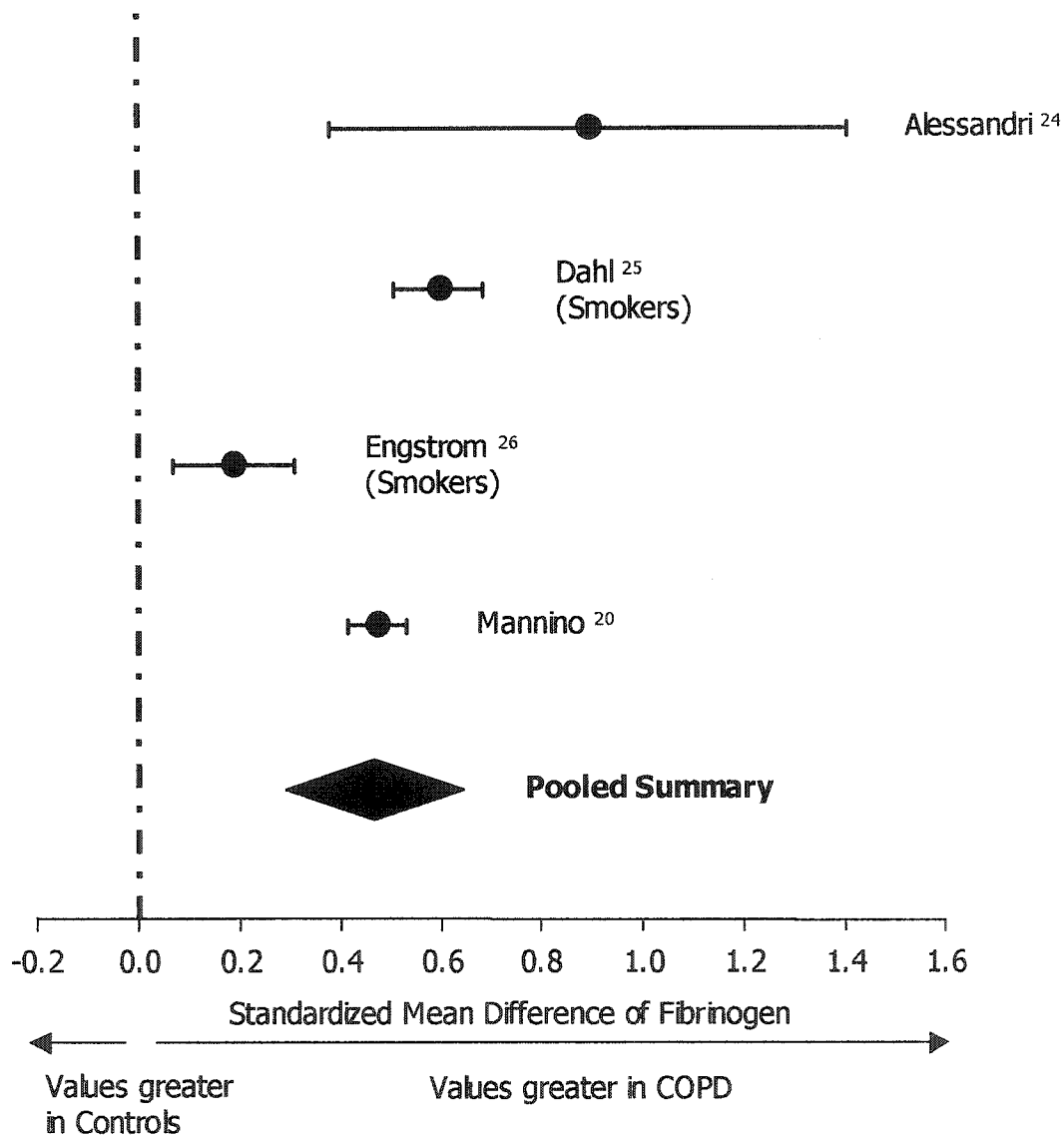
Figure 2.4. The Relationship of Leukocyte and COPD

Abbreviations: COPD, chronic obstructive pulmonary disease.

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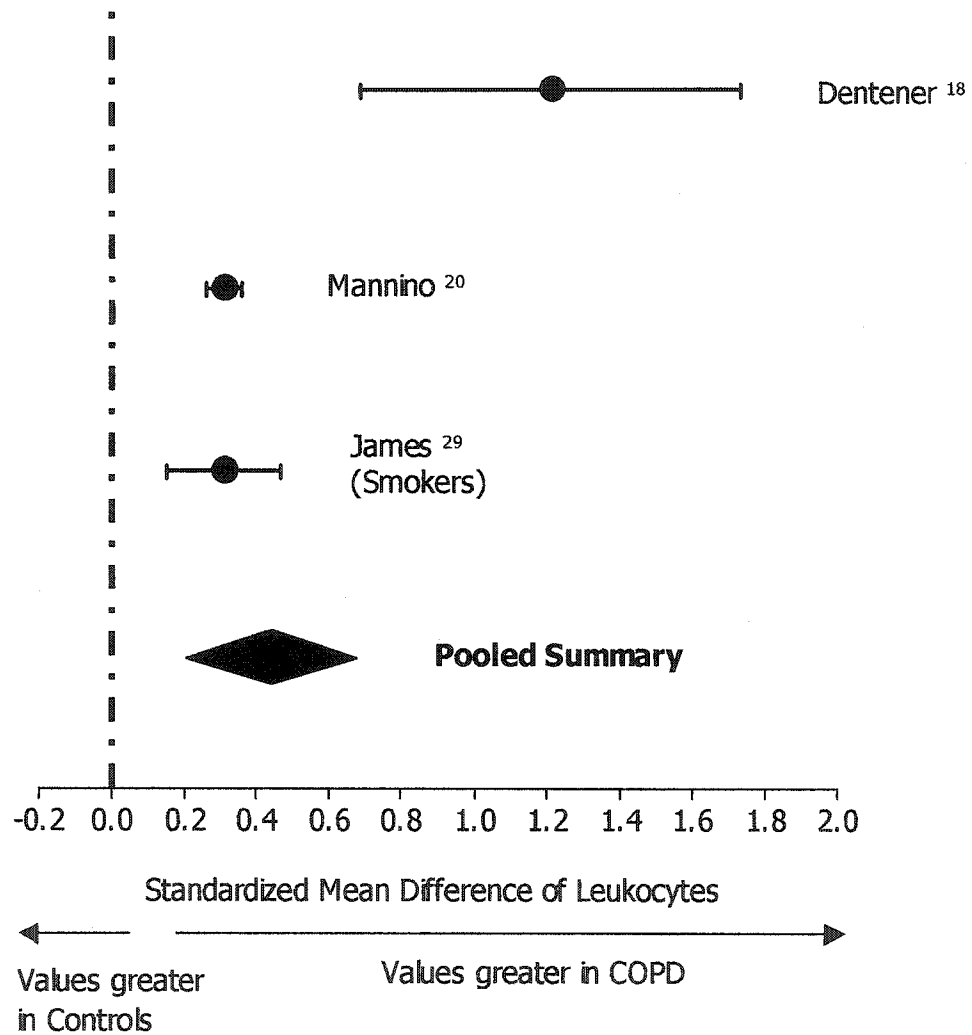
Figure 2.5. The Relationship of Tumor Necrosis Factor-α and COPD

Standardized Mean Difference of TNF-alpha

◄ Values greater in Controls

Values greater in COPD ►
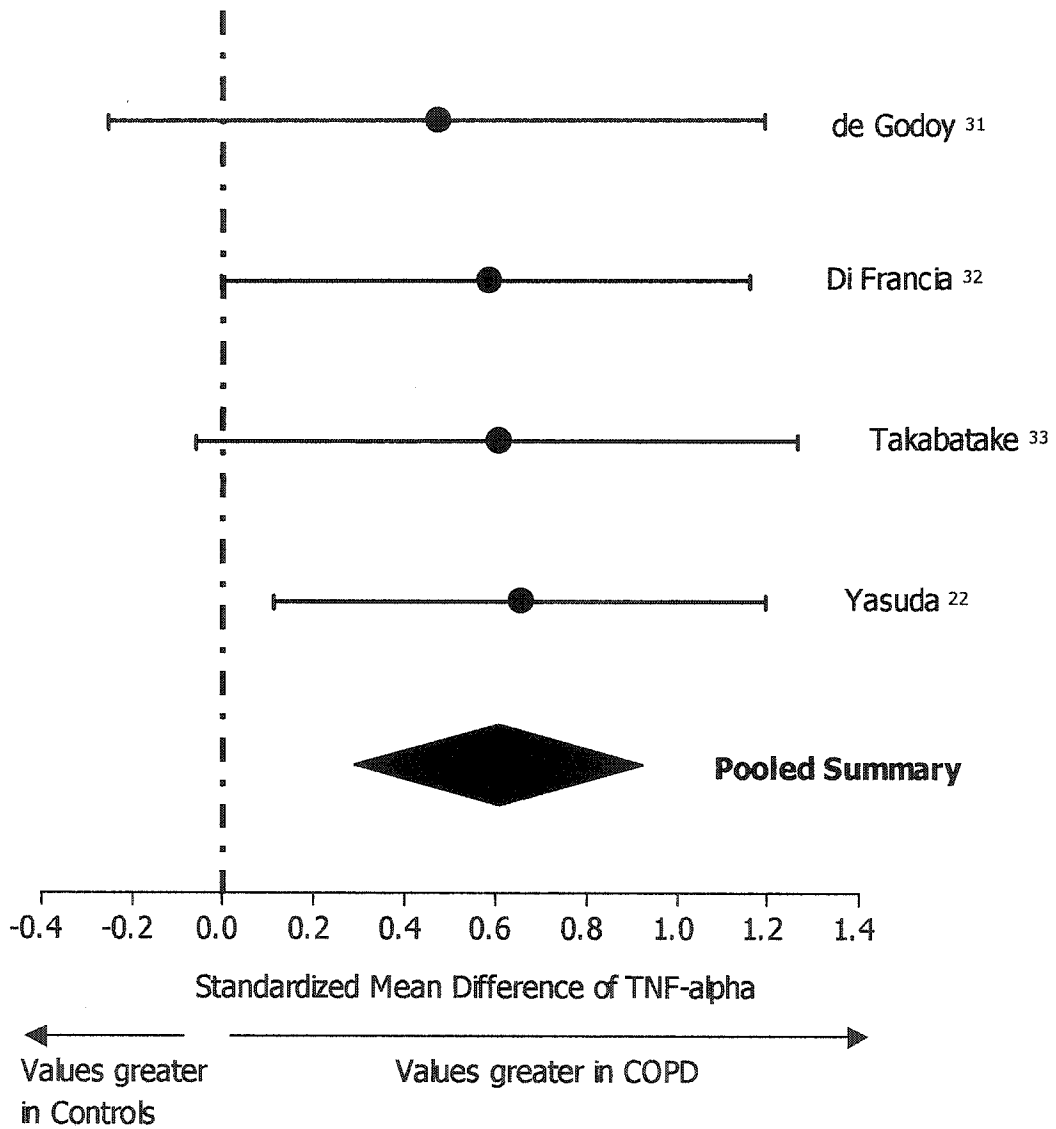
Abbreviations: COPD, chronic obstructive pulmonary disease;
TNF-α, Tumor necrosis factor-α.

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Chapter 3

The Interactions Between Cigarette

Smoking And Reduced Lung Function

On Systemic Inflammation

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3.1 INTRODUCTION

Individuals who have reduced forced expiratory volume in one second (FEV_1) are at increased risk of morbidity and mortality from various disorders including ischemic heart disease, stroke, arrhythmias, respiratory failure, and cachexia.[1-4] Although the exact mechanism for these relationships is still unknown, reduced FEV_1 is associated with persistent low-grade systemic inflammation,[5] which is a known risk factor for atherosclerosis, muscle loss and cachexia.[6-8] In the community, the single most important risk factor for reduced FEV_1 is cigarette smoking.[9] Since cigarette smoking by itself can also lead to systemic inflammation,[10-11] the observed relationship between reduced FEV_1 and systemic inflammation may be confounded by cigarette smoke exposure. We would like to examine the potential interaction of cigarette smoking and reduced FEV_1 on systemic inflammation which is unknown. We used data from the Third National Health and Nutrition Examination Survey (NHANES III) to determine firstly whether reduced FEV_1, independent of active cigarette smoking, is associated with systemic inflammation in the adult population, 40 years of age and older, and secondly whether cigarette smoking has an additive (or even a synergistic) effect on systemic inflammation among those with reduced FEV_1.

3.2 METHORDS

3.2.1 Study Sample

NHANES III was conducted from 1988 to 1994 in the United States by the National Center for Health Statistics of the Centers for Disease Control and

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Prevention. This was a cross-sectional, multistage probability representative sample of the civilian non-institutionalized U.S. population.[12] We restricted the present analysis to participants of NHANES III, who were 40 years of age and older to minimize the influences of age on markers of systemic inflammation. Of the 11,448 participants aged 40 years and older, we excluded those not having reported values for smoking status, body mass index (BMI), FEV_1, or serum cotinine. This process left 7,685 participants for the present study. Of these, 4,291 participants were either active or ex-smokers (as indicated by the participants on history).

3.2.2 Measurements

Laboratory procedures used in the NHANES III have been described previously.[13] Briefly, pulmonary functions were performed on study participants according to the standards of the American Thoracic Society.[14] Each study participant performed 5 to 8 forced expiratory maneuvers. To adjust for height, age, and gender, we used published prediction equations for FEV_1 and FVC, derived from the NHANES III population.[15] Serum cotinine level was measured by using high-performance liquid chromatography atmospheric-pressure chemical ionization tandem mass spectrometry.[16] A serum cotinine level of ≥ 10 ng/ml was used to indicate active cigarette smoking.[17] Because most participants had CRP levels below the lowest detectable limit for this assay (<2.1 mg/L), CRP levels were categorized as undetectable (<2.2 mg/L) or elevated (≥ 2.2 mg/L). Serum fibrinogen, leukocyte and platelet counts were also determined using standard

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assays as previously described.[13]   Serum leukocyte count, platelet count, and fibrinogen levels were deemed to be elevated if their values exceeded the 85th percentiles of respective markers. For leukocytes, the 85th percentile was $\geq$ 9.1×10^9/L; for platelets, it was $\geq 339.0 \times 10^9$/L; and for fibrinogen, it was ≥ 3.9 g/L.

3.2.3   Statistical Methods

The population was divided into four equal groups (quartiles) based on the predicted FEV_1% predicted values. Statistical comparisons of baseline characteristics of the study population in quartiles of FEV_1 were performed, using a χ^2 test for binary variables and a t test for continuous variables. To evaluate the effects of active cigarette smoke exposure on the relationship between FEV_1 and various systemic inflammatory markers, we further divided the study population according to their serum cotinine levels (active smoker, ≥ 10 ng/ml; nonsmokers, <10 ng/ml). The latter group comprised of life-time nonsmokers and ex-smokers. Using those with serum cotinine < 10 ng/ml and best FEV_1 (quartile 4) as the referent, we performed multiple logistic regression analyses. To this model, we added age, sex, race and body mass index as covariates. The latter was divided into quintiles and expressed as kg/m^2. We also performed similar analyses using serum leukocytes, platelets, and fibrinogen as the dependent variables. To test the robustness of the findings, we also conducted multiple linear regression analyses using the same covariates employed in the multiple logistic regression models. CRP could not be analyzed using multiple linear regression techniques, as it was non-normally distributed and values below 2.1 mg/L were undetectable. All tests

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were 2-tailed in nature and were performed using SAS version 8.2 and SUDAAN Release 8.0 (Research Triangle Park, NC). Analyses were performed with and without NHANES III weights. As the results were similar, we presented data from the unweighted analysis for parsimony.

3.3 RESULTS

The baseline characteristics of the study population are summarized in **Table 3.1**. Quartile 1 (lowest FEV_1) contained more Caucasians, more active smokers, and more men than quartile 4 (highest FEV_1). Individuals in quartile 1 tended to be older than those in quartile 4. There were no significant differences in the body mass index (BMI) across the quartiles. Crudely, those in quartile 1 had higher leukocyte, fibrinogen and CRP levels than those in quartile 4 **(Table 3.1)**. Adjustments of various factors including age, sex, BMI, race, and smoking status made little difference to the overall results **(Table 3.2)**. There was a clear gradient in the levels of leukocytes, fibrinogen, and CRP across the FEV_1 quartiles, such that those in quartile 4 had the lowest values, while those in quartile 1 had the highest values for both active smokers and non-smokers **(Table 3.2)**. More importantly, there appeared to be an additive effect between serum cotinine values and FEV_1 quartile groups. For instance, using those in quartile 4 (best FEV_1) and with serum cotinine < 10 ng/ml (non-smokers) as the referent group, active smoking (i.e. serum cotinine $\geq$ 10 ng/ml) was associated with an odds ratio, OR, of 1.63 for having elevated CRP. The OR for quartile 1 (worst FEV_1) but with serum cotinine < 10 ng/ml was 2.27. However, in quartile 1, for those who had a

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serum cotinine $\geq$ 10 ng/ml, the OR was 3.31, indicating an additive effect of reduced FEV_1 and active smoking on CRP levels (**Figure 3.1**). Similar findings were observed for serum leukocytes, and fibrinogen. Consistently, those in quartile 1 and with serum cotinine $\geq$ 10 ng/ml had the highest odds of having elevated systemic markers of inflammation. When we restricted the above analysis by using 4,291 active smokers and ex-smokers (as indicated on the participant's history), the results were similar to the main analysis (**Table 3.3**). Finally, the use of multiple linear regression technique yielded similar results to those obtained in logistic regression models (**Table 3.4**). In all cases, there was an additive effect of active cigarette smoking and reduced FEV_1 on markers of systemic inflammation.

3.4   DISCUSSION

The most important and novel finding of this study was that active cigarette smoking and poor FEV_1 had an additive effect on the markers of systemic inflammation. Individually, active smoking (as defined by serum cotinine $\geq$10 ng/mL) and reduced FEV_1 (as defined by FEV_1 $\leq$ 83.2% of predicted) were associated with 1.6 and 2.3 increased odds of elevated CRP, respectively. For individuals with both of these risk factors, the odds increased by 3.3 fold, indicating an additive response. Similar findings were also observed for serum leukocytes and fibrinogen. These findings are consistent with previous observations, demonstrating that cigarette smoking contributes significantly to persistent low-grade systemic inflammation in susceptible individuals.[10, 11] As well,

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our findings suggest that independent of active smoking, poor lung function is an important risk factor for low-grade systemic inflammation.

The mechanism(s) for the latter observation are not entirely clear. However, there is compelling evidence to suggest that disorders such as chronic obstructive pulmonary disease, the most common cause of reduced FEV_1 in the general population, have a strong inflammatory component in the airways,[18, 19] which persists even after smoking cessation.[20, 21] It is highly plausible that this inflammatory component may "spill over" into the systemic circulation, leading to a state of low-grade systemic inflammation. [22-25] The intensity of the systemic inflammation is further amplified by active smoking.

The present study has several strengths. First, it was conducted using a large representative sample of the U.S. population, providing sufficient statistical power to evaluate the potential interaction between active cigarette smoking and reduced FEV_1 on different markers of systemic inflammation. Second, due to very nature of NHANES III, we were able to use a validated biochemical marker of tobacco exposure, serum cotinine, thereby minimizing smoke exposure misclassification, seen in studies that exclusively rely on patient history. Third, we were able to control for important confounders such as age, sex, race and BMI, making our findings reliable and valid.

There were several limitations to the current study. First, because NHANES III was a cross-sectional study, the temporal nature of the relationship between cigarette smoking, reduced lung function and elevated inflammatory markers is uncertain. It is plausible though unlikely that systemic inflammation may lead to

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reduced lung function and not the other way around. Future prospective studies are needed to better understand the temporal relationships. Second, although the study adjusted for many factors, due to the observational nature of the study, residual confounding by these and other variables might still play a role.

In conclusion, our study findings suggest an additive effect of active cigarette smoking and reduced FEV_1 on various markers of systemic inflammation. Since persistent low-grade systemic inflammation is associated with various complications, including cachexia, cardiovascular events and mortality, our findings may explain why certain disorders, such as COPD, are associated with these systemic complications and why active smoking accelerates the risk of such complications in these patients. These data further emphasize the value of smoking cessation in patients with reduced lung function. However, our findings also suggest that smoking cessation alone helps but may be insufficient to fully normalize blood levels of CRP and other inflammatory biomarkers if compromised lung function has already developed.

3.5 TABLES

Table 3.1. Characteristics of Participants by Quartiles of FEV_1 % Predicted

	Quartiles of FEV_1 % Predicted				
	4th quartile >107.1%	3rd quartile 95.6–107.1%	2nd quartile 83.2–95.6%	1st quartile ≤ 83.2%	P* values
Age, year	58.7±14.0	57.6±13.3	59.3±13.0	64.8±12.6	<0.001
Male sex, %	35.1	45.5	52.9	59.5	<0.001
White, %	61.6	75.3	78.7	79.0	<0.001
Current smoker, %	16.0	19.2	23.2	32.0	<0.001
BMI, kg/m²	27.6±5.3	27.8±5.3	27.9±5.6	27.6±6.0	0.709
FEV_1, L	2.98±0.83	2.83±0.76	2.58±0.67	1.91±0.64	<0.001
FEV_1 % predicted	117.7±9.6	101.1±3.3	89.7±3.5	68.2±13.6	<0.001
FVC, L	3.76±1.04	3.66±1.02	3.46±0.95	2.89±0.93	<0.001
FVC % predicted	117.9±11.5	104.2±8.7	95.1±9.4	80.7±14.8	<0.001
Pack-years	17.5±17.0	20.2± 21.9	24.6±25.5	35.2±34.0	<0.001
Leukocytes, $\times10^9$/L	6.7±2.0	7.1±2.7	7.2±2.1	7.6±2.5	<0.001
Elevated leukocyte level, %[†]	10.2	13.7	16.4	21.2	<0.001
Platelet, $\times10^9$/L	270.4±69.3	271.3±72.5	269.9±74.4	268.7±77.3	0.477
Elevated platelet level, %[†]	14.5	15.0	14.9	15.8	0.239
Fibrinogen, g/L	3.05±0.81	3.06±0.86	3.14±0.86	3.34±0.94	<0.001
Elevated fibrinogen level, %[†]	11.6	12.7	14.3	21.1	<0.001
Serum CRP[‡], mg/L	3.0	3.3	3.5	4.0	<0.001
Elevated CRP level, %[†]	31.5	35.6	39.1	48.3	<0.001

Continuous variables are shown as mean ± SD.

* 1st quartile compared with the 4th quartile.

† Elevated levels of serum leukocyte, platelet, and fibrinogen were defined as ≥ 85th percentile of each inflammatory marker. Elevated CRP level was defined as a value ≥2.2 mg/L (see methods).

‡ Geometric mean.

Abbreviations: CRP, C-reactive protein; BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.

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Table 3.2. Odds Ratios and 95% Confidence Intervals for Elevated Blood Leukocyte, Platelet, Fibrinogen and C-Reactive Protein by Quartiles of FEV_1 % Predicted and Serum Cotinine Levels[*][‡]

		Quartiles of FEV_1 % Predicted			
		4th quartile >107.1%	3rd quartile 95.6–107.1%	2nd quartile 83.2–95.6%	1st quartile ≤ 83.2%
Leukocytes[†]	cotinine<10 ng/mL	1 (reference)	1.41 (1.10-1.81)	1.50 (1.17-1.93)	2.36 (1.84-3.03)
	cotinine≥10ng/mL	3.40 (2.47-4.69)	3.77 (2.80-5.07)	4.97 (3.79-6.52)	5.11 (3.97-6.59)
Platelets[†]	cotinine<10 ng/mL	1 (reference)	1.10 (0.89-1.36)	1.16 (0.94-1.44)	1.50 (1.20-1.88)
	cotinine≥10 ng/mL	1.14 (0.83-1.56)	1.40 (1.05-1.88)	1.50 (1.14-1.96)	1.60 (1.25-2.05)
Fibrinogen[†]	cotinine<10 ng/mL	1 (reference)	1.20 (0.96-1.52)	1.36 (1.08-1.71)	1.84 (1.47-2.31)
	cotinine≥10 ng/mL	1.58 (1.13-2.21)	2.02 (1.47-2.76)	2.03 (1.52-2.70)	2.96 (2.32-3.78)
CRP[†]	cotinine<10 ng/mL	1 (reference)	1.31 (1.12-1.54)	1.56 (1.32-1.84)	2.27 (1.92-2.70)
	cotinine≥10 ng/mL	1.63 (1.28-2.09)	2.12 (1.69-2.67)	2.35 (1.90-2.92)	3.31 (2.73-4.02)

* All values have been adjusted for age, sex, race and BMI and are presented as odds ratios (95% confidence intervals). A serum cotinine level ≥10 ng/mL indicates active smoking. Participants in the 4th FEV_1 quartile group with a serum cotinine Level <10 ng/mL constitute the reference category.

‡ Please see page 52A for relevant modification.

† Elevated leukocytes, platelets, and fibrinogen were defined as ≥ 85th percentile of each inflammatory marker. Elevated CRP was defined as a value ≥2.2 mg/L (see Methods).

Abbreviation: CRP, C-reactive protein; FEV_1, forced expiratory volume in one second.

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Table 3.3. Odds Ratios and 95% Confidence Intervals for Elevated Blood Leukocytes, Platelet, Fibrinogen and C-Reactive Protein by Quartiles of FEV_1 % Predicted and Serum Cotinine Levels among Current and Former Smokers*

		Quartiles of FEV_1 % Predicted			
		4th quartile >103.8%	3rd quartile 92.1–103.8%	2nd quartile 78.9–92.1%	1st quartile ≤ 78.9%
Leukocytes[†]	cotinine<10 ng/mL	1 (reference)	1.39 (0.90-2.12)	1.53 (0.99-2.35)	2.82 (1.88-4.24)
	cotinine≥10ng/mL	3.32 (2.19-5.06)	4.81 (3.24-7.14)	5.07 (3.46-7.43)	5.04 (3.45-7.38)
Platelets[†]	cotinine<10 ng/mL	1 (reference)	1.05 (0.75-1.47)	1.38 (0.98-1.93)	1.57 (1.11-2.23)
	cotinine≥10 ng/mL	1.09 (0.76-1.56)	1.34 (0.95-1.88)	1.49 (1.07-2.06)	1.48 (1.07-2.05)
Fibrinogen[†]	cotinine<10 ng/mL	1 (reference)	1.18 (0.82-1.71)	1.39 (0.97-1.99)	1.63 (1.15-2.33)
	cotinine≥10 ng/mL	1.73 (1.17-2.56)	1.84 (1.25-2.70)	2.09 (1.47-2.99)	2.64 (1.89-3.68)
CRP[†]	cotinine<10 ng/mL	1 (reference)	1.48 (1.15-1.90)	1.59 (1.22-2.05)	2.75 (2.11-3.57)
	cotinine≥10 ng/mL	1.85 (1.40-2.44)	2.18 (1.66-2.86)	2.64 (2.04-3.43)	3.44 (2.67-4.44)

* All values have been adjusted for age, sex, race and BMI and are presented as odds ratio (95% confidence interval). Participants in the 4th FEV_1 quartile group with a serum cotinine Level <10 ng/mL constitute the reference category.

† Elevated leukocytes, platelets, and fibrinogen were defined as ≥ 85th percentile of each inflammatory marker. Elevated CRP was defined as a value ≥2.2 mg/L (see Methods).

Abbreviation: CRP, C-reactive protein; FEV_1, forced expiratory volume in one second.

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Table 3.4. The Impact of FEV_1 % Predicted and Serum Cotinine on the Blood Levels of Leukocyte, Platelet, and Fibrinogen Based on Multiple Linear Regression*

		Quartiles of FEV_1 % Predicted			
		4th quartile >107.1%	3rd quartile 95.6–107.1%	2nd quartile 83.2–95.6%	1st quartile ≤ 83.2%
Leukocytes ($\times 10^9$/L)	cotinine<10 ng/mL	0 (reference)	0.29 (0.13-0.46)	0.29 (0.12-0.46)	0.75 (0.57-0.93)
	cotinine≥10 ng/mL	1.04 (0.78-1.29)	1.21 (0.96-1.45)	1.53 (1.31-1.76)	1.58 (1.38-1.79)
Platelets ($\times 10^9$/L)	cotinine<10 ng/mL	0 (reference)	3.30 (-1.91-8.50)	4.36 (-0.97-9.69)	8.14 (2.48-13.80)
	cotinine≥10 ng/mL	3.15 (-4.90-11.19)	7.70 (0.07-15.32)	9.94 (2.83-17.04)	14.34 (7.90-20.77)
Fibrinogen (g/L)	cotinine<10 ng/mL	0 (reference)	0.06 (-0.003-0.12)	0.10 (0.04-0.17)	0.23 (0.16-0.30)
	cotinine≥10 ng/mL	0.18 (0.09-0.28)	0.19 (0.10-0.29)	0.27 (0.19-0.36)	0.40 (0.32-0.48)

* All values have been adjusted for age, sex, race and BMI and are presented as regression coefficients (95% confidence intervals). Participants in the 4th FEV_1% predicted quartile group with a serum cotinine Level <10 ng/mL constitute the reference category.

Abbreviation: FEV_1, forced expiratory volume in one second.

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3.6   FIGURES

Figure 3.1. The Impact of Active Cigarette Smoking and Reduced $FEV_1\%$

Predicted on Circulating C-Reactive Protein Levels
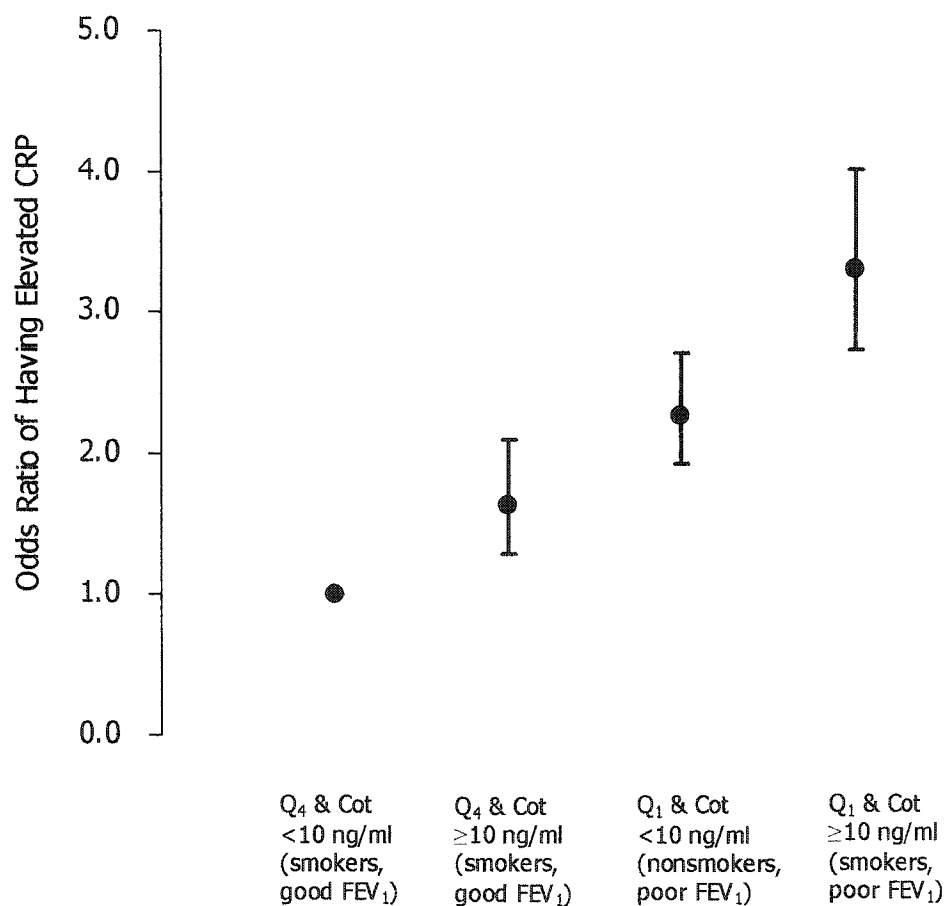
Serum cotinine ≥ 10 ng/mL indicates active cigarette smoking.

Abbreviations: CRP, C-reactive protein; Cot, serum cotinine (ng/mL); Q, Quartile

Based on $FEV_1\%$ Predicted (Q_1, lowest $FEV_1\%$ predicted; Q_4, highest $FEV_1\%$ predicted).

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Appendix

According to the suggestion of the Final Oral Examining Committee, I introduced the interaction term of predictive $FEV_1\%$ and serum cotinine (both were used as continuous variables) to the logistic regression model to examine the effect of the interaction on the level of systemic inflammatory markers (Log odds $= \alpha + \beta_1$ cotinine $+ \beta_2$ $fev_1\%pred + \beta_3$ cotinine$\times fev_1\%pred + \cdots$). The results indicate that the effect of the interaction was not statistically significant for each inflammatory marker (leukocyte, $p = 0.526$; platelet, $p = 0.137$; fibrinogen, $p = 0.407$; C-reactive protein, $p = 0.853$). Therefore, the model used in our initial analysis (see chapter 3) was appropriate.

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Chapter 4

Effects Of Inhaled Corticosteroids On Airway

Inflammation In Stable Chronic Obstructive Pulmonary

Disease: A Systematic Review And A Meta-Analysis

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4.1 INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive and irreversible airflow limitation, likely in response to the harmful effects of environmental irritants such as tobacco exposure.[1] Increasingly, COPD is being recognized as an inflammatory disorder of the airways. COPD airways have demonstrated increased numbers of neutrophils, macrophages, T lymphocytes and other pro-inflammatory cells.[1-3] Importantly, the severity of airflow limitation is associated with the intensity of the airway inflammation such that those with the most severe disease have the highest concentrations of these inflammatory cells, especially for neutrophils.[4-6] It is, therefore, hypothesized that some (if not all) of these inflammatory cells may, in part, be responsible for the ongoing tissue remodeling and destruction, which are the hallmarks of COPD.[1, 2]

Since airway inflammation may be at the heart of COPD pathogenesis, some have speculated that inhaled corticosteroids, which are potent but non-specific anti-inflammatory agents, may modify the inflammatory process and, in turn, improve lung function and health outcomes of COPD patients. In vitro and in vivo studies have demonstrated that inhaled corticosteroids can, indeed, downregulate certain aspects of the inflammatory cascade;[7, 8] however, it remains controversial whether these effects can be observed in the COPD patients.[9, 10] To date, the clinical studies, addressing this issue, have produced heterogeneous findings. However, all of these studies had relatively small sample sizes, raising the possibility that none of these studies by themselves had sufficient statistical power to detect subtle but potentially relevant changes in the inflammatory

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responses to inhaled corticosteroids. We, therefore, conducted a systematic review and a meta-analysis to determine whether inhaled corticosteroids do or do not suppress airway inflammation in patients with stable COPD.

4.2 METHODS

4.2.1 Search for Relevant Studies

MEDLINE (1966-2003), EMBASE (1980-2001), CINAHL (1982-2003), and the Cochrane Databases were searched for randomized, controlled clinical trials that used induced sputum to evaluate the effects of inhaled steroids on airway inflammation in stable COPD. The search was restricted on articles published in the English language, using human participants. Subject headings included disease-specific search terms (lung diseases, pulmonary diseases, airway obstruction, obstructive pulmonary disease, chronic obstructive pulmonary disease, bronchitis, emphysema, pulmonary emphysema, mediastinal emphysema, or subcutaneous emphysema), drug-specific search terms (glucocorticosteroids, corticosteroids, beclomethasone, budesonide, fluticasone, or triamcinolone), and laboratory method-specific search terms (biopsy, bronchoalveolar lavage, or sputum). We also scanned the reference lists of retrieved articles to supplement the electronic searches. We contacted the primary authors for additional data and/or clarification of data.

4.2.2 Study Selection and Data Abstraction

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The primary objective of this meta-analysis was to compare the changes in sputum inflammatory indices among stable COPD patients before and after treatment with inhaled corticosteroids, using the control group as the referent. The inflammatory indices included total inflammatory cell count, neutrophils, macrophages, eosinophils, lymphocyte, epithelial cells, and interleukin-8. Studied that used oral corticosteroids or that did not report on sputum inflammatory indices were excluded. From each selected article, two investigators abstracted the following baseline information: the source of data, study design, inclusion and exclusion criteria, concomitant drugs, demographics of study participants including sample size, age, sex, current smoking status, pack-years of smoking history, predicted forced expiratory volume in one second (FEV_1), the ratio of FEV_1 to forced vital capacity (FVC), reversibility with inhaled bronchodilator, the specific brand of inhaled corticosteroids as well as the dose, and the duration of therapy. Any questions or discrepancies were resolved through iteration and consensus.

4.2.3 Statistical Methods

To reflect both different inflammatory levels at baseline and different treatment effects in treated group and control group, we first calculated the mean differences and their standard deviations between after and before treatment in treated group and control group, respectively. Then we compared the mean differences and standard deviations in treated groups with that in control groups to determine the effects of inhaled corticosteroids on various inflammatory mediators in the sputum. To accommodate differences in laboratory techniques

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and the units of measurement across original studies, we used standardized mean difference to conduct the comparisons between treated and control groups. Standardized mean difference was derived by dividing the mean difference of inflammatory cell count between treated and control groups of each study by the pooled standard deviation of two groups.[11] For each outcome, we tested the heterogeneity of results across original studies, using a Cochran Q test. If significant heterogeneity was present ($p < 0.10$), then a random effects model, which assigns a weight to each study that is based on both individual study variance and between-study variance, was employed to pool the separate results together. In the absence of significant heterogeneity, a fixed effect model was used.[12] All analyses were conducted using Review Manager version 4.1 (Revman; The Cochrane Collaboration, Oxford, England) and were two-tailed in nature.

4.3 RESULTS

A summary of the search strategy is shown in **Figure 4.1**. The original search yielded 155 and 63 citations in MEDLINE and EMBASE, respectively. CINAHL and the Cochrane Databases did not contribute to the search results. The abstracts of these articles were selected and reviewed. Of these, 25 articles were retrieved for detailed review. This process left 6 original studies meeting the inclusion and exclusion criteria, which were used for the analyses.[13-18] Baseline Information concerning study designs is summarized in **Table 4.1**. The relevant demographic data are summarized in **Table 4.2**. All 162 patients were current smokers or ex-smokers with postbronchodilator $FEV_1 < 70\%$ predicted, FEV_1 to FVC

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ratio <0.7, and reversibility with bronchodilator of <15%. The medications used included budesonide, beclomethasone dipropionate, and fluticasone propionate with duration, which ranged from 2 to 12 weeks and doses, which ranged from 0.8 to 1600 mg/day.

After treated with inhaled steroids, total inflammatory cell counts decreased. Overall, the standardized mean difference between steroid and control groups was -0.43 units (95% confidence interval, CI, -0.75 to -0.11), indicating that inhaled corticosteroids reduced total number of inflammatory cells compared with control (**Figure 4.2**). Homogeneity was present across the 6 studies (test for heterogeneity, p= 0.35).

Among differential cell counts, inhaled corticosteroids had a salutary effect on neutrophil counts in the sputum. As compared with the control group, the standardized mean difference in those treated with inhaled corticosteroids was -2.16 units (95% CI, -3.81 to -0.50) (**Figure 4.3**). There was some heterogeneity in the results across the studies (test for heterogeneity, p<0.001). Inhaled corticosteroids also reduced the lymphocyte counts in the sputum (standardized mean difference, -0.39 units, 95% CI, -0.74 to -0.05; test for heterogeneity, p=0.58) (**Figure 4.4**). These medications were also effective in reducing epithelial cell counts compared with the controls (standardized mean difference, -0.51 units, 95% CI, -0.98 to -0.05; test for heterogeneity, p=0.20) (**Figure 4.5**). There was an insignificant trend towards reducing eosinophil counts in the sputum with inhaled corticosteroid therapy (standardized mean difference, -0.28 units, 95% CI, -0.62 to 0.07; test for heterogeneity, p=0.22) (**Figure 4.6**). Inhaled

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corticosteroids did not appear to have any significant effect on macrophage concentrations in the sputum (standardized mean difference, -0.02 units, 95% CI, -0.34 to 0.29; test for heterogeneity, p=0.65)(**Figure 4.7**). Inhaled corticosteroids did not have significant effects on sputum interleukin-8 (IL-8) levels (standardized mean difference, -0.22 units; 95% CI, -0.77 to 0.32; test for heterogeneity, p=0.84).

After treatment with inhaled steroids, lung function was improved slightly but neither the improvement in FEV_1 nor FVC reached statistical significance. For predicted FEV_1, the overall standardized mean difference was 0.26 units, 95% CI, -0.06 to 0.57 (test for heterogeneity, p=0.62) (**Figure 4.8**). For predicted FVC, the overall standardized mean difference was 0.31 units; 95% CI, -0.09 to 0.70 (test for heterogeneity, p=0.23) (**Figure 4.9**).

4.4 DISCUSSION

By combining data across these clinical studies, we had enlarged statistical power to demonstrate a salutary effect of moderate to high doses of inhaled corticosteroids on some inflammatory indices in the sputum of patients with stable COPD. Over a short term treatment, these medications reduced neutrophil, lymphocyte and epithelial cell counts in the sputum of stable COPD patients. They had smaller (and insignificant) effect on sputum eosinophils and IL-8. They had little effect on sputum macrophages.

Although corticosteroids delay neutrophil apoptosis and may increase neutrophil survival,[19, 20] they also have significant inhibitory action on neutrophil

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performance, likely through the annexin-I (lipocortin-1) pathways. Corticosteroids interfere with neutrophil chemotaxis, adhesion, transmigration, oxidative bursts, and phagocytosis, thereby downregulating the overall inflammatory cascade.[7, 8, 21] Llewellyn-Jones and co-workers, for example, showed that 4 weeks of inhaled fluticasone therapy significantly reduced sputum chemotactic activity for neutrophils and increased its elastase inhibitory capacity in patients with well-characterized COPD.[22] These data suggest that inhaled corticosteroids can reduce recruitment of neutrophils to the airways of COPD patients, thereby lowering the overall concentration of these cells in COPD airways.

Superficially, the present data appear to be inconsistent with the known effects of corticosteroids in general on eosinophils. Many experiments have shown that eosinophils are exquisitely sensitive to corticosteroids. The current data, however, suggest otherwise. In the patients with stable COPD, the sputum eosinophil count was only marginally elevated.[6, 23, 24] This could have introduced a "floor" bias wherein the overall signal to the noise ratio for eosinophils may have been too small to detect subtle but important effects of inhaled corticosteroids on these cells. To a lesser extent, this bias could have been present with the macrophage analysis because macrophages generally account for approximately 20% of the total cell population. In contrast, neutrophils account for 70 to 80% of the total cell population in sputum of COPD patients, making the measurements for these cells more reproducible and valid. Moreover, sputum neutrophil counts appear to be more robust (i.e. greater reproducibility) than sputum eosinophils, macrophages or lymphocytes, making the former measurement much more stable

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than the latter ones[25]. Although by combining data from these published studies we increased the power of the present analysis to detect salient changes in the inflammatory indices of the sputum, we may still have had insufficient power for analyses of cells with a relatively small signal. This may have also been the case for FEV_1. Although there was a trend towards improvement, we did not find a statistically significant effect of inhaled corticosteroids on FEV_1. Larger randomized trials have demonstrated, however, that inhaled corticosteroids significantly improve FEV_1 over the first three to six months of therapy,[26-30] suggesting that for certain endpoints our present analysis still lacked sufficient power. Therefore, the "negative" associations must be interpreted cautiously.

There are certain limitations with the present analysis. Although we used stringent entry criteria in order to minimize the heterogeneity across the selected studies, there were some variations in the study design, the nature of the exposure medications, and the target population evaluated across the original studies. However, the differences in the characteristics of the original studies were relatively small and unlikely to have materially affected the overall findings of the study. We contacted the primary authors to clarify any ambiguities or to obtain additional data, where necessary, to further minimize the "noise" inherent in our analyses. Moreover, to accommodate various differences in the methodology of data collection and laboratory techniques across the original studies, we converted the individual data into standardized mean estimates, which enhanced the comparability of data across the original studies. Meta-analyses, such as this one, are never meant to replace well-conducted large randomized controlled trials, as

the former are more susceptible to bias and methodological constraint than the latter. However, when conducted carefully, they provide a better understanding of the inconsistent data across the original studies.[31] Moreover, by quantitatively combining the results of several small studies, meta-analyses can create more precise and reliable conclusions, which may not be apparent in original studies because of their small sample sizes.

In summary, the present meta-analysis suggests that inhaled corticosteroids can significantly reduce neutrophils and other inflammatory indices in the sputum of patients with stable COPD. Large randomized controlled trials are needed in the future to confirm these early findings and to determine whether these salutary effects persist longer than 3 to 4 months of therapy.

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4.5   TABLES

Table 4.1. Baseline Information on Original Studies Included in the Meta-Analysis

Source	Setting	Design	Inclusion Criteria	Exclusion Criteria	Concomitant drugs	Withdrawal
Confalonieri [13] 1998	Outpatient clinic	Randomised, controlled, open study. The clinical parts of the study was open, but all differential cell counting was in a double blind fashion.	FEV_1/FVC <88% of predicted in men and <89% in women. All patients were current smokers.	Patients who had taken inhaled or oral steroids or had suffered a respiratory tract infection in the previous three months were excluded.	None of the patients was taking theophyllines or long acting β_2 agonists.	No
Culpitt [14] 1999	Outpatient clinic	Randomized, double-blind, placebo-controlled crossover design with a run-in period of 2 weeks.	1. FEV_1/FVC < 0.7; 2. Postbronchodilator FEV_1<85% predicted; 3. Reversibility with inhaled β_2-agonist of <15% of predicted; 4. Smoking history of ≥20 pack-years.	Patients who had taken inhaled or oral steroids or who had suffered an exacerbation of their airway disease in the previous 6 weeks, or patients with any history of asthma or atopy or variability in symptoms were excluded.	Three used albuterol (200 μg bid/d) and ipratropium bromide (40 μg bid/d), one used albuterol when needed.	12 subjects
Keatings [15] 1997	Outpatient clinics in different hospitals	Randomized, single-blind, crossover design. The clinical part of the study was single-blind, but all differential cell counting and assay were in a double blind fashion.	1. FEV_1/FVC< 0.7; 2. FEV_1 <70% predicted; 3. Reversibility with inhaled albuterol of <10% of predicted FEV_1; 4. Smoking history of ≥10 pack-years; 5. Negative results on skin prick testing on 4 common aeroallergens.	Patients who had taken inhaled or oral steroids or who had suffered an exacerbation of their airway disease in the previous 6 weeks, or patients with any history of asthma or variability in symptoms were excluded.	Albuterol was allowed.	2 subjects

Mirici [16] 2001	Outpatient clinic	Randomized, double-blind, placebo-controlled parallel design.	1. FEV_1 < 70% predicted; 2. No self-reported asthma; 3. Reversibility with inhaled terbutaline of <15% of predicted FEV_1; 4. Current smokers.	Long-term treatment with oral or inhaled steroids whihin 6 months of study entry; A respiratory tract infection in previous 3 months; pregnancy or lactation, or presence of other serious systemic diseases.	β_2 –agonists of all kinds, theophylline, and mucolytics were allowed.	10 subjects
Sugiura [17] 2003	NR	Randomized, placebo-controlled parallel design.	FEV_1/FVC < 0.7. All patients were ex-smokers who had stopped smoking for at least 1 year before the study.	A history of allergic rhinitis; positive allergen skin prick tests and RAST assay; a history of periodic wheezing; an improvement in FEV_1 of >12 % predicted or an absolute increase of 200 ml after inhalation of 200 μg salbutamol; had bronchial or respiratory tract infections recently; had taken systemic steroids in the 2 months before the study or inhaled steroids in the month before the study.	NR	No
Yildiz [18] 2000	Outpatient clinic	Randomized, placebo-controlled parallel design with a run-in period of 2 weeks.	1. FEV_1/FVC< 0.7; 2. FEV_1 <70% predicted; 3. reversibility with inhaled albuterol of <10% of predicted; 4. smoking history of ≥10 pack-years.	Patients with any history of asthma or variability in symptoms, and patients who had taken inhaled or oral steroids or had suffered a respiratory tract infection or exacerbation in the previous 6 weeks were excluded.	All patients continued to use salbutamol and ipatropium bromide. 9 patients also used theophylline.	No

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; NR, not reported.

Table 4.2. The Characteristics of COPD Patients at Baseline and Steroid Administration

Source	N	Age (yr)	Men (%)	Current Smokers(%)	Pack-years	FEV_1 (%pred)	Ratio (%)	Reversibility (%)	Drug	Dose (mg/d)	Duration (weeks)
Confalonieri [13]	34	58 (5)	59	100	NR	59.7(37.1)	66.5(4.7)	NR	Beclomethasone	1.5	8
Culpitt [14]	26	43-73	62	69	>20	49.5(16.6)	<70	<15	Fluticasone	1.0	4
Keatings [15]	26	45-78	60	46	>10	35.1(4.7)	<70	<10	Budesonide	1600	2
Mirici [16]	40	53(10)	75	100	26.5(16.1)	62.0(7.4)	NR	<15	Budesonide	0.8	12
Sugiura [17]	18[‡]	70(7)	89	0[*]	NR	1.2(0.4)[†]	<70	<12	Beclomethasone	0.8	4
Yildiz [18]	18	64(7)	78	89	52.0(23.4)	44.5(2.7)	56.8(2.7)	<10	Fluticason	1.5	8

† FEV_1, L.

‡ 6 patients in control group.

* All subjects were ex-smokers and stopped smoking for at least 1 year.

Abbreviations: FEV_1, forced expiratory volume in 1 second; ratio, the ratio of FEV_1 to FVC; Pred, predicted; NR, not reported/not calculable.

4.6 FIGURES

Figure 4.1. Study Selection Process

Search results: N=218
  MEDLINE: n=155
  EMBASE: n=63

Did not meet criteria
or duplicate articles:
n=198

Studies retrieved:
n=20

Oral medication: n=4
No sufficient data: n=10

Studies included in analyses: n=6

72
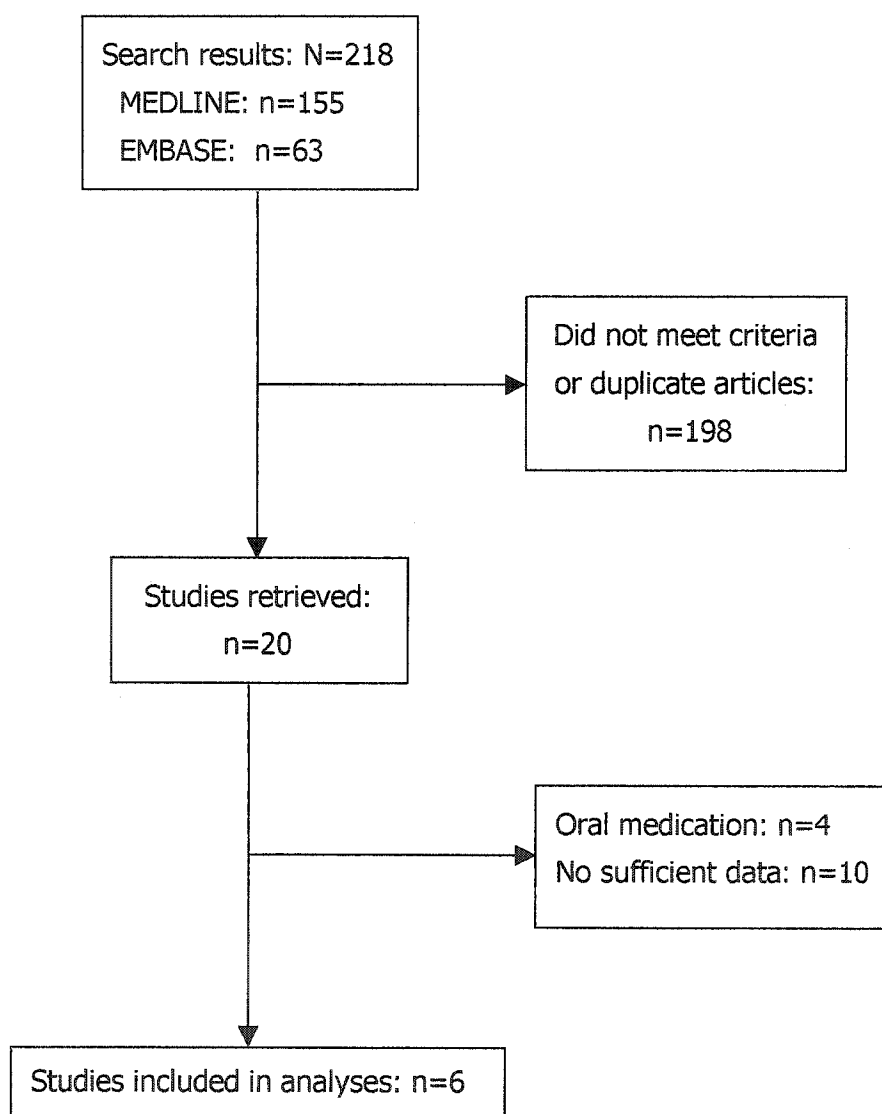
Figure 4.2. Effect of Inhaled Corticosteroids on Total Inflammatory Cell Counts in the Sputum of Stable COPD Patients

Confalonieri [13]

Culpitt [14]

Keatings [15]

Mirici [16]

Sugiura [17]

Yildiz [18]

Pooled Summary

-2.0   -1.5   -1.0   -0.5   0.0   0.5   1.0   1.5   2.0

Standardized Mean Difference (95% CI) of Total Cell Count

← Favors Steroids   Favors Controls →

73
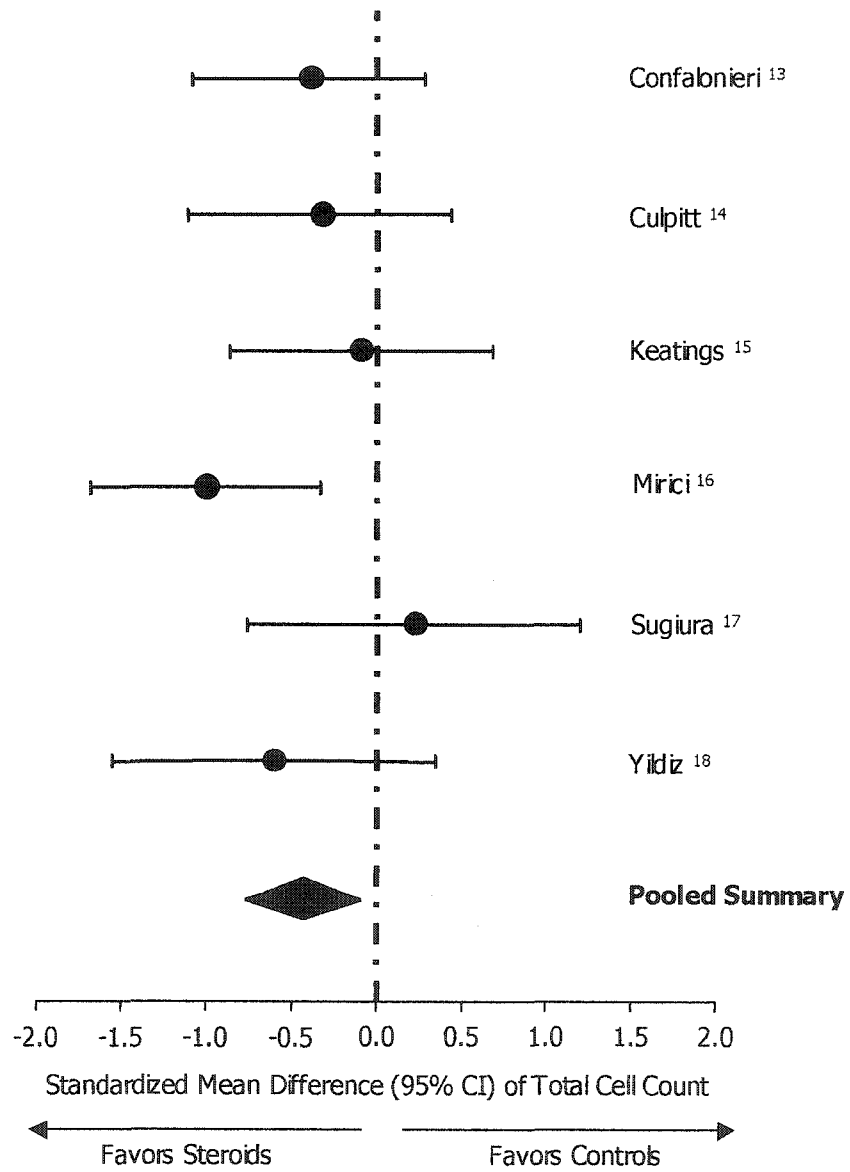
Figure 4.3. Effect of Inhaled Corticosteroids on Neutrophil Counts in the Sputum of Stable COPD Patients
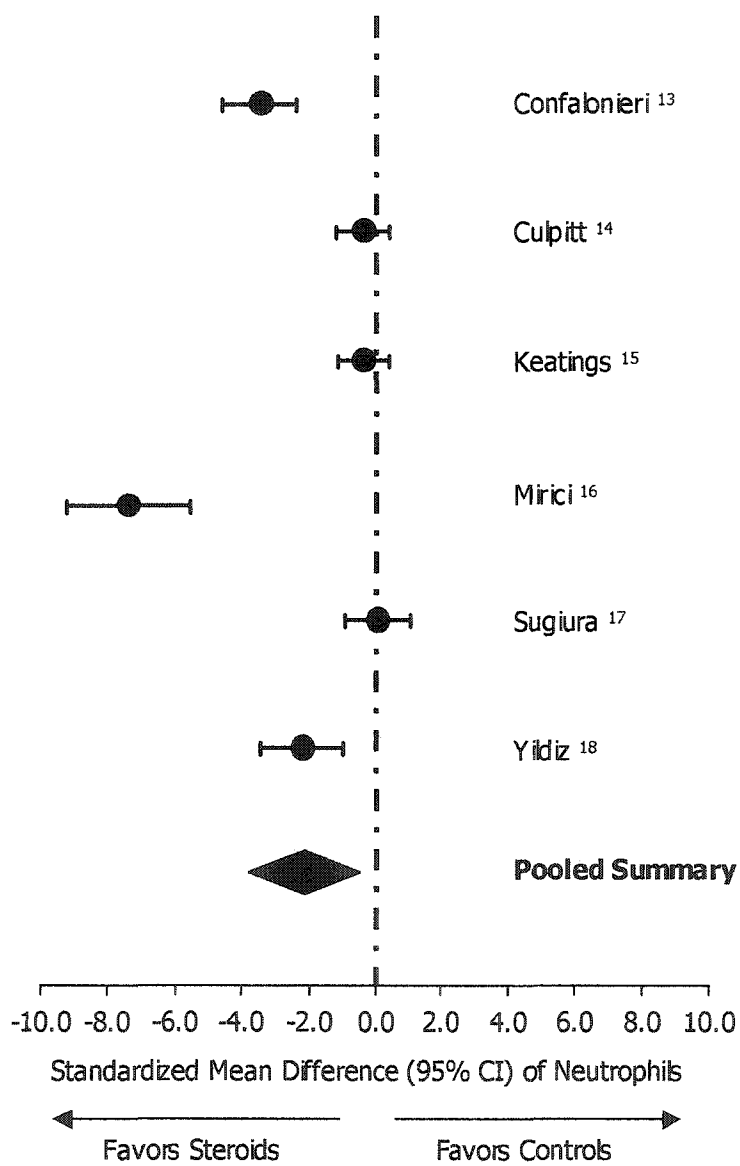
Figure 4.4. Effect of Inhaled Corticosteroids on Lymphocyte Counts in the Sputum of Stable COPD Patients

Confalonieri [13]

Keatings [15]

Mirici [16]

Sugiura [17]

Yildiz [18]

Pooled Summary

-2.0   -1.5   -1.0   -0.5   0.0   0.5   1.0   1.5   2.0

Standardized Mean Difference (95% CI) of Lymphocytes

Favors Steroids   Favors Controls

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Figure 4.5. Effect of Inhaled Corticosteroids on Epithelial Cell Counts in the Sputum of Stable COPD Patients

Confalonieri [13]

Mirici [16]

Pooled Summary

Standardized Mean Difference (95% CI) of Epithelial Cell Counts
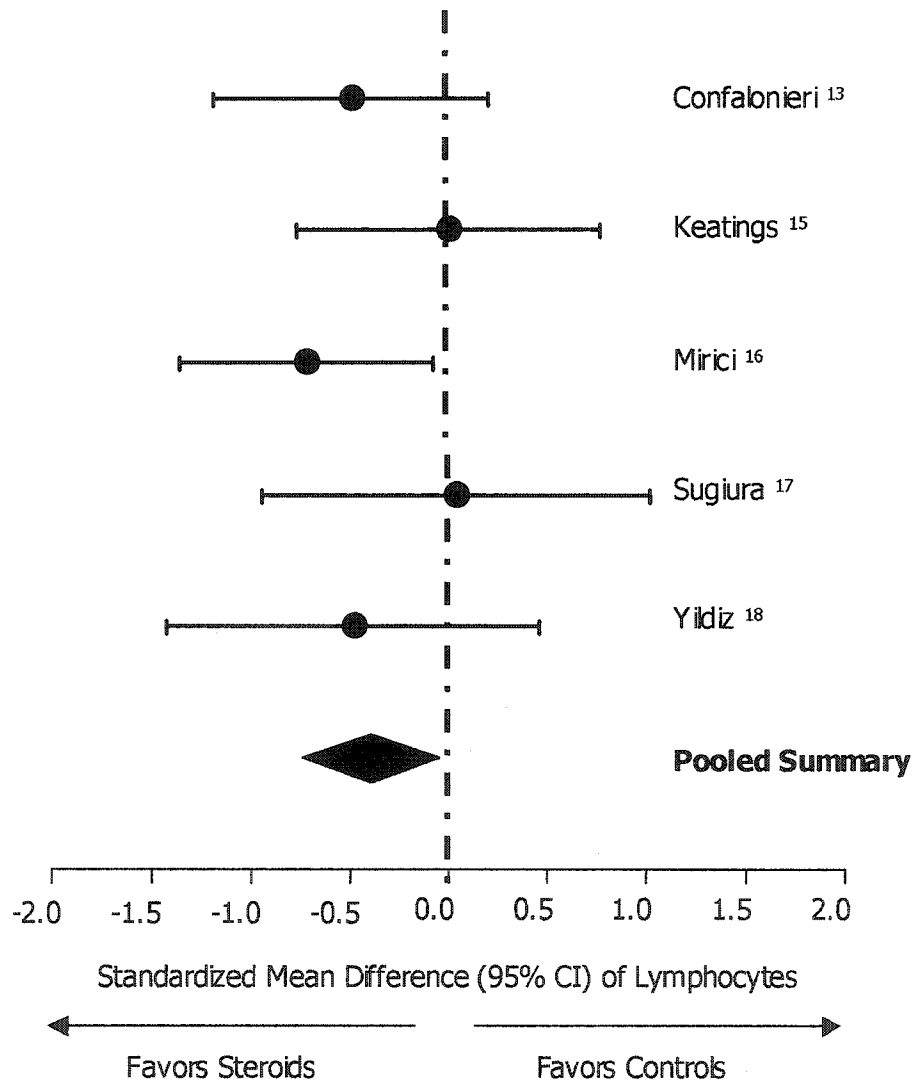
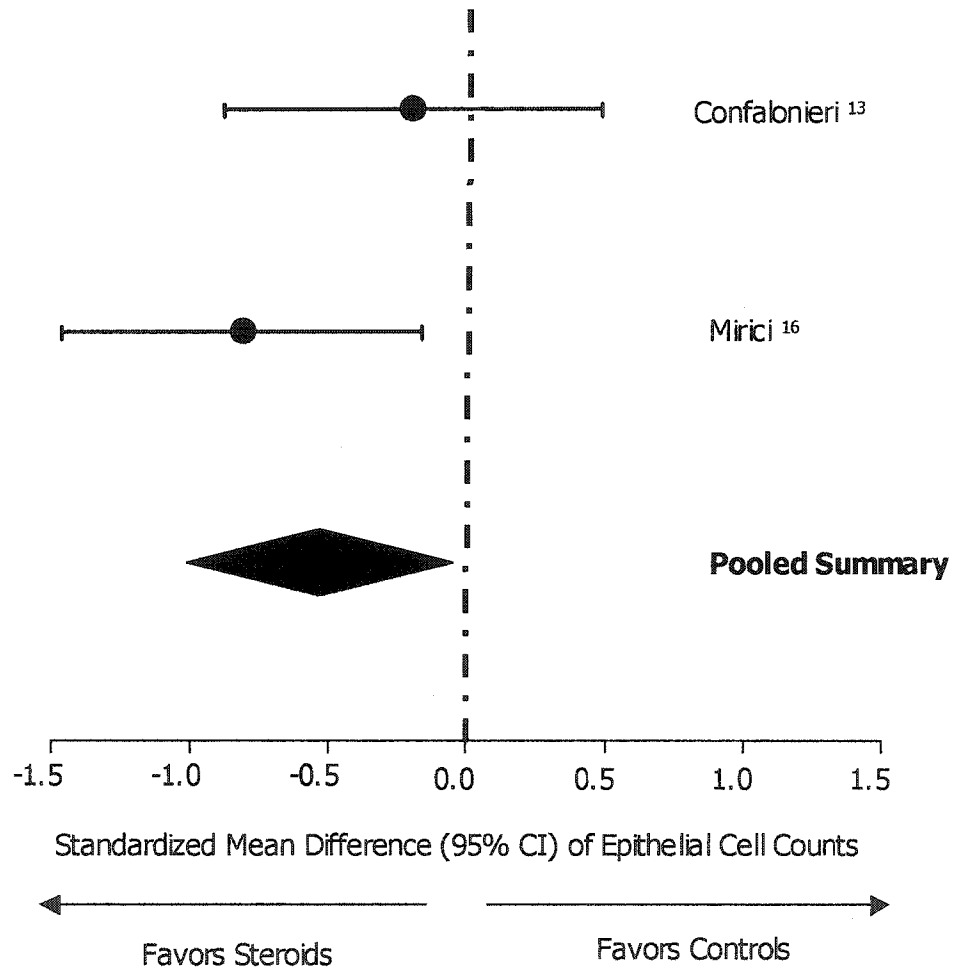Favors Steroids   Favors Controls

Figure 4.6. Effect of Inhaled Corticosteroids on Eosinophil Counts in the Sputum of Stable COPD Patients

Figure 4.7. Effect of Inhaled Corticosteroids on Macrophage Counts in the Sputum of Stable COPD Patients
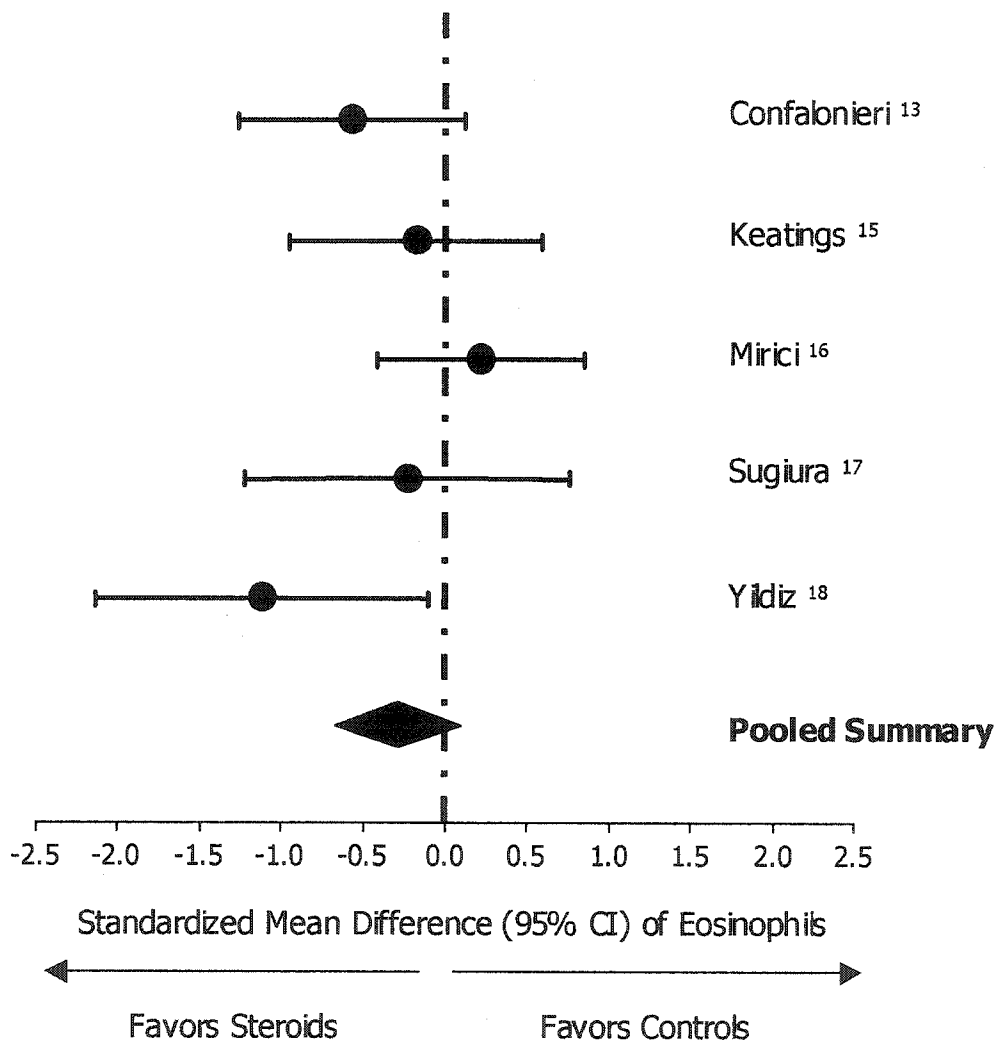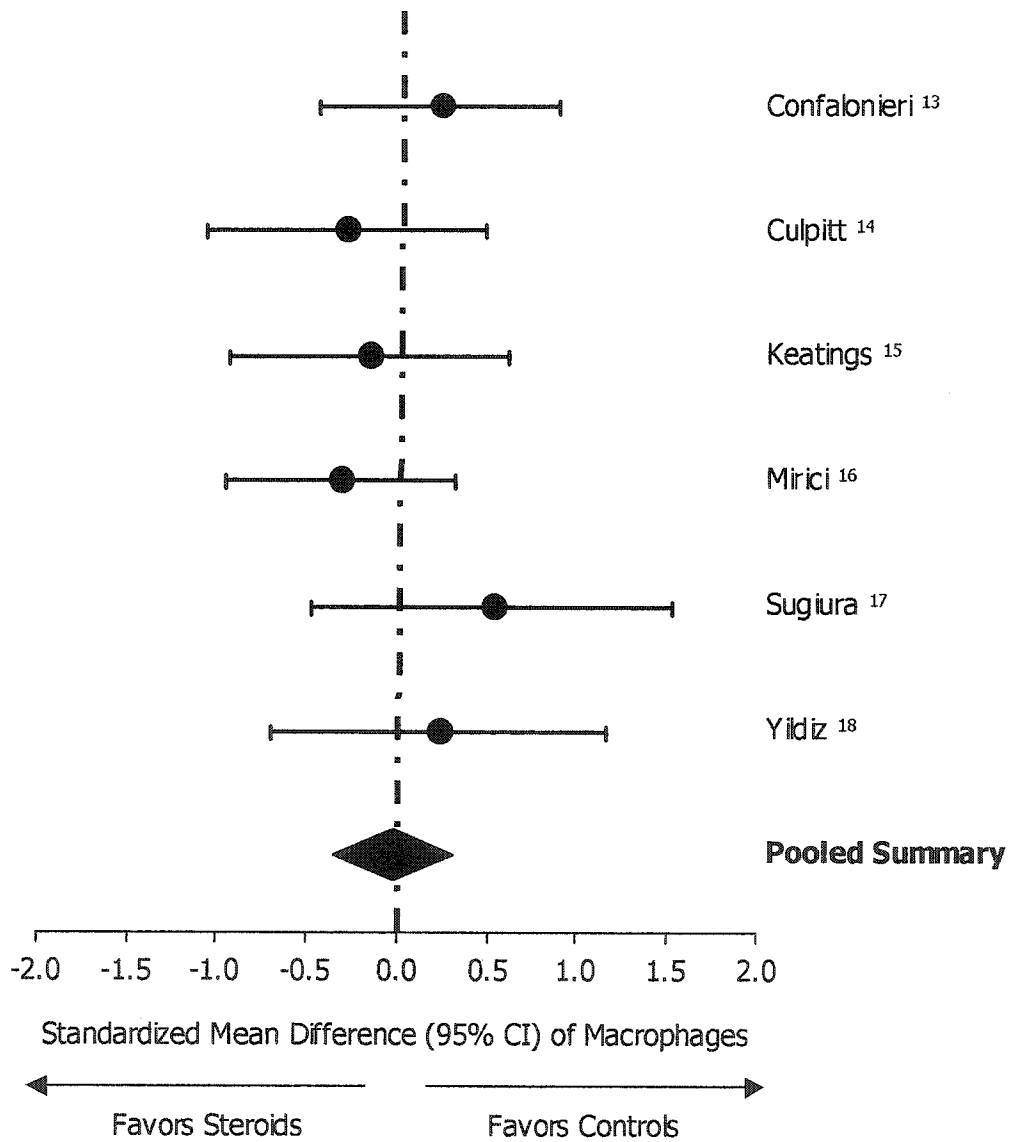
Figure 4.8. Effect of Inhaled Corticosteroids on FEV_1% Predicted of Stable

COPD Patients

Confalonieri [13]

Culpitt [14]

Keatings [15]

Mirici [16]

Sugiura [17]

Yildiz [18]

Pooled Summary

-2.0   -1.5   -1.0   -0.5   0.0   0.5   1.0   1.5   2.0

Standardized Mean Difference (95% CI) of FEV_1

Favors Controls   Favors Steroids

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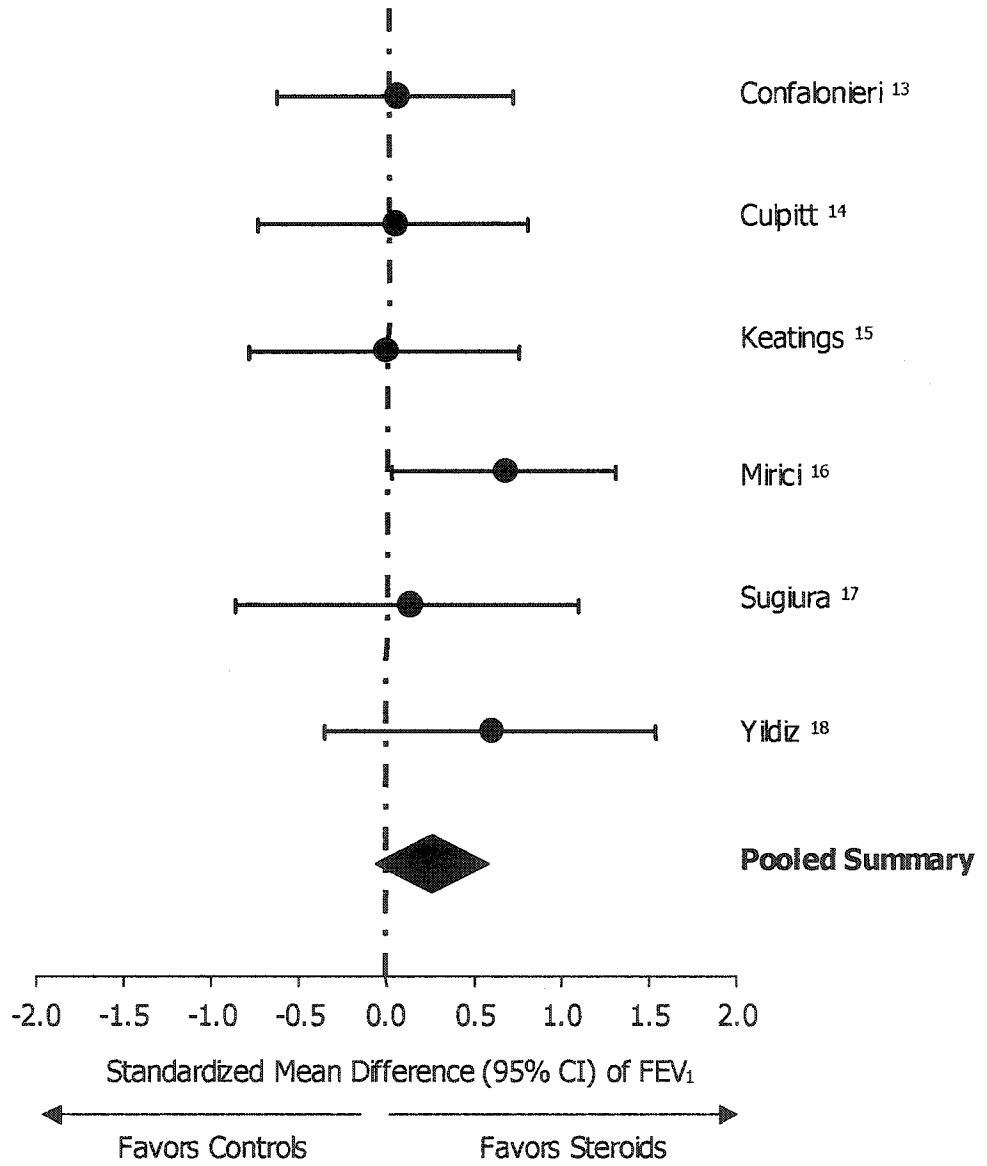
Figure 4.9. Effect of Inhaled Corticosteroids on FVC% Predicted of Stable COPD Patients

Confalonieri [13]

Keatings [15]

Mirici [16]

Pooled Summary

-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

Standardized Mean Difference (95% CI) of FVC

Favors Controls   Favors Steroids
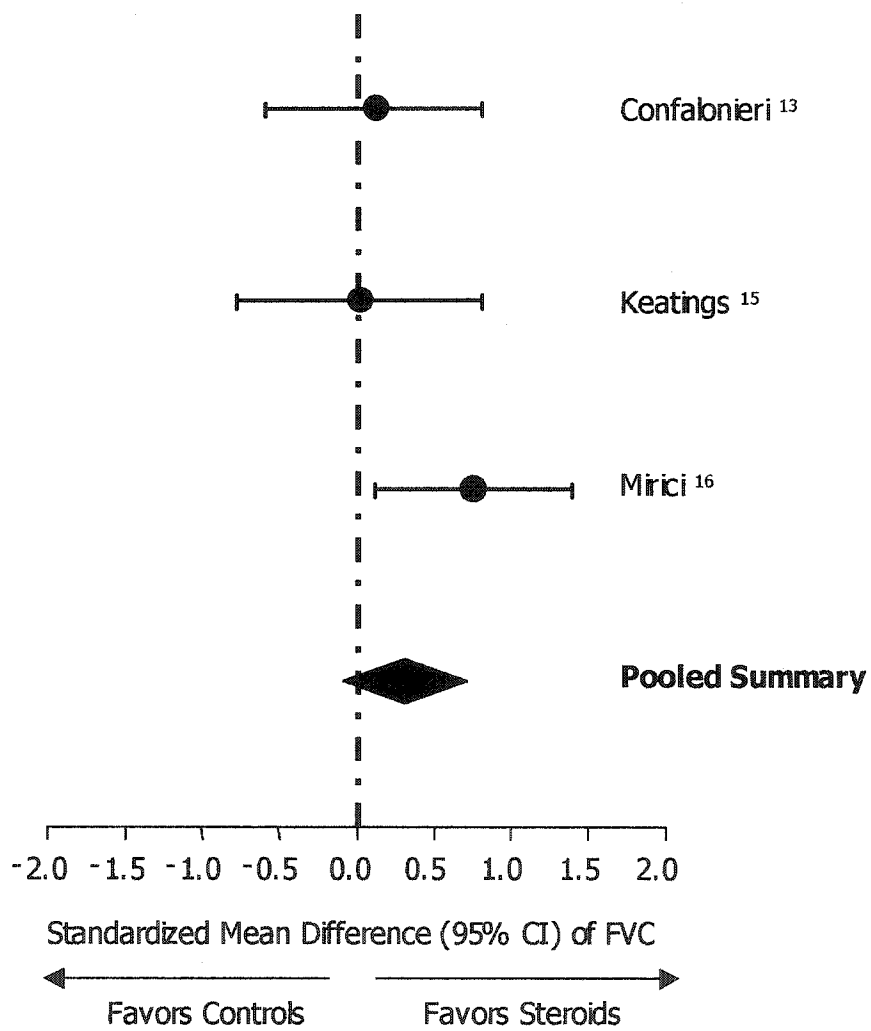
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Chapter 5

General Discussion And Conclusions

5.1 SUMMARY

In Chapter 2 (study #1), I did a systematic review and a meta-analysis to evaluate the association between COPD and systemic inflammation. I identified a total of 14 original articles that met the inclusion and exclusion criteria of the study. There was convincing evidence that COPD (and reduced lung function) is associated with elevated levels of C-reactive protein, fibrinogen, leukocytes and TNF-alpha in the systemic circulation.

Since COPD is associated with systemic inflammation, in Chapter 3 (study #2), I explored whether the relationship between COPD and systemic inflammation was severity-dependent and whether the systemic inflammation associated with COPD could be amplified by active cigarette smoking. Using NHANES 3 data, I found that the relationship between COPD and systemic inflammation was indeed severity dependent, such that those with the worst lung function impairment had the highest levels of CRP and other markers of systemic inflammation, while those with the least impairment had the lowest levels. Moreover, I found that active cigarette smoking further increased the levels of CRP in those with COPD.

Finally, in Chapter 4 (study #3), I sought to determine whether inhaled corticosteroids which are commonly used for the management of COPD, could down-regulate airway inflammation. In this systematic review, I identified six original articles. Although there was some heterogeneity of results, overall, it appeared that inhaled corticosteroids reduced total inflammatory cell and neutrophil count in the sputum of COPD patients.

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5.2 IMPLICATIONS

This project has demonstrated that persistent low-grade systemic inflammation is present in patients with stable COPD, suggesting that COPD is not only a local inflammatory condition in the lungs, but also a systemic inflammatory disorder. The finding may explain why systemic complications, for instance, cardiovascular disease, are commonly observed among patients with stable COPD, particularly among patients with moderate or severe disease.

My project has also shown that cigarette smokers are more likely to have elevated levels of systemic inflammatory markers. Smoking cessation would be expected to reduce systemic inflammation and to decrease incidence of various systemic complications in COPD. However, since impaired lung function is another independent variable significantly associated with elevated levels of systemic inflammatory markers, smoking cessation by itself may be insufficient to fully attenuate the excess occurrence of systemic complications in COPD among those with established lung function impairment.

Among the 6 randomized controlled clinical trials examining the effect of inhaled corticosteroids on pulmonary inflammation in patients with stable COPD, I found that inhaled corticosteroids reduce neutrophils, lymphocytes, and other inflammatory indices in the lungs of stable COPD patients. This finding may at least partly explain why corticosteroid therapy could ameliorate clinical symptom as well as reduce exacerbations, hospitalizations, and mortality rate in patients with stable COPD.

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5.3 FUTURE WORK

Since all relevant studies regarding the association between impaired lung function and systemic inflammation are cross-sectional in nature, the temporal relationships between these two variables remain uncertain. Future prospective studies are necessary to evaluate the temporal relationship between impaired lung function, and systemic inflammation as well as their independent and combined effects on the morbidity and mortality of cardiovascular diseases.

With respect to clinical therapy, since considerable variation of sputum inflammatory cell count is present in stable COPD, large randomized placebo controlled clinical trials with adequate statistical power are needed to confirm the early findings in the study and to reexamine the effects of inhaled corticosteroids on other inflammatory indices such as eosinophils and macrophages. Moreover, the effect of long-term inhaled corticosteroids on systemic inflammation in stable COPD should be examined, which may provide important pathological evidence to evaluate the long term effects of inhaled corticosteroids on stable COPD. Based on the findings of this project, systemic inflammation is a promising therapeutic target for future COPD management.

5.4 CONCLUSIONS

I have found that persistent low-grade systemic inflammation is present among individuals with stable COPD or impaired lung function. Systemic inflammatory level is independently associated with active cigarette smoking and impaired lung function. For active cigarette smokers with impaired lung function,

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the likelihood of having elevated levels of systemic inflammatory markers significantly increases, indicating an additive effect of active cigarette smoking with impaired lung function on systemic inflammation. Inhaled corticosteroids in moderate-to-high doses reduces neutrophils, lymphocytes, and other inflammatory indices in the sputum induced from stable COPD patients, which provided pathologic evidence of clinical improvement after treated with inhaled corticosteroids.