DISSERTATION

AN EPIDEMIOLOGIC EVALUATION OF RISK FACTORS ASSOCIATED WITH ASTHMA SEVERITY AND PHENOTYPES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ASHLEY A. BUSACKER ENTITLED AN EPIDEMIOLOGIC EVALUATION OF RISK FACTORS ASSOCIATED WITH ASTHMA SEVERITY AND PHENOTYPES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

AN EPIDEMIOLOGIC EVALUATION OF RISK FACTORS ASSOCIATED WITH ASTHMA SEVERITY AND PHENOTYPES

Asthma is an inflammatory disorder of the airways characterized by airway hyperresponsiveness, periodic episodes of bronchoconstriction and airway obstruction. Severe asthma accounts for a minority of asthma but utilizes a disproportionate amount of healthcare costs associated with asthma. Severe asthma is increasingly recognized as a very heterogeneous disease, for which further studies are needed to identify risk factors that differentiate severe from non-severe asthma. In addition to being a very heterogeneous disease, asthma – particularly severe asthma – most likely consists of several different phenotypes. Limited epidemiologic studies have been conducted to identify risk factors specific to the development of severe asthma. Factors associated with proposed asthma phenotypes have not been evaluated in a multivariate manner. This cross-sectional study was designed to investigate the risk factors associated with severe asthma, the risk factors associated with specific phenotypes of asthma, and the association between a potential biomarker, C-reactive protein (CRP), and a previously described asthma phenotype, aspirin intolerant asthma. The research project was a collaborative effort between the University of Pittsburgh (Pitt), National Jewish Medical and Research Center (NJMRC), and Colorado State University (CSU). The project utilized data that have been collected from National Institutes of Health funded research studies in the laboratory of Dr. Sally Wenzel (Pitt and NJRMC) and data collected as part

of an electronic chart review. Data from questionnaires, histological, radiological and physiological studies were used to determine univariate associations between these data and asthma severity and then to determine associations between the data and different asthma phenotypes. Multiple logistic regression analysis was then used to evaluate the differences between severe and non-severe asthma, early and late onset asthma, asthma subjects who exhibited air trapping and those who did not, and aspirin intolerant and tolerant asthma with particular attention to CRP levels.

This dissertation research project resulted in several important findings. Gastro-esophageal reflux disease, air flow obstruction, history of pneumonia, history of sinusitis, and atopy were identified as independent factors that differentiated severe from non-severe asthma. A parental history of asthma, duration of asthma, atopy and airway eosinophils were identified as independent factors that differentiated early onset from late onset asthma. Duration of asthma, history of pneumonia, high levels of neutrophils in the airway, air flow obstruction and atopy, were identified as independent risk factors associated with the air trapping phenotype. Increased CRP levels were associated with increased odds of aspirin intolerant asthma. Additionally, forced vital capacity and blood eosinophils were found to be important variables in the relationship between CRP and aspirin intolerance. This investigation found important clinical differences between severe and non-severe asthma that should be further evaluated as risk factors that may give insight into severe-asthma mechanisms to be targeted in asthma treatment. The analysis of asthma phenotypes also yielded important findings. Specifically, whereas early onset asthmatics appear to be a relatively homogeneous group with strong genetic influences and presence of allergic responses, late onset disease is a more heterogeneous

group. The analysis of the air trapping phenotype demonstrated that quantitative CT-determined air trapping in asthmatic subjects identifies a group of individuals with a high risk of severe disease, particularly those with intensive health care utilization. In the asthmatics studied, several independent risk factors for the presence of this phenotype were identified, perhaps most interestingly history of pneumonia, neutrophilic inflammation, and atopy. Lastly, this study provides evidence that C-reactive protein may be elevated in aspirin intolerant subjects and, consequently, that C-reactive protein deserves further study as a potential biomarker for the aspirin intolerant phenotype of asthma.

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DEDICATION

For my family:
My parents, Jacque and Riley Busacker; and my brother Chauncey and his family,
Ashley, Emery and Joshua Busacker

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Chapter 1

Specific Aims and Hypotheses

Introduction

Asthma is an inflammatory disorder of the airways characterized by airway hyperresponsiveness, periodic episodes of bronchoconstriction and airway obstruction. The Centers for Disease Control and Prevention (CDC) and the National Centers for Health Statistics estimate asthma prevalence in the US to be 8%.[1] However, among these patients, there is a wide range of asthma severity. Most asthmatic patients can be adequately controlled with low to moderate doses of medication. However, there are still many patients who have frequent symptoms and exacerbations despite high doses of medication. These severe asthmatic patients are difficult to treat as standard therapies fail to completely control symptoms.[2] Therefore, severe asthma patients are forced to take high doses of medications, which still may not fully control their disease. These patients are at an increased risk of near fatal asthma events and of morbidity from not only their severe asthma but also the medications (namely oral corticosteroids) used to treat the disease.[3-5] Severe asthma likely affects less than 10% of all asthmatics[6] but utilizes a disproportionate amount of healthcare costs associated with the disease, accounting for at least half of the total costs (direct and indirect) for asthma.[7] Severe asthmatics are 15 times more likely to use emergency medical care compared to mild to moderate asthmatics and are 20 more as likely to require hospital admission.[7] Those suffering from severe asthma are likely to be impacted by their disease on a daily basis which leads to a

significantly decreased quality of life compared to mild/moderate asthmatics.[8] Limited epidemiologic studies have been conducted to identify risk factors specific to the development of severe asthma. Additionally, few studies have evaluated the risk factors in a multivariate manner. Studies are needed that can examine a multitude of risk factors while both adjusting for confounding and evaluating effect modification. To complicate matters further, asthma, particularly severe asthma, is a very heterogeneous disease with several different phenotypes. These phenotypes should also be examined in a multivariate manner.

This cross-sectional study was designed to investigate the risk factors associated with severe asthma, the risk factors associated with specific phenotypes of severe asthma, and the association between a potential biomarker, C-reactive protein (CRP), and an already described asthma phenotype, that of aspirin intolerant asthma (AIA). The research project was a collaborative effort between the University of Pittsburgh (Pitt), National Jewish Medical and Research Center (NJRMC) and Colorado State University (CSU). The project analyzed existing and accumulating data that have been collected from National Institutes of Health (NIH) funded research studies in the laboratory of Dr. Sally Wenzel at Pitt and NJRMC.

Specific Aims/Hypotheses

There were several aims of this cross-sectional epidemiologic study, the related hypotheses of which are presented below.

1. The identification and description of the association of various clinical variables with the overall phenotype of severe asthma as compared to the mild/moderate referent group were primary aims of this study.

a. Describe the population of non-severe and severe asthmatics.

b. Determine clinical factors that differentiate severe from non-severe asthma, including the development of a multivariate model based on clinical data collected from NIH studies.

Hypothesis: Decreased lung function, increased airway inflammation, increased duration of asthma, current co-morbid conditions (such as obesity, aspirin sensitivity, sinusitis) are associated with an increased probability of severe asthma. Additionally, a history of pneumonia, history of allergies, and a family history of allergies and/or asthma are associated with increased odds of severe asthma.

2. Since severe asthma is heterogeneous, consisting of several phenotypes, this study aimed to determine if risk factors differ for the development of particular phenotypes – specifically, for a previously described asthma phenotype (based on age at asthma onset), as well as one new phenotype among all asthmatics (those who exhibit air trapping measured quantitatively by multi-detector CT-scan).

a. Determine which risk factors are associated with early onset disease compared to late onset disease, including the development of a multivariate model based on clinical data collected from NIH studies.

Hypothesis: Risk factors differ among asthma phenotypes. Specifically, a family history of disease, increased atopy, higher lung function and increased eosinophils are associated with an increased odds of early onset disease. In contrast, presence of sinusitis and a history of pneumonia is associated with a decreased odds of having early onset disease.

b. Determine which risk factors are associated with air trapping asthma vs. non-trapping disease, including the development of a multivariate model based on clinical data collected as part of the NIH Severe Asthma Research Program multi-site study.

   Hypothesis: Atopy, decreased lung function, a longer duration of asthma, increased airway inflammation, gastro-esophageal reflux disease (GERD), and chronic sinusitis are associated an increased odds of air trapping.

3. Aspirin intolerant asthma (AIA) is a well recognized asthma phenotype. CRP levels have been shown to be elevated in adult onset asthma, and AIA is more common among late onset asthmatics. Additionally, aspirin sensitive asthmatics have high levels of inflammation that is likely to be systemic. A primary aim of this study was to investigate if CRP levels are elevated in aspirin sensitive asthma as compared to non-intolerant asthma.

   Hypothesis: CRP is elevated, after adjustment for covariables, among aspirin intolerant asthma subjects compared to non-sensitive asthma subjects.

This dissertation has been organized into eight chapters. The results obtained in this research project are presented in manuscript form in Chapters 4 through 7. Because the background and methods within those chapters are relatively abbreviated in this format, a detailed background and literature review is provided in Chapter 2, and a detailed description of the dissertation methods is presented in Chapter 3. A detailed description of clinical methodologies is provided in Appendix A. The conclusions from this research project are summarized in Chapter 8, which includes discussion of the research findings, as well as suggestions for future research.

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Chapter 2

Background and significance

Definition of Severe Asthma

Severe asthma is increasingly recognized as a very heterogeneous disease. For this and other reasons, there has been much difficulty in agreeing upon a strict, complete definition. The most comprehensive definition of severe asthma came about as a result of an American Thoracic Society (ATS) sponsored workshop, the proceedings of which were published in 2000.[1] This definition is based on a combination of major and minor criteria which aim to identify subjects with inadequate asthma control despite appropriate treatment. The definition requires patients to be optimally treated, taking either continuous high dose inhaled corticosteroids or oral corticosteroids for more than 50% of that in the previous year, and fulfill 2 of 7 additional criteria: the use of additional controller medications, the presence of daily symptoms requiring the use of a rescue inhaler, reduced lung function, urgent care by a physician, recurrent exacerbations requiring oral corticosteroids, clinical deterioration with steroids withdrawal and a history of near fatal events.[1] This definition's ability to identify severe asthma subjects was recently validated[2] and is now endorsed by an international consortium of experts in severe asthma.[3]

Although the definition of severe asthma is becoming more refined, little is known about the development of severe asthma. It is not clear if severe asthma develops slowly over time due to unknown genetic and environmental factors or if an acute event occurs near

the onset of disease that irreversibly alters the structure of the lungs to promote severe asthma. Further studies examining possible risk factors associated with severe asthma are needed to help elucidate possible mechanisms for the development of severe asthma, which can be further examined in stronger study designs.

Severe Asthma Cohorts

To date, three large cohorts for the study of severe asthma cohorts have been established and described in the literature. These groups include the European Network For Understanding the Mechanisms for Severe Asthma (ENFUMOSA), the Severe Asthma Research Program (SARP) and the Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR). The ENFUMOSA and SARP studies are cross-sectional studies comparing a group of severe asthma subjects to a group of mild/moderate asthmatic referent subjects. The TENOR study is a prospective, observational study that followed a group of severe or difficult to treat asthmatics over 3 years. So far, most of the reports from these cohorts are descriptive accounts of the cohorts' clinical data.

The European Network For Understanding Mechanisms of Severe Asthma. The European Network For Understanding Mechanisms of Severe Asthma (ENFUMOSA) was established with the goal of investigating mechanisms of severe asthma. The study was a multi-center, international, cross-sectional study that compares clinical, physiological and laboratory measures in a group of adult severe asthma subjects and a group of adult asthmatics who were well controlled with low to moderate doses of inhaled corticosteroids (ICS). A total of 344 subjects were enrolled. All were taking ICS and had documented asthma. Subjects were classified as severe asthmatics if they had a

history of at least one asthma exacerbation in the last year despite high dose ICS therapy or oral corticosteroid (OCS) therapy. Subjects completed a clinical questionnaire, had a physical examination, height and weight measured, skin prick allergy testing, pulmonary function testing, serum IgE testing and white blood cell counts. A subset of subjects underwent sputum induction, exhaled nitric oxide testing and had urinary leukotriene and eosinophil protein levels measured.[4,5]

The primary findings from the cohort included more females, increased levels of airway neutrophils, and less atopy among the severe asthma group compared to the well-controlled asthma group. Females with severe asthma had a higher BMI than the females with mild/moderate asthma, but similar differences were absent among the males. Factors triggering severe asthma were also sex-specific. Serum IgE levels, adjusted for age and sex, were lower among the severe asthma group ($p<0.05$) and severe asthmatics had a lower mean number of positive skin prick tests than the comparison group. Total serum IgE was not associated with asthma severity after adjusting for the number of positive skin prick tests, age and sex. Those with severe disease had a significantly greater number of neutrophils in their sputum, but there was no difference in eosinophil number in sputum or in circulating blood between the two groups. Factors associated with severe asthma were then evaluated using a multivariate model. Results from multiple regression analysis identified the following factors that were independently associated with severe asthma: female sex (Odds Ratio (OR): 2.69, 95% Confidence Interval (CI): 1.62-4.49), perennial symptoms (OR:2.9, 95% CI: 1.8-4.5) and exacerbations during the autumn (OR: 2.42, 95% CI:1.19-4.94), mother's history of atopy

(OR: 0.46, 95% CI: 0.27-0.79), and a history of allergic rhinitis (OR: 0.59, 95% CI: 0.38-0.91) as.[4]

In the second report from this cohort, severe asthma subjects had a lower prevalence of family history of disease, but the association was not statistically significant at the 0.05 level (OR: 0.6, p=0.07). Maternal history of allergy was less prevalent among severe asthmatics (OR: 0.6, 95% CI: 0.3-0.9), and there were no differences in paternal history of asthma or allergy or sibling history of asthma between the two groups. The authors reported no association between serious respiratory tract infections, play school attendance and exposure to allergens/animals in childhood, and current severe asthma.[5]

There are limitations to the ENFUMOSA study. Most of the results are from univariate analyses. There were several multiple comparisons made in the studies. When multivariate analysis was used, confounding and interactions were not explored. Additionally, the authors cited a linear regression analysis but presented odds ratios from the analysis. The cross-sectional study design does not allow for the inclusion of temporality into the analysis. Many of the variables were assessed through subject questionnaires that were based on retrospective self-reporting and therefore subject to reporting bias. Some of the null findings, such as no effect of severe respiratory infections during childhood on severe asthma, may be attributed to non-differential misclassification of exposure, which would likely bias the results towards the null. The strengths of the ENFUMOSA study include the multi-center study design, which increased power and external validity. A common protocol and strict definition for severe asthma was used at all sites, thereby decreasing the likelihood of site variation in both testing procedures and classification of asthma severity. Additionally, the likelihood

of misclassification of asthma severity was reduced. Clinical variables of interest were measured as part of the study rather than collected from questionnaires, thereby limiting the likelihood of reporting bias from the subjects. The subjects were well characterized as they underwent a large number of clinical measurements.

Severe Asthma Research Program. The Severe Asthma Research Program (SARP) is a National Heart Lung and Blood Institute (NHLBI) funded multi-site study. One of the primary goals was to identify and characterize a large number of subjects with severe asthma. The collaborative program was established to investigate the mechanistic basis for severe asthma and how the severe asthma phenotype differs from mild/moderate asthma.[6] In the group's first report,[7] the SARP cohort was described in detail. Clinical characteristics, health care utilization and pulmonary function of the subjects were described. Subjects were classified as severe asthmatics if they met the American Thoracic Society (ATS) definition of severe asthma that was developed by an ATS workshop on refractory asthma.[1] Those with "not severe asthma" represented a spectrum of asthma from mild to moderate which were further classified *post hoc* based on a classification scheme used to define asthma severity in national and international guidelines. Normal control subjects were also enrolled. Subjects underwent a comprehensive phenotypic characterization that included standardized questionnaires, pulmonary function testing, atopy evaluations, measurement of exhaled nitric oxide, and collection of blood.[2]

A total of 438 subjects were included in the initial report, with 204 severe asthmatics. Subjects 12 years of age and older were included. There was no difference in age of asthma onset between severe and not severe asthmatics. However, severe asthma

subjects were older and had the longest duration of disease. There was no difference in race or sex distribution among the groups. The frequency of asthma symptoms increased with increasing severity. Urgent healthcare utilization was more frequent in severe asthma. More severe asthma subjects had a baseline FEV_1 less than 60% predicted. Fewer subjects with severe asthma had positive skin tests, but the number of positive responses among those who tested positive did not differ between the groups. In contrast, IgE levels or blood eosinophils were not significantly different between the two groups. Level of exhaled nitric oxide did not differentiate the mild, moderate or severe groups. Aspirin sensitivity was more common among the severe asthma group than the other two groups. Gastroesophageal reflux (GERD) and history of sinopulmonary infections were also reported more frequently in severe asthmatics. A multivariate model was built, using a backward selection process, to find variables associated with severe asthma. Five variables were reported to independently increase the likelihood that a subject would be classified with severe asthma: pre-bronchodilator FEV_1 percent predicted (OR: 1.36 for every 5% fall in FEV_1); history of pneumonia (OR 3.30; 95% CI: 1.92-5.69); lower number of blood basophils (OR: 2.55; 95% CI: 1.46-4.47); asthma symptoms during routine physical activities (OR: 2.28; 95% CI: 1.25-4.15); and lower number of positive allergy skin test reactions (OR: 1.11; 95% CI: 1.00-1.22).[2]

There are also limitations to the SARP report. Most results are based on univariate analyses, so it is not possible to assess or adjust for confounding. Interactions are likely due to the heterogeneity of asthma, and were not examined. Additionally, multiple comparisons were made. A multivariate approach was taken to explain the differences between the groups; however, the process of model building for the multivariate models

is suspect, as it appears to have been based on only statistical significance. It does not appear that confounding was evaluated. Additionally, the scale on the continuous independent variables (lower number of positive skin test reactions and lower number of basophils) is unclear. It would be useful to see the unadjusted along with the adjusted results. The goal of the paper was to describe the population under study and not to test a specific hypothesis; so, the methods are appropriate. However, the cross-sectional study design limits the study conclusions. Further analyses are warranted to examine the results in the context of confounding and interactions, as well as to determine if the results can be verified or if they are chance findings. The SARP study also has several strengths. The study was a multi-site study which typically increases power and external validity. A strict, comprehensive definition of severe asthma was used at all sites, thereby decreasing both the likelihood of misclassification of disease and the variability of asthma severity classification by site. Subjects were well characterized, and all of the clinical data were collected reducing the possibility of information bias.

The SARP and ENFUMOSA reports share some similar results, as well as some markedly different results. In the SARP study, severe asthmatics were older than the non-severe comparison groups, but the ENFUMOSA study did not report a significant age difference between severe and non-severe asthma. The ENFUMOSA study reported a 4:1 ratio of females to males among severe asthmatics. The SARP study reported more females across all groups but found no difference in the proportion of females in the severe group. Body mass index was reported to be increased among female severe asthmatics in the ENFUMOSA study, while no such difference was reported in the SARP study when compared to moderate asthma. Sinusitis was increased among female severe

asthmatics in the ENFUMOSA study and was found to be increased in both female and male severe asthmatics in the SARP study. In both studies, atopy was decreased in severe asthma, and aspirin sensitivity was increased in severe compared to non-severe asthma.[6] Both studies conducted a multivariate regression analysis, and variables found to be associated with severe asthma were markedly different with the exception of atopy (allergic rhinitis in the ENFUMOSA study and allergy skin tests in the SARP study). The SARP study used a more strict and complete definition of severe asthma, which likely increased both the sensitivity and specificity of asthma severity classification, thereby decreasing the likelihood of misclassification of asthma severity.

Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens. The goal of the Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study was to track health care outcomes, quality of life and school or work productivity prospectively over three years so that asthma control and associated health outcomes can be analyzed over time. Subjects whose asthma was identified as difficult to treat by their treating physician were enrolled, categorized by the physician as mild, moderate, or severe and then studied in an observational manner over 3 years. Subjects were at least 6 years of age, had a smoking history of 30 pack years or less, and had either high medication use or high health care utilization in the year prior to enrollment. A total of 4756 subjects were enrolled and completed a baseline study visit. A majority of the subjects were adults (78%), but children (16%) and adolescents (10%)were also enrolled. A majority of the adult subjects were female while a majority of the children and adolescents were male. About half of the cohort (48%) was classified as having severe asthma, with the remaining half having moderate (48%) or mild asthma

(3%). When stratified by asthma severity, overall IgE geometric means values were lowest among patients with mild asthma (99.9 IU/mL) compared to moderate (102.1 IU/mL) and severe asthma (112.0 IU/mL). This trend was shown in children and adolescence but was not shown in adults. A smaller number of patients with mild asthma had IgE levels above 100 IU/mL than moderate or severe subjects. Mild/moderate disease was more common among children than adolescents or adults. This observation supports the theory that the longer one has asthma the more likely the disease will be considered severe.[8]

The group has published many reports on the cohort. However, most do not compare the severe asthmatic group to the non-severe group, but a few reports do examine disease severity. Gender differences were evaluated. Females had lower quality of life, greater healthcare utilization (including steroid bursts, unscheduled office visits, and misted work/school) and more problems controlling their asthma as compared to males. However, the females had significantly better lung function compared with males (post-bronchodilator $FEV_1\%$ – as percent predicted –and FEV_1/FVC ratio). There were no differences between the males and females on asthma severity, medication use or overall physician evaluation of treatment difficulty.[9] Aspirin intolerant asthma (AIA) was also examined in the cohort. A greater percentage of subjects with AIA were classified as severe asthmatics, and these subjects had increased levels of healthcare use despite increased use of systemic and inhaled corticosteroids and of leukotriene modifiers.[10] Another report examined the prevalence of skin testing and characterized the differences in subjects who were skin test positive, skin test negative, and subjects who had not had a skin test. Most of the subjects reported having a skin test (86%), and there was a high

prevalence of subjects who tested positive (93.5%). The frequency of unscheduled office visits in the past 3 months and use of long-term control medications was higher among skin test positive subjects than negative subjects, suggesting that atopy was increased among those with more severe asthma. There was a wide range of total serum IgE a finding which suggests that measurement of total serum IgE alone may not be an adequate indicator of the allergic component of asthma.[11] In contrast to both the ENFUMOSA and SARP studies, this report suggests that the presence of allergies is an important risk factor for the development of asthma.

There are several limitations to the TENOR study. Many data were self-reported so that reporting bias may have affected the results. Severity was evaluated by study physicians but not standardized among physicians; so, variability among the severe subjects is likely as physicians may not have been classifying the same. In addition, it is likely that some subjects' severity was misclassified. This method of classification likely had lower sensitivity and specificity compared to either the ENFUMOSA or SARP studies. Atopy was based on self- reported data, which is less accurate than the skin testing data used in the ENFUMOSA and SARP studies. Because of the small amount of clinical testing done on the subjects, they are not as well characterized as subjects in the ENFUMOSA or SARP studies, therebyleading to potential misclassification in some of the variables. This misclassification could bias results in either direction, depending on the variable. The study utilized the prospective study design. However, the study could examine only factors leading to asthma exacerbations in a prospective manner. The study could not make conclusions in a prospective manner about the development of severe asthma as the subjects were classified at study enrollment; they were not followed for the development

of severe disease. Most of the multiple comparisons were based on univariate analyses resulting in the inability to adjust for important confounding variables. . The TENOR study has several strengths. The prospective cohort design allowed the investigators to prospectively follow exacerbations and to obtain repeated lung function over 3 years. This design avoids temporal variability in lung function measurements and can include temporality in the evaluation of asthma exacerbations. The study was of a large cohort of severe asthmatics over several sites, increasing the power and external validity.

The ENFUMOSA studies identified female sex, increased neutrophils, lower levels of atopy, decreased lung function, and family history of atopy to be associated with severe asthma. They reported no association between childhood infections and exposures to allergens/animals during childhood and severe asthma. The SARP study identified an increased duration of disease, decreased lung function, lower levels of atopy, aspirin sensitivity, a clinical history of GERD, and a history of pulmonary and sinus infections as associated with severe asthma. They reported no difference between severe and non-severe asthmatics on the basis of sex, race, IgE, blood eosinophils or exhaled nitric oxide. The TENOR studies identified female sex, age, aspirin sensitivity, and higher IgE levels as associated with severe asthma. Although each study used a different definition of severe asthma, some similar factors were found. We used the findings of these studies, as well as findings from other studies discussed in the next section, as a guide for the proposed study.

Other factors related to severe asthma

Clinical Risk Factors: Lung function is a measure often used to define asthma severity. Lung function measures include: forced expiratory volume in one second (FEV_1), the

volume of air exhaled in the first second of a forced expiratory volume maneuver; forced vital capacity (FVC), the volume of air exhaled after a complete and forceful expiration after maximal inhalation; and the ratio of the two volumes FEV_1/FVC, a measure of airway obstruction. The ATS includes reduced lung function as one of the minor, not major, criteria in the severe asthma definition.[1] Both the SARP and ENFUMOSA studies reported decreased lung function as measured by FEV_1 (% predicted) in the severe group compared to the non-severe group. Longitudinal studies examining severe asthma have focused primarily on lung function, specifically FEV_1 to determine the cause of severe asthma. Although typically used to diagnose or define asthma, the correlation between lung function and asthma symptoms is poor.[12] Two large cohorts of asthmatic and control subjects from Australia and New Zealand have been followed for 17-35 years.[13,14] Data from these cohorts suggest that children with reduced lung function early in life are likely to have reduced lung function in adulthood. However, "progressive decline" in lung function was shown to be modest compared to the initial loss in this group, and compared to control groups. Lange et al. found contrasting results. They reported a more rapid decline in FEV_1 over time in "all" asthmatics compared to controls.[15] In that study, no attempt was made to break asthmatics into severity groups. Although decline in FEV_1 contributes to more severe disease, it is likely that additional factors are required for disease progression. These additional factors may include worsening levels of inflammation, hyperresponsiveness, lung compliance, or even levels of asthma symptoms. Although limited in usefulness, FEV_1 is currently the only outcome measured longitudinally to which development of severe asthma can be linked. Further examination of other lung function measurements such as FEV_1/FVC is also warranted.

Obesity has also been associated with an increased incidence of asthma and severe asthma. The ENFUMOSA study suggested that body mass index (BMI) increases with increasing severity of disease (76% of the severe cohort was overweight or obese).[4] However, data from the SARP study did not identify obesity as a risk factor for severe asthma.[2] Being overweight or obese has been associated with an increased incidence of asthma in childhood and in adult men and women.[16] Another study reported an association between body mass index and asthma prevalence in adult men and women.[17] Schachter and colleagues assessed obesity as a risk factor for diagnosed asthma, asthma symptoms, use of asthma medication or airway hyperresponsiveness by pooling data from three large epidemiological studies. They found that, after adjusting for atopy, age, sex, smoking history and family history, severe obesity was a significant risk factor for recent asthma, wheeze in the previous 12 months, and asthma medication use. However, obesity was not associated with airway hyperresponsiveness (a hallmark of asthma). The authors concluded that, because of the similarities in atopy, airway hyperresponsiveness and airway obstruction between the obese and non-obese group, there was not evidence to support the idea of increased asthma prevalence in obese populations.[18] Thus, evidence for an association between obesity and asthma is inconsistent. Some of these inconsistencies may be due to widely varying definitions of asthma. Clinical (only) definitions of asthma may be difficult to interpret as shortness of breath with exertion is often seen in obese patients in the absence of asthma. Additionally, obesity is a side effect associated with corticosteroids, a common treatment for severe asthma. It is further possible that subjects with severe asthma patients, because of their disease, are unable to participate in physical activity, thereby possibly contributing to obesity.

Sinusitis is extremely common in severe asthma with evidence for some disease in >80% of this population. The severity of the sinusitis has been associated with both inflammation and lung function abnormalities.[19] Unfortunately, little effective long-term therapy for sinusitis exists to allow determination of whether this is a parallel or a causative process.

Respiratory infection may also contribute to severe asthma. Respiratory syncytial virus infections have been thought to contribute to childhood asthma while pathogens, such as mycoplasma and chlamydia, may play a role in adult onset disease.[20-22] In the SARP study, a history of pneumonia was also found to be a risk factor for severe asthma.[2] It is not evident if the association between pneumonia and severe asthma is a reflection of increased asthma duration, which may increase susceptibility to pneumonia, or if severe asthma develops as a result of the pneumonia.

The ENFUMOSA and TENOR studies reported that severe asthma was more prevalent among women than men.[4,10] The TENOR study confirmed other population-based asthma prevalence reports [23] in finding that most of the adult subjects were female, while most of the children and adolescents were male.[24] In another TENOR report, females reported significantly greater healthcare utilization, more asthma control problems, and a lower quality of life, but there was no difference in physician-assessed asthma severity when compared to males.[9] The higher prevalence in women may be a result of hormonal and poorly identified environmental factors. However, it remains unknown if gender is an independent risk factor for severe asthma.

Asthma, regardless of the severity, is a chronic inflammatory airway disorder. Airway inflammation contributes to airflow limitation, airway hyperresponsiveness,

bronchoconstriction, airway wall remodeling, and respiratory symptoms.[25] A trigger causes the release of inflammatory mediators from mast cells, macrophages, T lymphocytes, and epithelial cells which then direct the migration and activation of other inflammatory cells, such as eosinophils and neutrophils, to the airway. These cells then can cause a wide range of tissue injury including alterations to the epithelium, abnormalities in autonomic neural control of airway tone, hypersecretion of mucus, changes in mucociliary function, and increased smooth muscle responsiveness. The importance of these pathways is confirmed by the correlation of markers of inflammation with bronchial hyperresponsiveness, symptoms and lung function.[25] Eosinophils are thought to have an important pro-inflammatory role in asthma pathogenesis and have been consistently identified in asthmatic lungs while nearly absent in healthy lungs. Additionally suppression of eosinophils is usually associated with decreased asthma symptoms.[26] Because of their decreased response to therapy, severe asthma patients may demonstrate a different inflammatory process than that seen in asthmatics with milder disease. Pathologic studies of severe asthma airways indicate that one-half to two-thirds of severe asthmatics have persistent large airway tissue eosinophils despite high doses of corticosteroids.[27] Other studies have indicated that neutrophils are elevated in severe asthma subjects compared to asthmatics with milder disease.[28,29] Because of the phenotypic differences noted in eosinophil positive and eosinophil negative asthma, assessment of inflammatory phenotypes has gained much attention. It is apparent that eosinophils and neutrophils are associated with severe asthma. However, their effect has not been evaluated in the presence of other risk factors.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, trigger asthma exacerbations in a large subset of severe asthma patients. NSAID or aspirin intolerant asthma is more common among severe asthma patients compared to mild/moderate patients.[2,24] The patients with this intolerance experience severe asthma without chronic exposures to these drugs. The TENOR study found that aspirin intolerance predicted an increased level of persistent airflow obstruction.[10] Although an increased prevalence of NSAID sensitivity has been documented in severe asthma, it is not known if NSAID sensitivity is an independent risk factor for severe asthma. The aspirin intolerant asthma phenotype will be discussed at length below in the asthma phenotypes section.

Atopy is often thought of a major risk factor for asthma, and especially severe asthma. The TENOR study results suggested that atopy was increased in severe asthma.[11] However, both the ENFUMOSA and SARP studies demonstrated less atopy in severe than milder asthma.[4,7] These data support the concept of a disconnect between disease severity and the presence of allergic reactions. The development of severe asthma may therefore be attributed to other factors in addition to allergen exposure, and should be evaluated in a way that allows adjustment for other factors.

Family History: Familial clustering of asthma and allergies has been repeatedly noted. Evidence also suggests that genetics plays an important role in asthma etiology. No single gene has been implicated in asthma, and most likely a multitude of genes acting either alone or in combination are responsible for development of asthma, and specifically severe asthma. Numerous candidate genes have been identified on the basis of functional properties or positional cloning in large population studies.[30] However, not all asthmatics have a positive family history of asthma and/or allergies. This may

indicate that those with a positive family history may have a different type of asthma than those without. Efforts are needed to identify phenotypes which appear to have familial linkages. Once identified, these phenotypes should be used to guide genetic studies of asthma. Family history is further discussed below in the age of asthma onset phenotype section.

Asthma Phenotypes

Phenotype is defined as the observable properties of an organism that are produced by the interaction of the genotype and the environment.[31] It has been widely recognized that asthma is a very heterogeneous disorder which is likely governed by interactions between genes and the environment. Therefore, asthma should be examined by phenotype, and once these phenotypes are established and validated, treatments should be tailored to asthma patients based on their clinical phenotype. Additionally, phenotypes may be used to guide the genetic assessment of asthma. Numerous classifications of potential phenotypes of asthma and severe asthma have been proposed based on age of onset, type of inflammation, pattern of severity, sensitivity to aspirin, allergy presence or absence, and lung function values. Although proposed, these phenotypes are poorly characterized, and none has been evaluated using multivariate modeling approaches, which allow for both the adjustment for confounding factors and the examination of potential interactions. This study will focus on modeling of phenotypes that have been previously proposed: early vs. late onset asthma; and a newly proposed phenotype of air trapping vs. non-trapping asthma (quantified by CT measurements).

Age at asthma onset. Although many epidemiologic studies of asthma focus on childhood onset disease, a large percentage of asthma may develop in adolescence or

adulthood.[32] Allergic, pathologic, and physiologic differences between early and late onset asthma have been reported and strengthen the case for phenotypic differences.[33] In an NJRMC study, subjects who developed asthma early in life (before the age of 12) were compared to those who developed asthma later in life (after age 12). Subjects with late onset disease had lower lung function, measured by FEV_1, as an adult than the childhood onset group, despite the fact that the adult onset group had the disease for substantially fewer years.[33] Similar studies from Europe support the concept that adult onset asthma is associated with a more rapid decline in lung function.[20,34,35] Looking at onset in a slightly different manner, a study comparing clinical data between children and adults reported that children had significantly less airflow limitation than the adults. Additionally, lung function impairment was associated with asthma duration in children and adults with childhood onset of asthma, but there was no relationship between duration and disease severity in the adults with adult onset asthma.[36]

Allergic responses also seem to differentiate the two groups. The two onset groups from the NJRMC study differed markedly in allergic responses; 98% of early onset asthmatics had positive allergy skin tests, while 76% of late onset asthmatics had positive tests ($p=0.007$). In response to questions about asthma symptoms associated with common allergic triggers, 70-75% of early onset asthmatics answered that symptoms occurred most or all of the time, compared to 40-50% of late onset asthmatics. Finally, 40% of early onset severe asthmatics had a history of atopy, while only 4% of late onset asthmatics gave a similar history.[33] Liang et al. also reported that food and milk allergen sensitization were more common in early-onset asthma as compared to later onset disease.[37] In the TENOR study, adults with childhood-onset disease had higher IgE

levels than subjects with adult onset disease,[38] also suggesting greater atopy among asthmatics with early onset disease.

Family history of allergy or asthma seems to also differ between the two groups. Miranda et al. reported that a family history of asthma was significantly more common in the early onset group.[33] London et al. also reported that early-onset asthma was more strongly associated with parental asthma than late onset.[39] Additionally, Liang et al. reported that having a sibling with either a history of asthma or uticaria was associated with early onset disease. However, parental history of allergy/asthma was not associated with early onset.[37]

Pathologic differences have also been reported between the two groups, although the results are not consistent. Miranda et al. reported that late onset disease was associated with the highest numbers of lung eosinophils ($p=0.007$) while early onset disease was associated with lymphocytic/mast cell processes.[33] Liang et al. reported that subjects with early onset disease had higher blood eosinophil counts than subjects with late onset disease ($p=0.04$).[37] Inconsistencies may be due to differing locations of eosinophils.

Age of asthma onset was also examined in the SARP study. Early onset was defined as asthma occurring before age 12 with late asthma occurring at or after age 12. Similar to the findings of Miranda et al., those with late onset disease were older with a significantly shorter duration of asthma, but with a lower FVC. There was no difference in frequency of positive skin tests between the two groups, but the early onset group had a higher mean number of positive skin tests and more symptoms to allergic exposures, particularly cats. In the SARP cohort, a history of pneumonia and sinusitis was more common in late onset asthma. Life-long health care utilization was greater in the subjects with early-

onset asthma, but there was not a difference when healthcare utilization was examined in the 12 months before study entry. This observation may be indicative of a longer duration rather than a true difference between groups.[2]

Similarities exist across many of these studies despite a much different definition of early onset disease (before age 3 vs. before age 12, or children vs. adults). Early onset asthmatics appear to be a more homogeneous group with strong genetic influences and presence of allergic responses. In contrast, late onset disease appears to be a more heterogeneous group, with evidence for both allergic and non-allergic disease. However, these associations need to be examined in a multivariate manner. Most previously reported associations are based on univariate anlyses and need to be assessed in relation to one another to determine if the associations are independent. Additionally, confounding by other factors, such as sex and age, should be examined. Also, interactions between factors should also be examined.

Air trapping phenotype. Physiologically defined air-trapping has been considered a risk factor for more severe forms of asthma [27,40] and is thought to be the result of involvement of the small airways in the asthma process.[41] Physiologically, air trapping is often defined by the increase in residual volume, or the relationship of residual volume to total lung capacity. However, air trapping can now also be defined and objectively quantified using high resolution multi-detector (MD) computed tomography (CT) imaging and quantitative software analysis.

Each pixel, or picture element, of a CT image has a CT attenuation value. These values are expressed in Hounsfeld units (HU), ranging from 3095 HU for dense bone to -1000 HU the CT density of air. Lower (negative) values represent the least dense (more air-

like) areas, while higher numbers represent more dense areas, such as blood and bone.[42-58] The normal density of lung is between -700 and -800 on inspiration. Pixels with low attenuation values are highlighted using "density mask" software and by measuring the number of pixels below a given density, a pixel index can be calculated. The pixel index is defined as the percentage of pixels in the lungs on a single scan that are less that or equal to a certain density (for instance -850 HU).[58,59] This index has been utilized to quantify air trapping in the lung. Previous studies in emphysema patients have suggested that areas of lung <-950 HU are representative of empysematous regions as identified on pathologic specimens. On the other hand, the normal specific volume of the lung at total lung capacity (TLC) is 6.0 ml/gm, which corresponds to a CT density of - 856 HU.[45,46] The notion that at FRC the normal specific volume and hence CT density should normally be less than the TLC value suggests that -850 HU may also be a reasonable threshold for air trapping when scans are done at FRC. This CT density has been previously used to quantify air trapping in asthmatic children.[60]

Severity of asthma has been associated with air trapping measured plethysmographically, but very little is understood regarding the factors which might predispose to this condition. In asthma, there is often a strong relationship between FEV_1 values and residual volume, suggesting that airway obstruction is strongly related to distal lung air trapping. Additionally, subjects with more air trapping are more likely to have a history of severe asthma exacerbations including intensive care visits, intubations and asthma related hospitalizations,[61] indicating that asthmatics who air trap may be different from those who do not. No previous studies have integrated a range of possible risk factors,

including those related to allergy, past medical history, co-morbid conditions and inflammatory processes to examine air trapping as measured by CT scan in asthma.

CRP as a Biomarker for Aspirin intolerant asthma.

Aspirin intolerance. Shortly after the introduction of aspirin therapy more than 100 years ago, violent episodes of bronchospasm were reported following aspirin ingestion. In 1922, an association between aspirin sensitivity, asthma and nasal polyps was described and later termed the aspirin triad.[62] This triad has since gained attention as a separate asthma phenotype, aspirin intolerant asthma (AIA), with an estimated prevalence among diagnosed asthmatics ranging from 5-19%.[63,64]

Aspirin intolerant asthma is a poorly understood asthma phenotype with a typically aggressive course and continuous inflammation of the airways. One prominent feature is a respiratory reaction manifested by exacerbations of both asthma and rhinitis following ingestion of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs).[62] AIA includes aggressive and continuous inflammation of the airways combined with exacerbation of asthma and rhinitis following the ingestion of aspirin and most non-steroidal anti-inflammatory drugs (NSAIDs).[62] It begins with persistent rhinitis which often follows a typical viral infection and is followed by recurrent then chronic sinusitis and nasal polyposis. Asthma and sensitivity to aspirin typically manifest 1-5 years following the onset of rhinitis.[62] Reactions associated with AIA can be severe. Up to 25% of hospital admissions for acute asthma that require mechanical ventilation in adults may be due to NSAID ingestion.[65] AIA patients are more likely to have been intubated as compared to non-aspirin sensitive asthma subjects.[10] A hallmark of AIA is chronic, persistent inflammation. AIA patients exhibit raised blood eosinophil counts,[64] and up to a four-

fold increase in eosinophils on bronchial biopsy specimens.[62] AIA is more common in late onset asthma and among severe asthmatics.[10,66] AIA may be under-diagnosed in the asthmatic population as asthmatics are often counseled to deliberately avoid NSAIDs and some patients may lack a recognition of mild reactions.[62] Definitive diagnosis of AIA has traditionally required a provocation test using increasing doses of aspirin. These tests may illicit severe, life threatening reactions. Because of the severity of these reactions, a biomarker to aid in diagnosis would be extremely helpful.

C-reactive protein. Research has shown that inflammation plays a key role in coronary artery disease.[67] Immune cells are present in early atherosclerotic lesions, effector molecules accelerate the progression of the lesions, and activation of the inflammatory process can lead to acute coronary syndromes.[68] C-reactive protein (CRP) is an acute-phase plasma protein that is a marker of systemic inflammation. It is mainly produced in the liver in response to IL-6, an important mediator of the acute phase response.[69] Numerous studies have reported an association between elevated CRP levels and the risk of developing cardiovascular disease and metabolic syndrome (obesity, insulin resistance, diabetes, hypertension, and low HDL cholesterol levels).[69-72] Because of the inflammatory properties associated with coronary artery disease, this circulating factor related to inflammation is used as a marker for risk of coronary artery disease and stroke.

CRP has been shown to be a relatively stable protein with long-term stability similar to blood pressure or serum cholesterol. One study evaluated within-person fluctuations in inflammatory markers using paired blood samples taken on average 12 years apart from 379 participants. Within-person correlation coefficients were calculated. Correlation coefficients were similar among CRP (0.59, 95% CI: 0.52-0.66) and other more

established risk factors, such as systolic blood pressure (0.66, 95% CI: 0.60-0.72), diastolic blood pressure (0.53, 95% CI: 0.46-0.60), and total serum cholesterol (0.60, 95% CI: 0.54-0.66).[73]

It has been hypothesized that CRP levels may also be elevated in other inflammatory diseases. Associations have been found in diseases thought to have an inflammatory component, such as colon cancer,[74,75] ovarian cancer,[76] lupus,[77] and rheumatoid arthritis.[78] A handful of studies have also examined CRP levels in asthma. Positive associations have been reported with some studies reporting that CRP levels may be elevated only in a specific subset of asthmatics. As part of the follow up study to the cross-sectional study, the European Community Respiratory Health Survey, Kony et al. reported that increased CRP levels were associated with bronchial hyperresponsiveness (OR:2.27, 95% CI: 1.20-4.28) independently of age, gender, BMI, smoking status, SES, hypercholesterolaemia, and hypertension. The authors also reported that FEV_1 was significantly lower in subjects with high CRP levels as compared to those with low levels independent of the same confounding variables. This study included both asthma subjects and non-asthmatic subjects, and after adjustment for asthma, the relationship remained.[79] Another study in men examining the relationship of CRP to a number of factors associated with mortality reported an association between elevated CRP and reduced lung function.[80] Because the primary goal of that study was not to examine the relationship of CRP and lung function, the relationship was reported as an incidental finding. Another study reported increased CRP levels among steroid naïve patients compared to controls but not among patients taking inhaled steroids. In the steroid naïve subjects, CRP correlated with pulmonary function and sputum eosinophil count. These

relationships were not present among subjects taking inhaled steroids.[81] This study did not adjust for confounding variables; so, it is difficult to conclude that the elevated CRP levels were a result of pulmonary inflammation and not due to a confounding factor, such as cardiovascular disease. A study examining the role of CRP in *Chlamydia pneumoniae* infection and the immune response to the *C. pneumoniae* heat shock protein 60 reported that asthma patients had higher CRP levels than the control groups. Additionally, the authors reported that CRP levels were higher among moderate asthmatics than mild asthmatics (p for trend <0.01).[82] Again, this study did not adjust for confounding variables. Another study compared CRP levels among asthmatics with exacerbation and among asthmatics without a recent exacerbation. A multi-center epidemiological study examined the relationship of CRP levels and respiratory symptoms, bronchial responsiveness, asthma and atopy. Asthmatic subjects had higher CRP levels than non-asthmatic participants. The authors stratified asthma subjects by atopy. Non-allergic asthma subjects had significantly higher CRP levels than non-asthmatic subjects, while allergic asthma subjects had similar levels as non-asthmatic subjects. The association between non-allergic asthma and CRP remained after adjustment for age, sex, smoking, BMI and study center (OR=2.19, 95% CI: 1.04-4.63).[83] However, a new study also examining the association between non-allergic asthma and CRP reported that the association was likely due to confounding factors.[84]

The non-allergic asthma phenotype is associated with late onset disease while late onset disease is associated with AIA. AIA patients have increased levels of eosinophilic inflammation compared to non-AIA patients. Therefore, we hypothesized that CRP levels may be associated with aspirin sensitivity. In a recent NJRMC pilot study of

asthmatic patients (n=40), late onset asthmatics with aspirin sensitivity had a significantly higher CRP level than non-sensitive asthmatics (p=0.023). These preliminary data suggested that CRP should be further examined as a potential biomarker of AIA and warranted further investigation to determine if CRP is associated with the AIA phenotype after adjusting for confounding factors.

Summary

Patients with severe asthma are at an increased risk for morbidity and mortality, not only of dying from their asthma but also the drugs used to treat the disease. Limited epidemiologic studies have been conducted to identify risk factors specific to severe asthma, and studies have generally not examined confounding variables or interactions. This study provides a comprehensive epidemiologic evaluation of associations between severe asthma and possible risk factors, alone and in combination. A previously proposed phenotype of severe asthma, based on age of onset, has not been evaluated in a multivariate manner. The study includes an evaluation of severe asthma in general, as well as the age at asthma onset phenotype, using a multivariate approach including evaluation and subsequent adjustment of potential confounding variables and examination of interactions. Variables identified in previous reports as significantly associated with the phenotypes (at the univariate level) will be included in the analysis. In addition, these studies focus on a relatively newly proposed asthma phenotype, that of subjects who exhibit air trapping who are apparently at increased risk of severe exacerbations. Little is known about these subjects in relation to clinical factors associated with air trapping. The study uses a multivariate approach to identify a set of clinical factors associated with air trapping. Finally, AIA, a well known phenotype, does

not have a diagnostic biomarker. Such a marker would be very useful clinically as the current method for evaluating AIA involves a dangerous aspirin challenge.

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Chapter 3

Research Design and Methods

Approach

Study Design. A cross-sectional study design was used to epidemiologically evaluate severe asthma and asthma phenotypes and to examine a potential biomarker for aspirin intolerant asthma (AIA). Specific aims 1 and 2 utilized data that were collected as part of ongoing National Institutes of Health (NIH) funded studies to evaluate risk factors associated with severe asthma and severe asthma phenotypes. Specific aim 3 consisted of a chart review of National Jewish Research and Medical Center (NJRMC) asthma clinic patients to determine whether or not C-reactive protein (CRP) was elevated in AIA compared to aspirin tolerant asthma. The studies were approved by the NJRMC and Pitt Institutional Review Boards (IRB) and were monitored by an independent Data Safety Monitoring Board. Secondary data analysis was approved by the Colorado State University IRB.

Clinical Testing. A detailed description of clinical testing methods is included as Appendix A. Briefly, subjects underwent a battery of testing. This battery included: spirometry, methacholine challenge, allergy skin testing, multi-detector CT scan (subset of subjects), sputum induction to gather white blood cell quantity, measurement of the fractional concentration exhaled nitric oxide (FE_{NO}), and bronchoscopy including endobronchial biopsy and collection of bronchoaveolar lavage (BAL) fluid for determination of white blood cell quantity. Subjects also completed a detailed

questionnaire which collected information about age at asthma onset, family history of allergies and asthma, co-morbid conditions, history of pneumonia and a variety of other data points (see Appendix B). Spirometric data utilized in the current study were limited to forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the ratio of the two volumes (FEV_1/FVC). FEV_1 and FVC were converted to the percent of predicted based on Hankinson equations.[1] Provocative concentration causing a 20% fall in FEV_1 (PC_{20}) was the only information utilized from methacholine challenge. Spirometry testing, methacholine challenge and FENO measurement were done according to American Thoracic Society Guidelines.[2-4] All other testing was completed according to the study protocols and was applicable the Severe Asthma Program Manual of Procedures. Study coordinators were trained according to a standardized protocol and competency tested.

Study population. Table 1.1 illustrates the subjects by specific aim.

Specific aims 1 and 2: All subjects in this study had been enrolled in Dr. Sally Wenzel's studies at NJRMC/PITT. All procedures were part of IRB approved clinical research studies, and all subjects had signed informed consent at NJRMC/PITT. All data came to us de-identified, with each subject having a unique study identifier (SARP/SEW number). Subjects were either self-selected by responding to advertisements and word-of-mouth publicity or had been referred from clinic visits at NJRMC or PITT. All subjects were classified as stated below by Dr. Wenzel at their initial screening visit. To be eligible for study inclusion, subjects had to be between 12-65 years of age. All asthmatic subjects had to demonstrate either a positive methacholine challenge or

reversibility after bronchodilator to be included. Further inclusion criteria are addressed below.

Asthma Severity Classification

Severe Asthmatics: Severe asthmatics were classified based on the American Thoracic Society definition of severe asthma.[5] Subjects had an asthma diagnosis and had to be on either continuous high dose of inhaled steroids or oral corticosteroids for at least 50% of the last year. In addition, they had to meet two of the seven minor criteria: 1) requirement for additional daily treatment with a controller medication, such as long-acting beta agonist, theophylline, or leukotriene antagonist; 2) asthma symptoms requiring short acting beta agonist use on a daily or near daily basis; 3) persistent airway obstruction (FEV_1 < 80% predicted; diurnal PEF variability > 20%); 4) one or more urgent care visits for asthma per year; 5) three or more oral steroid "bursts" per year; 6) Prompt deterioration with $\leq$ 25% reduction in oral or inhaled corticosteroid dose; and 7) near fatal asthma event in the past.[5]

Non-severe Asthmatics: Asthma subjects who were not classified as severe asthma subjects were considered non-severe. This cohort included both moderate and mild subjects. The classification for the two groups is listed below for informatory purposes only. The two groups were combined in all analyses to maximize power.

Moderate Asthmatics: Moderate asthmatics had to have: baseline FEV_1 60-80% predicted and PC20 < 16 mg/ml, treatment with low to moderate doses of inhaled corticosteroids, and no asthma related hospitalizations, urgent care visits, or oral steroid bursts in last 6 months.

Mild Asthmatics: Mild asthmatics had to have baseline $FEV_1 > 80\%$ predicted and PC20 < 16 mg/ml, treatment with beta agonists alone, and no asthma related hospitalizations, urgent care visits or oral steroid bursts in last 12 months.

Exclusion criteria. Subjects with any of the following were excluded: any history of clinically significant non-respiratory disease, physician diagnosis for other significant respiratory disease such as sarcoidosis or chronic obstructive pulmonary disease, more than 5 pack-year smoking history, or smoking within the year prior to enrollment. Subjects could not have had an infection in the four weeks prior to enrollment.

Specific aim 3. Data came from an IRB approved chart review at NJRMC. Patients from Dr. Wenzel and Dr. Katial's asthma clinics were enrolled. All data were de-identified.

Inclusion criteria: To be included, subjects had to have: physician-diagnosed asthma with a history of either a positive methacholine challenge or a 12% improvement in FEV_1 (% predicted) following administration of bronchodilator; CRP level drawn for clinical purposes and recorded in chart; spirometry reading consistent with asthma within one month of C-reactive protein measurement; CBC or circulating blood eosinophil levels taken at the same time as CRP level; be 18-60 years of age; and have a known aspirin intolerance status.

Exclusion criteria: Subjects with any of the following were excluded: current smoking; smoking within the last year; more than 20 pack year history of smoking; current infection; or current asthma exacerbation.

Data analyses.

Data were statistically analyzed using the Statistical Analysis System (SAS) computer program (SAS 9.1, SAS Institute Inc., Cary, NC). Descriptive statistics (e.g., mean,

standard deviation) were calculated for continuous data, and relative frequencies were calculated for categorical data. Normality of continuous variables is an assumption required in some of the statistical tests used in this process. The assumption of normality of continuous variables was assessed via histograms and the Shapiro-Wilk test. Non-normal variables were transformed and re-examined. If normality was satisfied, the transformed variables were used in analyses requiring normality. If normality was not achieved with a common transformation, non-parametric methods were used (such as the Wilcoxon rank sum test). Univariable methods, such as the chi-square test of association, the two-sample t-test for differences in means, and analysis of variance (ANOVA), were utilized as appropriate for categorical and continuous variables. The data analysis for this study included an initial univariate screening of all available variables that could affect the development of severe asthma, followed by multivariate modeling using multiple logistic regression analyses. Because of the heterogeneity of asthma and expected interactions, stratified analysis of odds ratios was conducted to examine potential effect modifiers. Effect modification was also evaluated in multivariate modeling by including an interaction term in the model. Any interaction with a probablity-value less than 0.05 was considered to be statistically significant. Factors of interest that were significantly associated with the outcome at the univariate level were included in the multivariate analysis to develop explanatory models using logistic regression analysis. The dependent variable differed by analysis: asthma severity (severe/non-severe) in the first analysis; asthma onset (early/late) in the second analysis; air trapping status (yes/no); in the third analysis; and aspirin tolerance (tolerant/intolerant) in the fourth analysis. Confounding was assessed throughout the model building procedure with adjustment for confounder(s)

included as needed. Interactions of risk factors determined to be statistically significant and biologically important terms in the stratified analysis were explored (where the sample size permitted) via both stratified analysis and multiple logistic regression analysis.

Odds ratios and 95% confidence intervals were computed via logistic regression analysis. Potential confounders, which were chosen based on *a priori* selection, were evaluated by examining the change in the coefficient or effect estimate after adding the confounder to the model. . Variables that appreciably changed the estimate of interest were considered confounders.[6] Those variables that did not change the estimate were eliminated in a stepwise manner. The final models included all potential confounders that remained, as well as the *a priori* predictors. Logistic regression requires data to be linear in the logit (i.e., the natural logarithm of the odds). This assumption was evaluated via fractional polynomials.[6] If non-linear, potential transformations of continuous variables were examined to assure that the assumption of linearity was satisfied. The goal of this analysis was to obtain the least biased estimates of association.

(Specific Aim 1): The effect of various clinical and demographic variables on the presence of severe asthma were evaluated in a defined population of asthmatics of varying clinical severity. Subjects with severe asthma were compared to subjects with non-severe asthma. Statistical analysis was conducted as described above via logistic regression. A model based on clinical variables was created to determine factors that differentiated severe from non-severe asthma. Potential factors of interest for the this model included pulmonary inflammation, atopy, pulmonary function, PC_{20}, duration of

asthma, age at asthma onset, aspirin intolerance, sinusitis, BMI, aspirin intolerance, race, sex, family history of asthma and allergies, and history of pneumonia.

(Specific Aim 2): Risk factors for previously identified asthma phenotypes (age at onset, air trapping, and aspirin tolerance) were evaluated both among all asthmatics and specifically among severe asthma.

Specific Aim 2a Asthma Onset. Subjects were categorized by age at asthma onset. Subjects with onset before age 12 were compared to subjects with onset after age 12.[7] Statistical analysis was conducted as described above using logistic regression. Potential explanatory variables included: atopy (allergy testing and IgE), lung function (FEV_1% predicted, FVC % predicted FEV_1/FVC), pulmonary inflammation (eosinophils, neutrophils via biopsy, BAL, or sputum), family history of asthma/allergies, duration of asthma, sex, age, sinusitis, aspirin intolerance and history of pneumonia.

Specific Aim #2b Air trapping: Logistic regression analysis was used based on categorizing subjects in the top two quartiles of percent of lung less than -850 HU as those who exhibited air trapping ("air trappers") compared to subjects in the bottom two quartiles who did not exhibit air trapping (non-trappers). Potential explanatory variables included: lung function: FEV_1 (% predicted), FVC (% predicted), FEV_1/FVC, airway inflammation (including eosinophils, neutrophils, and FE_{NO}), airway hyperresponsiveness (PC_{20}), family history of allergies/asthma, age, sex history of pneumonia, duration of disease, race, BMI, onset of disease, allergies/IgE, and oral steroid use.

(Specific Aim 3 CRP as a biomarker of AIA): Subjects with aspirin intolerant asthma were compared to those without aspirin intolerant asthma. Statistical analysis was conducted as described above via logistic regression. AIA status was the outcome, and

CRP level was the exposure of interest and thus forced into the model. Potential confounding variables included: BMI, age, sex, blood eosinophils, hypertension, smoking history, hypercholesterolemia, diabetes, corticosteroid use (both inhaled and oral), atopy, and lung function. Age at asthma onset and atopy were examined as potential effect modifiers.

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Table 3.1. Source of study subjects by specific aim

Specific Aim	Source of Study Population
1	NJRMC/Pitt subjects enrolled in NHLBI sponsored SARP and previous NIH funded studies (n=238)
2a	NJRMC/Pitt subjects enrolled in NHLBI sponsored SARP and previous NIH funded studies (n=223)
2b	SARP subjects from NJRMC, University of Virginia, University of Wisconsin, Washington University (n=94)
3	Clinical subjects seen by RK or SW at NJRMC (n=95)

Chapter 4

A multivariate analysis of risk factors for severe asthma

Abstract

Background: Severe asthma accounts for a minority of asthma but utilizes a disproportionate amount of healthcare costs associated with asthma. Severe asthma is increasingly recognized as a very heterogeneous disease; further studies are needed to identify risk factors which differentiate severe from non-severe asthma.

Rationale: The current study was undertaken to identify clinical factors that collectively, best described a severe asthma cohort in comparison to a cohort of non-severe asthma subjects.

Methods: Severe asthma was classified using the American Thoracic Society's severe asthma definition. Subjects with severe asthma (n=159) were compared to non-severe (n=78) asthma subjects based on select clinical data using both univariate and multivariate statistical analyses.

Results: Gastro-esophageal reflux disease (GERD), FEV_1/FVC, history of pneumonia, history of sinusitis, and atopy were identified as independent factors which were associated with asthma severity.

Conclusions: In the 237 asthma subjects studied, several differences were identified between severe and non-severe asthma subjects. Some of the most interesting results included the increased odds of severe asthma associated with GERD and history of pneumonia, and the decreased odds of severe asthma found among subjects with atopy.

Introduction

Severe asthma likely affects less than 10% of all asthmatics[1] but utilizes a disproportionate amount of healthcare costs associated with the disease, accounting for at least half of the total costs (direct and indirect) for asthma.[2] Severe asthmatics are 15 times as likely to use emergency medical care compared to mild to moderate asthmatics and are 20 times as likely to require hospital admission.[2] Those suffering from severe asthma are likely to be impacted by their disease on a daily basis which leads to a significantly decreased quality of life compared to mild/moderate asthmatics.[3] Limited epidemiologic studies have been conducted to identify risk factors specific to the development of severe asthma. Additionally, few studies have evaluated the risk factors in a multivariate manner.

Severe asthma is increasingly recognized as a very heterogeneous disease. For this and other reasons, there has been much difficulty in agreeing upon a strict, complete definition. The most comprehensive definition of severe asthma came about as a result of an American Thoracic Society (ATS) sponsored workshop, the proceedings of which were published in 2000. This definition is based on a combination of major and minor criteria which aim to identify subjects with inadequate asthma control despite appropriate treatment.[4] This definition's ability to identify severe asthma subjects was recently validated[5] and is now endorsed by an international consortium of experts in severe asthma.[6] Although the definition of severe asthma is becoming more refined, little is known about the development of severe asthma. It is not clear if severe asthma develops slowly over time due to unknown genetic and environmental factors or if an acute event occurs near the onset of disease that irreversibly alters the structure of the lungs to

promote severe asthma. Further studies examining possible risk factors associated with severe asthma are needed to help pinpoint possible mechanisms for the development of severe asthma. Once possible risk factors are identified, prospective studies should be undertaken to evaluate their role in the development of severe asthma.

This cross-sectional study was undertaken to identify those clinical factors that collectively best described a severe asthma cohort in comparison to a cohort of non-severe asthma subject. Data are from clinical testing that was undertaken as part of a National Institutes of Health (NIH) funded severe asthma study and were examined using multivariate logistic regression.

Methods

Study design

As part of clinical studies, subjects underwent a clinical history, physical examination, allergy skin testing, laboratory tests (including sputum analysis and IgE levels), pulmonary function tests, exhaled nitric oxide (FE_{NO}) testing, completed questionnaires on demographic factors, medication use and medical history, and had a multi-detector CT (MDCT) of the chest prior to fiberoptic bronchoscopy. Details and descriptions of the cohort have been previously described.[7-9] The clinical studies were approved by the National Jewish Medical and Research Center (NJRMC) and University of Pittsburgh (PITT) Institutional Review Board and monitored by an Independent Data and Safety Monitoring Board; the secondary data analysis was approved by the Colorado State Institutional Review Board (Human Research Committee).

Human subjects

Subjects were either self-selected by responding to advertisements and word of mouth publicity or had been referred from clinic visits at NJRMC or PITT. Subjects were 12-64 years old and non-smokers (smoking history <5 pack-years and no smoking within past year). All subjects had physician-diagnosed asthma, no concurrent lung disease, and a positive methacholine bronchoprovocation ($PC_{20} \leq 16$ mg/ml) or $\geq 12\%$ improvement in FEV_1 post-bronchodilator. Severe asthma subjects met ATS workshop refractory asthma criteria.[4] All asthmatics who did not meet criteria for severe asthma were classified as non-severe asthmatics.[5] All subjects signed informed consent, and the study was approved by the National Jewish and University of Pittsburgh IRB.

Clinical testing

Data on demographic variables, such as age at asthma onset, duration of asthma, age, sex, race and family history of asthma and/or allergy, and co-morbid conditions, were obtained via questionnaire. Subject height and weight were obtained by either clinical research coordinators or pulmonary function technicians using a calibrated stadiometer and scale. Allergy skin tests were performed by trained technicians with 14 common allergens using positive (histamine) and negative (saline) controls. Methacholine challenges and spirometry testing were performed according to ATS guidelines.[10, 11] Results of positive methocholine challenges are reported as the provocative concentration causing a 20% decrease in FEV_1 (PC_{20}). Predicted values for FEV_1 and FVC were calculated using Hankinson values.[12] Fractional exhaled nitric oxide concentration (FE_{NO}) was measured online by chemiluminescence at a constant expiratory flow (50 mL/s), consistent with published guidelines.[13] Bronchoscopy was performed as

previously described.[7,8,14] Briefly, the bronchoscope was passed orally or nasally through the vocal cords and into the trachea/bronchi. Endobronchial biopsies were taken from the first or second subcarinae of the right or left lower lobes. The bronchoscope was then repositioned in the opposite lung where bronchoaveolar lavage (BAL) was performed in subsegments of the lingula or right middle lobe using four 60-ml aliquots of warmed sterile saline, with sequential instillation and manual aspiration.[7,8,15] Tissue and lavage fluid were processed as previously described.[7,8]

Sputum cells were obtained via induced sputum induction. A 3% saline solution mist and ultrasonic nebulizer was used for the induction. Peak flow rates were monitored throughout the induction. For processing, sputum was diluted to 50% with a solution of 0.1% dithiothreitol. Cytospins were made for differential cell counts, which were performed by two separate counters and recorded as white blood cell percentages. For further details of the clinical procedures or laboratory methods, see Appendix A.

Lung function, predicted values

Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) values are presented and analyzed in this study as the percent of predicted values (% predicted), which were based on Hankinson 1999 equations.[12] In subjects who completed the study before the initiation of the Severe Asthma Research Program (SARP) studies, predicted values were according to Cherniack equations.[16] Where possible the predicted values were recalculated according to Hankinson equations. However, some elements of the equation (either age or height) were missing for some of these subjects (n=86); however, the Cherniack predicted values in the data set allowed us to estimate the Hankinson values. Specifically, Hankinson predicted values of FEV_1 and FVC were imputed based

on respective regression equations of the Hankinson predicted values versus the Chernaiack predicted values (regression equations were based on 174 subjects with both predicted values). The R-squared values of the regression equations were 94.6% for the FEV_1 prediction and 93.8% for the FVC prediction. These estimations of FEV_1 and FVC Hankinson predicted values were completed so that all of the predicted values were similar.

Subject classification

Airway neutrophil and eosinophil variables were calculated based on sputum, endobronchial biopsy, and bronchoavelor lavage (BAL) data. The cut-point for positive (eosinophil or neutrophil) was based on the mean plus two standard deviations in the normal control population[8] for all measures except sputum eosinophils. Extreme outliers were removed for the cut-point analysis. The cut-point for classifying sputum eosinophil positive has been studied and is generally accepted to be 2%.[17] Atopy was defined by the presence of one or more positive allergy skin tests. Late onset asthma was defined as asthma diagnosis at or after the age of 12.[5,7] If subjects reported either or both parents having a history of asthma, they were considered to have a parental history of asthma. The same was true for parental history of allergy.

Statistical Analysis

The chi-square test of association and the Wilcoxon rank sum test (data were not normally distributed) were used to evaluate associations between severe asthma and potential covariables. Logistic regression analysis was used to evaluate univariate associations among variables and severe asthma and, thus, to determine a group of risk factors potentially associated with severe asthma among asthmatic subjects. In particular,

the multivariate logistic regression (MLR) model was built using as candidate variables only those variables previously reported to be associated with severe asthma or any variable with a univariate odds ratios significant at the 25% level (i.e., $p<0.25$). Only variables with at least 100 responses were used. Purposeful selection was used for model building.[18] Variables that had been eliminated were re-entered into the final model and retained if statistically significant ($p<0.05$). The odds ratio (OR) and its 95% confidence interval (CI) was calculated between severe asthma and each significant covariate ($p<0.05$). Potential confounding of the association between variables of interest and asthma severity (based on biological evidence) was examined on the basis of change in magnitude of the estimates of the variables that were included in the model.[18] The model building process was also completed using a sample size scheme. Purposeful selection was carried out first with variables with at least 200 responses then 175, 150, 100. Final results did not differ between the two techniques. All analyses were conducted with SAS computer program (SAS 9.1, SAS Institute Inc., Cary, NC).

Results

Descriptive Statistics

Severe asthma subjects (n=140) were compared to non-severe subjects (n=74). The non-severe cohort included both mild (n=50) and moderate subjects (n=24). Data on co-variables of interest are summarized by asthma severity in Tables 4.1 and 4.2. Severe asthma subjects were significantly older ($p=0.041$), had higher body mass index (BMI) ($p=0.006$), lower lung function (FEV_1, FVC and FEV_1/FVC), and more airway hyperresponsiveness (PC_{20}) than non-severe asthma subjects ($p<0.001$ for all lung function parameters and PC_{20}). Atopy was more prevalent in non-severe asthma subjects

($p<0.001$), but the mean IgE level was not significantly different between the two groups ($p=0.369$). As to be expected, more severe asthma subjects took inhaled and oral corticosteroids than non-severe asthma subjects. Severe asthma subjects were also more likely to report a clinical history of pneumonia, sinusitis, gastro-esophageal reflux disease (GERD), and aspirin intolerance. Family history of asthma, but not allergy, was also more common among severe asthma subjects. Severe asthma subjects were significantly more likely to be classified as having high levels of neutrophils in biopsy tissue and BAL. There were no statistically significant differences between severe and non-severe asthmatics in either eosinophils from any compartment or neutrophilic inflammation from sputum. Duration of asthma was slightly longer among severe asthma subjects, but the difference in mean duration did not reach statistical significance ($p=0.106$). Neither the mean FE_{NO} ($p=0.683$) nor mean age at asthma onset ($p=0.391$), were significantly different between severe and non-severe asthma subjects.

Logistic Regression

Univariate odds ratios are presented in Table 4.3. Not surprisingly, since they are part of the criteria for severe asthma, both inhaled and oral steroid use were associated with severe asthma. Because of its use in the classification of severe asthma, oral corticosteroid use was not further examined. PC_{20} and airway hyperresponsiveness are inversely related (a lower PC_{20} indicates increased airway hyperresponsiveness). The odds of severe asthma decreased as PC_{20} increased; therefore, severe asthma was associated with increasing airway hyperresponsiveness (OR: 0.68, 95% CI: 0.54-0.86). This variable had a large amount of missing data, in part due to safety requirements in obtaining FEV_1, and therefore was not considered in the MLR analysis. Neither age at

asthma onset nor sex was significantly associated with asthma severity (OR : 1.19, 95% CI: 0.68-2.09; and OR : 1.28, 95% CI: 0.76-2.15, respectively). Eosinophilic inflammation, in sputum, BAL, or bronchial biopsy, was also not significantly associated with asthma severity. Although non-significant, the odds of severe asthma was increased among subjects with eosinophilic inflammation (sputum eosinophil OR: 1.43, 95% CI: 0.69-2.98; BAL eosinophils OR: 2.17, 95% CI: 0.69-6.82; and tissue biopsy eosinophil OR: 1.39, 95% CI: 0.63-3.06). Neutrophilic inflammation, in biopsy tissue and BAL, was significantly and strongly associated with severe asthma (biopsy tissue neutrophils OR: 9.22, 95% CI: 1.19-71.28; and BAL neutrophils OR: 11.96, 95% CI: 1.56-91.90). However, the confidence intervals are imprecise. Neutrophilic inflammation in sputum was not significantly associated with asthma severity (OR: 1.38, 95% CI: 0.66-2.87). Severe asthma subjects had significantly lower lung function ($p<0.001$, all spirometric measures).

Co-morbid Conditions

The relationship between GERD and severe asthma was strong and highly significant (crude OR: 15.59, 95% CI: 7.06-34.42). Adjustment for BMI slightly attenuated the odds ratio for GERD; however, the estimate was still statistically significant (crude OR among subjects with BMI value: 9.65, 95% CI: 4.03-23.08; adjusted OR: 8.90, 95% CI: 3.67-21.56). Similar results were obtained based on adjustment for obesity (BMI $\geq$ 30). The odds of severe asthma subjects were approximately four times higher among subjects who reported sinusitis. Atopy was associated with decreased odds of severe asthma (crude OR: 0.24, 95% CI: 0.11-0.55). In contrast, increasing levels of IgE appeared to be associated with increased odds of severe asthma – albeit not statistically significant

possibly because these levels were available in only a sub-set of subjects (crude OR: 1.002, 95% CI: 1.000-1.004 based on one unit increase in IgE). Severe asthma subjects were significantly more likely to report aspirin sensitivity than non-severe asthma subjects (OR: 4.90, 95% CI: 1.64-14.65). The odds of severe asthma were significantly elevated for both overweight (BMI≥25) and obese (BMI≥30) subjects compared to non-overweight/obese subjects (crude OR overweight: 2.14, 95% CI: 1.12-4.07; crude OR obese: 2.72, 95% CI: 1.35-5.48).

Family History

Parental history of asthma and allergies seemed to differentiate the two groups. The relationships between severe asthma and maternal/paternal history of asthma were similar; so, these two variables were combined into a single parental history variable. The same was true for maternal/paternal history of allergies. A parental history of asthma was significantly associated with increased odds of severe asthma (OR: 2.15, 95% CI: 1.06-4.38). Conversely, parental history of allergies was associated with decreased odds of severe asthma (OR: 0.55, 95% CI: 0.28-1.09), although the relationship was only marginally significant (p=0.085).

Multivariate Analysis

The following variables were considered as candidates for the multivariable logistic regression (MLR) model: BMI (dichotomized into overweight yes/no), duration of asthma, race (white/non-white), history of GERD, history of sinusitis, atopy, parental history of asthma, eosinophil (+/-) based on either sputum and/or BAL, neutrophil (+/-) based on either sputum and/or BAL, eosinophil (+/-) based on bronchial biopsy tissue, neutrophil (+/-) based on bronchial biopsy tissue, history of pneumonia, and FEV_1/FVC.

Results of the MLR analysis are summarized in Table 4.4. The final model included GERD, FEV_1/FVC, history of pneumonia, history of sinusitis, and atopy. Linearity of FEV_1/FVC was examined using fractional polynomials,[18] and because the assumption was satisfied, FEV_1/FVC was kept as a continuous variable in the MLR model. Subjects with GERD were much more likely to be severe asthmatics (OR: 7.50, 95% CI: 2.26 - 24.88). Although the confidence interval was wide, one can see that, even at the lower limit of the confidence interval, the odds of being a severe asthmatic is more than two times greater among subjects with GERD. Lower lung function, as measured by FEV_1/FVC, was also associated with severe asthma; for every 10% decrease in FEV_1/FVC, the odds of having severe asthma increased by 2.72 times (95% CI: 1.63-4.53). A history of pneumonia was also associated with increased odds of severe asthma. Subjects with a history of pneumonia were almost six times as likely to be severe asthmatics (OR: 5.70, 95% CI: 1.75 - 18.58). Again, the confidence interval is relatively imprecise; however, even at the lower limit of the 95% confidence interval, the odds of severe asthma was still increased by 75% compared to subjects who did not have a history of pneumonia. Results were similar for subjects reporting sinusitis; the odds of severe asthma was 4.5 times greater for these subjects compared to subjects not reporting sinusitis (OR: 4.53, 95% CI: 1.37 - 14.97). The lower limit of the odds ratio confidence interval – although not as strong as for other estimates in the model – still shows a 37% increase in odds for severe asthma among subjects with sinusitis. Finally, atopic subjects were significantly less likely to have severe asthma (OR: 0.10, 95% CI: 0.02 - 0.66). Again at the conservative limit of the confidence interval, atopic subjects were about 34%

less likely than non-atopic subjects to have severe asthma. There was no evidence of confounding by age, sex, or use of inhaled steroids.

Discussion

This study used a well characterized cohort of asthma subjects with a wide range of asthma severity to examine clinical and demographic differences between severe and non-severe asthma subjects. Using multivariate logistic regression analysis, we developed a model containing five variables to explain severe asthma as compared to non-severe disease.

Most of the risk factors included in the model were co-morbid conditions (GERD, sinusitis, atopy). GERD has long been reported to be associated with asthma[19] with one of the first reports of co-occurrence over a century ago.[20] In spite of this well established association, the mechanism of the association is not clear. Esophageal acid exposure likely exacerbates asthma symptoms possibly through increased bronchial reactivity,[21] tracheal acidification,[22] or direct alterations in ventilation.[23] GERD has also been linked to obesity, which has been reported as a risk factor for severe asthma.[24] Because of this reported association between GERD and severe asthma, it might be thought that obesity is acting as a confounding factor. However, in this study, adjusting GERD for obesity attenuated the odds ratio for severe asthma, but the odds ratio remained elevated and statistically significant. This finding suggests that, in this population, GERD is a risk factor for severe asthma independent of obesity. Perhaps another explanation for the association between GERD and severe asthma might be the relationship between GERD and air trapping. Decreasing FVC (% predicted) was significantly associated with increased odds of GERD – for each 10% decrease in FVC, there was a 44% increase in

odds of GERD (OR: 1.44, 95% CI: 1.22-1.70). Subjects with increased air trapping (here based on decreased FVC) might be prone to GERD due to increased pressure on the stomach from enlarged lungs. Adjusting the univariate odds ratio for FVC (% predicted) did not eliminate the effect but did slightly attenuate the odds ratio. Additionally, the MLR model was adjusted for FEV_1/FVC (which can also be a reflection of air trapping); so, the relationship in the MLR model between severe asthma and GERD is not likely due to air trapping. It is also possible that the use of oral steroids contributes to GERD by weakening sphincter muscles, which may result in increased reflux. However, adjustment for oral steroid use in the model is not feasible because, in this cohort, oral steroid use is essentially a surrogate for severe asthma. The SARP study has also reported, albeit by univariate analysis, that GERD was more common in severe versus non-severe asthma.[5] Further research should examine the link between GERD and severe asthma, including both adjustment for body mass index and ascertained GERD diagnosis instead of self reported disease. Subjects who reported GERD may have had GERD currently or have had GERD that had been successfully treated and therefore was not active; unfortunately, the questionnaire did not distinguish between the two. The influence of active vs. controlled GERD on severe asthma should be examined. Unlike some of the co-morbid conditions, such as chronic sinusitis, effective treatment for GERD exists. The effect of successful GERD treatment on asthma severity could serve to determine if GERD is a causative or parallel process of severe asthma. One study that has examined treatment effects on asthma found that 24 weeks of treatment with a proton pump inhibitor did not improve daily asthma symptoms, albuterol use, or lung function. Asthma exacerbations were decreased in the treatment arm.[25] The effect of treatment on

asthma severity was not assessed. Additionally, the length of treatment was likely not long enough to detect a difference. Further research should be conducted to further evaluate GERD as a factor associated with severe asthma.

The presence of sinusitis was also found to be an independent risk factor for severe asthma. The severity of the sinusitis has been previously associated with both inflammation and lung function abnormalities.[26] Additionally, the initial SARP report found the prevalence of sinusitis to be elevated among their cohort.[5] Unfortunately, little effective long-term therapy for sinusitis exists to allow determination of whether this is a parallel or a causative process.

Atopy is often considered a major risk factor for asthma and especially severe asthma. The TENOR results suggested that atopy was increased in severe asthma, but skin testing was not performed.[27] However, as with this study, both ENFUMOSA and SARP demonstrated less atopy in severe asthma than in milder asthma.[24,28] These data support the concept of a disconnect between disease severity and the presence of allergic reactions. The development of severe asthma may therefore be attributed to other factors. However, it should be noted that IgE values were available in a only subset of subjects, and although the relationship was not statistically significant, the mean IgE level was more than two times higher in severe asthma subjects. Allergy symptoms should also be examined. It is possible that symptoms and asthmatic responses to these symptoms are more prevalent and likely more severe in the severe asthma population and that this parameter may more accurately reflect the effect of atopy on severe asthma. Additionally, differences in atopy between the two severities should be examined with particular attention to the response on skin testing to specific allergens, as well as the

severity of symptoms associated with specific allergens. Allergen exposure has been identified as an important environmental risk factor for asthma and particularly severe asthma. The strongest supporting data for this exposure are exposures to house dust mite, cockroach and alternaria.[29-31] The evaluation of specific allergens, including the response on skin testing and associated triggers, should be further analyzed to gain additional insight into the role of atopy in asthma severity.

Severe asthma subjects had significantly lower lung function as compared to non-severe asthma subjects on all three of the spirometry values (FEV_1, FVC, FEV_1/FVC). For the multivariable analysis, FEV_1/FVC was used rather than FEV_1 or FVC (% predicted). FEV_1 is a minor criterion in the severe asthma definition and was therefore not ideal. Additionally, FEV_1 is low in restrictive, as well as obstructive, disease while FEV_1/FVC decreases only with increasing airflow limitation. FEV_1/FVC has also been reported to better correlate with air trapping measured both physiologically[32] and by multi-detector CT scan.[14] Subjects with severe asthma had a reduced FEV_1/FVC, indicating that these subjects have a significantly higher amount of obstruction, independently of the other terms in the model. Other studies have also reported FEV_1/FVC to be reduced in severe asthma.[33,34]

A self reported history of pneumonia was also identified as a risk factor for severe asthma. This relationship was also reported in the SARP study; however, the odds ratio was slightly lower than in the current study (OR=3.30, 95%CI: 1.92-5.69).[5] The temporality of this relationship has yet to be established. Kraft et al. reported that *Mycoplasma pneumoniae* was present in the airways of chronic stable asthma subjects with a greater frequency than in control subjects suggesting that it may play a role in

asthma pathogenesis.[35] Conversely, asthma has been previously reported to be a risk factor for pneumococcal disease,[36] which may suggest that the pneumonia may not occur before onset of disease. In several COPD studies, inhaled corticosteroid use has been associated with an increased risk for pneumonia in prospective studies.[37,38] All of the severe asthma subjects in this study were on very high ICS dose which could have contributed to a higher risk for development of pneumonia. However, only longitudinal studies will be able to more definitively confirm that relationship. Because this study is a cross-sectional study, it is impossible to determine if the pneumonia occurred before or after the onset of asthma. Pneumonia occurring in a mild/moderate subject may have contributed to progression of non-severe asthma to severe asthma. There was also a significant association between duration of asthma and pneumonia in this population; increasing duration was significantly associated with increased odds of reporting a history of pneumonia. These results suggest that, in this population, increased asthma duration may increase susceptibility to pneumonia rather than pneumonia contributing to the onset of asthma later in life. Recall bias may also affect this factor as it is possible that severe asthma subjects are more likely to remember having pneumonia than mild/moderate asthma subjects. If recall bias exists, it is likely to bias the estimate away from the null. It should also be noted that subjects may have misreported a history of pneumonia. Other diseases, such as bronchitis, may have been incorrectly reported as pneumonia. Misreporting on this factor would likely have been non-differential by asthma severity. However, the resulting misclassification may have biased the estimate in either direction. To further address history of pneumonia as a risk factor for severe

asthma, mild/moderate asthma subjects reporting a history of pneumonia, which should be clinically confirmed, should be followed for development of severe asthma.

Some covariables that we expected to be important risk factors were not significant in the multivariable model. Obesity has been reported to be associated with severe asthma in the ENFUMOSA study;[24] however, the SARP study did not report an association.[5] Increased body mass index was more common among severe asthma subjects than non-severe asthma subjects; however, when the odds ratio was adjusted for oral steroid use, the relationship lost statistical significance. This may suggest that, rather than being a risk factor for severe asthma, being overweight is an effect of treatment of severe asthma (and the associated decreased physical activity). Temporality cannot be assessed from this study. Overweight status was considered in the model building process but was not statistically significant in the presence of the other variables. Airway inflammation (measured via sputum or lavage cells), both eosinophilic and neutrophilic, was more common among severe asthma subjects. However, neither of these indicators of airway inflammation was statistically significant in the final model. It is possible that neutrophilic inflammation was also associated with another variable included in the model that served as a surrogate for neutrophilic inflammation, such as GERD or history of pneumonia. Duration of asthma was marginally significant in the univariate analysis but was not found to be statistically significant in the MLR analysis. It is also possible that these terms are important risk factors but were not identified due to reduced power.

There are some limitations to this study. The study is a cross-sectional study and therefore cannot assess temporality. The subjects were self-selected for the study and therefore may not be representative of the general asthma population. There were

multiple comparisons made. Some of the results may be attributed to chance rather than true associations with severe asthma. Some of the data were obtained via questionnaire, which introduces the possibility of recall bias. If severe asthma subjects are more likely to recall past infections and co-morbid conditions, the estimates could be biased either away from or towards the null. Comparing severe asthma subjects to other asthmatic subjects likely reduces the probability of recall bias (as compared to using non-asthma subjects for a comparison group) but does not eliminate the possibility of occurrence. In spite of a relatively large dataset, power was still limited. Missing data were prevalent. These limitations restricted our ability to assess more variables and interactions.

In spite of the limitations, the study did yield interesting results about the association between severe asthma and various clinical factors. Some of the most interesting results included the increased odds of severe asthma associated with gastro-esophageal reflux disease and history of pneumonia and the decreased odds of severe asthma found among subjects with atopy. These results should serve as a basis for further research to examine risk factors for severe asthma in hopes of improving treatment outcomes.

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Table 4.1. Descriptive statistics for continuous variables for all study subjects and by asthma severity

Variable	All asthma			Severe asthma			Non-severe asthma			p-value*
	Mean	Standard Deviation	N	Mean	Standard Deviation	N	Mean	Standard Deviation	N	
Age	37.32	12.79	214	38.59	13.34	140	34.92	11.39	74	0.0410
PC20	1.62	2.02	122	1.00	1.66	63	2.27	2.17	59	<0.0001
IgE	201.03	324.28	67	267.06	417.12	36	124.35	130.59	31	0.3685
Duration of asthma	23.55	13.80	208	24.85	14.22	133	21.24	12.77	75	0.1056
Age at asthma onset	14.11	14.90	221	14.84	16.10	143	12.77	12.38	78	0.3910
BMI	29.16	7.21	177	30.15	7.39	117	27.22	6.47	60	0.0058
FeNO	62.45	55.69	68	66.75	61.04	45	54.04	43.38	23	0.6830
FEV1 percent predicted	63.73	22.68	231	54.01	18.77	149	81.40	18.07	82	<0.0001
FVC percent predicted	81.28	21.26	230	74.28	20.08	148	93.92	17.17	82	<0.0001
FEV1/FVC	0.64	0.13	240	0.60	0.12	158	0.72	0.11	82	<0.0001
Neutrophils sputum	36.98	26.18	119	41.77	27.69	66	31.03	23.06	53	0.0308
Eosinophils sputum	7.59	15.92	119	10.12	18.61	66	4.44	11.13	53	0.1767
Eosinophils-BAL (SARP)	2.57	5.89	21	2.82	6.52	17	1.48	1.64	4	0.4409
Neutrophils-BAL (SARP)	4.87	6.61	21	5.46	7.21	17	2.33	1.87	4	0.3707
Eosinophils-BAL	1.62	4.13	124	2.04	4.87	86	0.65	0.93	38	0.4386
Neutrophils-BAL	2.78	6.37	124	3.69	7.48	86	0.72	0.49	38	<0.0001
Eosinophils- biopsy tissue	36.08	67.78	160	41.72	78.05	110	23.67	33.59	50	0.8726
Neutrophils-biopsy tissue	90.97	134.61	163	107.19	156.05	111	56.34	56.91	52	0.0756

*Probability value (p-value) from Wilcoxon Rank Sum test comparing severe to non-severe asthma

Table 4.2. Descriptive statistics for categorical variables for all study subjects and by asthma severity

Variable	All			Severe asthma			Non-severe asthma			p-value*
	Number	Percent	N	Number	Percent	N	Number	Percent	N	
Male	114	39.05	262	78	45.61	171	36	39.56	91	0.3467
Overweight	115	64.97	177	83	70.94	117	32	53.33	60	0.0201
Obese	67	37.85	177	53	45.30	117	14	23.33	60	0.0043
Non-white	40	18.35	218	31	21.53	144	9	12.16	74	0.0907
BAL/sputum eosinophil status	67	34.72	193	43	34.96	123	24	34.29	70	0.9247
BAL/sputum neutrophil status	70	36.27	193	49	39.84	123	21	30.00	70	0.1717
Biopsy tissue eosinophil status	42	26.25	160	31	28.18	110	11	22.00	50	0.4101
Biopsy tissue neutrophil status	18	11.04	163	17	15.32	111	1	1.92	52	0.0110
BAL eosinophil status	23	16.79	137	19	19.59	97	4	10.00	40	0.1722
BAL neutrophil status	23	16.79	137	22	22.68	97	1	2.50	40	0.0041
Sputum eosinophil status	53	44.54	119	32	48.48	66	21	39.62	53	0.3337
Sputum neutrophil status	50	42.02	119	30	45.45	66	20	37.74	53	0.3965
Inhaled corticosteroid use	140	68.63	204	121	86.43	140	19	29.69	64	<0.0001
Oral corticosteroid use	99	49.25	201	98	71.53	137	1	1.56	64	<0.0001
Atopy	178	77.06	231	103	69.59	148	75	90.36	83	0.0003
Sinusitis	120	62.18	193	96	71.64	134	24	40.68	59	<0.0001
GERD	101	47.20	214	92	64.79	142	9	12.50	72	<0.0001
History of pneumonia	106	62.72	169	85	73.91	115	21	38.89	54	<0.0001
Aspirin sensitivity	41	22.78	180	37	28.03	132	4	8.33	48	0.0007
Father allergy	60	35.50	169	41	33.88	121	19	39.58	48	0.1402
Mother allergy	68	40.24	169	46	38.98	118	22	43.14	51	0.0211
Parent allergy	100	56.50	177	66	52.38	126	34	66.67	51	0.0825
Father asthma	33	18.54	178	27	21.60	125	6	11.32	53	0.0297
Mother asthma	40	21.62	185	33	25.00	132	7	13.21	53	0.0672
Parent asthma	65	34.57	188	80	60.61	132	13	23.21	56	0.0329

*Probability values are from chi square tests of association comparing severe and non-severe asthma

Table 4.3. Odds ratios (OR), along with 95% confidence intervals (CI), from the univariate logistic regression analysis comparing co-variates and asthma severity among clinically diagnosed asthma subjects

Variable	N	Coefficient	Standard Error	OR	95% CI	p-value*	Unit Change
Asthma duration	208	0.020	0.011	1.103	0.991 - 1.226	0.0717	5 year increase
Onset (referent=early)	221	0.174	0.288	1.190	0.677 - 2.092	0.5449	1
Sex (referent=female)	262	0.248	0.264	1.281	0.764 - 2.148	0.3471	1
Race (referent=white)	218	0.684	0.409	1.981	0.888 - 4.420	0.0949	1
PC20	122	-0.387	0.122	0.679	0.535 - 0.862	0.0015	1
IgE	67	0.002	0.001	1.002	1.000 - 1.004	0.1044	1
Exhaled nitric oxide	68	0.005	0.005	1.046	0.947 - 1.155	0.3747	10 ppb increase
GERD	202	2.747	0.404	15.589	7.059 - 34.424	<0.0001	1
Age	214	0.023	0.012	1.258	1.003 - 1.578	0.0469	10 year increase
Oral corticosteroid use	201	5.065	1.026	158.298	21.211 - ∞	<0.0001	1
Inhaled corticosteroid use	204	2.713	0.368	15.081	7.325 - 31.049	<0.0001	1
Sinusitis	190	1.386	0.331	4.000	2.093 - 7.645	<0.0001	1
Atopy	231	-1.410	0.413	0.244	0.109 - 0.548	0.0006	1
Overweight	177	0.759	0.329	2.136	1.120 - 4.073	0.0212	1
Obese	177	1.001	0.357	2.721	1.351 - 5.481	0.0051	1
FEV1/FVC	240	-9.471	1.484	2.578	1.927 - 3.448	<0.0001	10% decrease
FEV1 percent predicted	231	-0.075	0.010	2.107	1.731 - 2.564	<0.0001	10% decrease
FVC percent predicted	230	-0.059	0.010	1.803	1.491 - 2.180	<0.0001	10% decrease
Sputum neutrophil status	119	0.319	0.241	1.375	0.658 - 2.873	0.7181	1
Sputum eosinophil status	119	0.361	0.374	1.434	0.690 - 2.982	0.3344	1
BAL neutrophil status	138	2.482	1.040	11.960	1.556 - 91.903	0.0171	1
BAL eosinophil status	138	0.772	0.586	2.165	0.687 - 6.822	0.1874	1
Biopsy tissue neutrophil status	163	2.222	1.044	9.221	1.193 - 71.284	0.0333	1
Biopsy tissue eosinophil status	160	0.330	0.402	1.391	0.633 - 3.058	0.4113	1
Aspirin sensitivity	168	1.590	0.558	4.904	1.641 - 14.649	0.0044	1
History of pneumonia	169	1.493	0.351	4.452	2.239 - 8.854	<0.0001	1
Father allergy	154	-0.417	0.369	0.659	0.320 - 1.360	0.2594	1
Father asthma	176	0.726	0.486	2.066	0.797 - 5.356	0.1353	1
Mother allergy	166	-0.281	0.346	0.755	0.383 - 1.487	0.4164	1
Mother asthma	184	0.762	0.453	2.143	0.881 - 5.210	0.0927	1
Parental history of allergies	177	-0.598	0.347	0.550	0.764 - 2.148	0.0845	1
Parental history of asthma	188	0.621	0.189	2.150	1.055 - 4.381	0.0010	1

*probability value from maximum likelihood ratio

Table 4.4. Odds ratios (OR), along with 95% confidence intervals (CI), from the multiple logistic regression analysis of severe asthma among 129 clinically diagnosed asthma subjects (86 severe and 43 non-severe asthma subjects)

Variable	Coefficient	Standard Error	OR	95% CI	p-value
Gerd	2.014	0.612	7.495	2.258 - 24.876	0.0010
FEV1/FVC*	0.999	0.709	2.716	1.628 - 4.530	0.0001
Pneumonia	1.741	0.603	5.702	1.750 - 18.584	0.0039
Sinusitis	1.512	0.609	4.536	1.374 - 14.972	0.0131
Atopy	-2.272	0.945	0.103	0.016 - 0.657	0.0162

*Odds ratio based on 10% decrease in FEV1/FVC

Chapter 5

A multivariate analysis of the age at asthma onset phenotype

Abstract

Background: Asthma has been widely recognized as a very heterogeneous disorder which is likely governed by interactions between genes and the environment. Differences based on age at asthma onset have been previously reported.

Rationale: Therefore, this study was undertaken to compare asthma subjects with an early onset of asthma to those with onset later in life with the goal of finding a multivariate model to explain clinical and demographic differences between the two phenotypes based on previous univariate findings.

Methods: Subjects were classified as having early onset asthma (n=131) if the onset occurred before age 12 and late onset asthma (n=92) if asthma onset occurred after age 12. Subjects were then compared based on select clinical and demographic data using both univariate and multivariate statistical analyses.

Results: A parental history of asthma, duration of asthma, and atopy were identified as independent factors that were associated with decreased odds of having late onset asthma. Having airway eosinophils was associated with an increased odds of having late onset asthma.

Conclusions: In the 223 asthma subjects studied, several differences were identified between early and late onset asthma subjects. Perhaps the most interesting included the

family history of asthma, atopy and eosinophilic information. Early onset asthmatics appear to be a more homogeneous group with strong genetic influences and presence of allergic responses. In contrast, late onset disease is more heterogeneous with evidence for both allergic and non-allergic disease.

Introduction

Asthma has been widely recognized as a very heterogeneous disorder which is likely governed by interactions between genes and the environment. Therefore, phenotypic differences in asthma should be examined, and once these phenotypes are established and validated, treatments and prevention strategies should be tailored to asthma patients based on their clinical phenotype. Numerous classifications of potential phenotypes of asthma and severe asthma have been proposed based on age of onset, type of inflammation, pattern of severity, sensitivity to aspirin, allergy presence or absence and lung function values. Although proposed, these phenotypes remain poorly characterized, and none has been evaluated using multivariate modeling approaches that include both adjustment for confounding factors and examination of interactions.

Although many epidemiologic studies of asthma focus on childhood onset disease, a large percentage of asthma may develop in adolescence or adulthood.[1] Phenotypic differences between early and late onset disease have been previously reported, by our group[2] and others. Significant differences in lung function, atopy (skin tests as well as symptoms), family history of disease and eosinophilic inflammation were found based on univariate analyses.[2] Similar studies from Europe have supported the association between adult onset asthma and decreased lung function.[3,4] Other studies have reported increased atopy among early onset asthmatics based on food allergies[5] and IgE levels.[6] The familial relationships were also confirmed by other studies either through history of parental asthma[7] or through a sibling with a history of asthma.[5] The relationship between eosinophils and early onset disease has not been replicated. The Severe Asthma Research Program, (SARP) recently examined the differences between early and late onset disease

and reported that late onset subjects had lower lung function and were more likely to have a history of both sinusitis and pneumonia. Early onset asthma subjects had a greater number of positive skin tests and asthma symptoms in response to allergic triggers.[8]

Early onset asthmatics appear to be a more homogeneous group with strong genetic influences and presence of allergic responses. In contrast, late onset disease is a more heterogeneous group, with evidence for both allergic and non-allergic disease. However, these associations need to be examined in a multivariate manner. Most previously reported associations have been univariate and need to be assessed in relation to one another to determine if the associations are independent. Therefore, this study was undertaken to compare asthma subjects with an early onset of asthma to those with onset later in life using multivariate analysis. The goal of the analysis was to find a multivariate model to explain clinical and demographic differences between the two phenotypes based on previous univariate findings.

Methods

Study design

As part of clinical studies, subjects underwent a history, physical examination, allergy skin testing, laboratory tests (including sputum analysis and IgE levels), pulmonary function tests and exhaled nitric oxide (FE_{NO}) testing, completed questionnaires on demographic factors, medication use and medical and family history, and had a chest multi-detector CT prior to fiberoptic bronchoscopy. Details and descriptions of the cohort have been previously described.[2,9] The clinical studies were approved by the National Jewish Medical and Research Center and University of Pittsburgh Institutional Review Board and monitored by an Independent Data and Safety Monitoring Board, and

the secondary data analysis was approved by the Colorado State Institutional Review Board (Human Research Committee).

Human subjects

Subjects were either self-selected by responding to advertisements and word of mouth publicity or had been referred from clinic visits at NJRMC or PITT. Study subjects were 12-64 years old and non-smokers (smoking history <5 pack-years and no smoking within past year). All subjects had physician-diagnosed asthma, no concurrent lung disease, and a positive methacholine bronchoprovocation ($PC_{20} \leq 16$ mg/ml) or $\geq 12\%$ improvement in FEV_1 post-bronchodilator. All subjects signed informed consent.

Clinical testing

Demographic variables, such as age at onset, duration of asthma, age, gender, race and family history of asthma/allergy, and co-morbid conditions, were obtained via questionnaire. Allergy skin tests were performed by trained technicians with 14 common allergens using positive (histamine) and negative (saline) controls. Methacholine challenges and spirometry testing were performed according to ATS guidelines.[10,11] Fractional exhaled nitric oxide concentration (FE_{NO}) testing was completed using the Niox NO-analyzer (Aerocrine, Stockholm, Sweden) or Sievers NO-analyzer (Sievers Ionics, Boulder, CO) according to ATS guidelines.[12] Forced vital capacity and forced expiratory volume in one second are presented as percent of predicted (FVC % predicted and FEV_1 % predicted). Predicted values were calculated using Hankinson equations.[13] Bronchoscopy was performed as previously described. Briefly, the bronchoscope was passed orally or nasally through the vocal cords and into the trachea/bronchi. The bronchoscope was positioned, and bronchoaveolar lavage (BAL) was performed in

subsegments of the lingula or right middle lobe using four 60-ml aliquots of warmed sterile saline, with sequential instillation and manual aspiration.[2,9,14,15] Lavage fluid was processed as previously described.[15]

Sputum cells were obtained via induced sputum induction. A 3% saline solution mist and ultrasonic nebulizer was used for the induction. Peak flow rates were monitored throughout the induction. For processing, sputum was diluted to 50% with a solution of 0.1% dithiothreitol. Cytospins were made for differential cell counts, which were performed by two separate counters and recorded as white blood cell percentages. For further details of the clinical procedures or laboratory methods, see Appendix A.

Subject classification

Subjects were classified into early onset asthma if their asthma diagnosis occurred before age 12 and late onset if at or after age 12.[2,8] Severe asthma subjects met ATS workshop refractory asthma criteria.[16] All asthmatics who did not meet criteria for severe asthma were classified as non-severe asthmatics.[8] Subjects were classified as having airway eosinophils (+/-) and neutrophils (+/-) based on sputum and/or bronchoavelor lavage (BAL) data. The cut-point for positive (eosinophil or neutrophil) was based on the mean plus two standard deviations in the normal control population[9] for all measures except sputum eosinophils. Extreme outliers were removed in the determination of each cut-point. The cut-point for classifying sputum eosinophil positive has been studied and is generally accepted to be 2%.[17] Atopy was defined by the presence of one or more positive allergy skin tests. If subjects reported either or both parents having a history of asthma, they were considered to have a parental history of asthma. The same was true for parental history of allergy.

Statistical Analysis

Descriptive statistics were calculated for the overall study population, as well as separately for early and late age at asthma onset subjects. Normality of continuous variables was assessed using the Shapiro-Wilk test. Associations between asthma onset and potential co-variables were initially evaluated via the chi-square test for association (categorical variables) and the Wilcoxon rank sum test (continuous variables when data were not normally distributed), respectively. Logistic regression analysis was used to evaluate univariate associations among variables and asthma onset and to determine those risk factors associated with asthma onset in asthmatic subjects. The multivariate model was developed using variables for which the univariate odds ratios was significant at the 25% level (i.e., p-value < 0.25) or were previously reported to be associated with onset of asthma. Only variables with at least 100 responses were used. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated between asthma onset and each co-variable found to be significant at the 5% level ($p<0.05$) in the multiple logistic regression analysis. Confounding was examined as the change in magnitude of the estimates.[18] Duration of disease was kept as a continuous variable in the final model on the basis of the results of analyses using fractional polynomials[18] which indicated that the assumption of linearity was satisfied. All analyses were conducted with SAS (SAS 9.1, SAS Institute Inc., Cary, NC).

Results

Basic demographic information comparing early to late onset asthma is summarized in Tables 5.1 and 5.2. Early onset asthma subjects were significantly more likely to be

male, atopic, and older and to have both higher IgE levels and longer duration of disease. Late onset asthma subjects were more likely to have airway eosinophils than early onset subjects. There were no statistically significant differences between the two groups in any of the lung function parameters, as well as in history of either pneumonia or sinusitis.

The univariate associations with onset of asthma and each of the variables summarized in Tables 5.1 and 5.2 are presented in Table 5.3 via the respective odds ratios. For all comparisons in Table 5.3, early onset is the referent group. The odds of having late onset asthma increased two-fold for subjects having high airway eosinophils, measured by BAL and/or sputum (OR: 2.46, 95% CI: 1.28-4.73). In spite of the relationship with eosinophils, the relationship between late onset disease and FE_{NO} (available for only a subset of individuals) was non-significant with the odds ratio for a 10 parts per billion increase in FE_{NO} being close to the null (OR: 1.01, 95% CI: 0.93-1.11). A parental history of asthma was found to decrease the odds of having late onset disease although the association was not statistically significant (OR: 0.55, 95% CI: 0.28-1.06). Additionally, although also not statistically significant, the odds of late onset asthma was reduced among subjects with a parental history of allergies (OR: 0.77, 95% CI: 0.41-1.50). The odds of having late onset disease were considerably lower for atopic individuals (OR: 0.22, 95% CI: 0.12-0.43; $p<0.001$). There was not an association between late onset disease and sinusitis (OR: 1.07, 95% CI: 0.57-2.02). Having a history of pneumonia was associated with a non-significant decrease in odds of late onset disease (OR: 0.59, 95% CI: 0.31-1.12). Asthma severity was not significantly associated with age at onset (OR: 1.90, 95% CI: 0.68-2.09).

Not surprisingly, the odds of having late onset asthma were lower for those subjects who had a longer duration of disease. Additionally, the odds of having late onset disease increased with increasing age. Although both age and duration were significantly associated with age at asthma onset, age and duration of asthma were co-linear and therefore could not be used in the same model. For clinical purposes, duration was chosen as a more meaningful variable in the disease process to be used in multivariate modeling.

Results of the multivariable logistic regression analysis are presented in Table 5.4; again early onset serves as the referent group. The final multivariate model included a parental history of asthma, duration of asthma, atopy and airway eosinophils. Linearity of asthma duration was examined using fractional polynomials,[18] and because the assumption was satisfied, asthma duration was kept as a continuous variable in the model. Subjects with a parental history of asthma were significantly less likely to have late onset asthma (OR: 0.16, 95% CI: 0.05-0.53). The odds of having late onset asthma decreased with increasing asthma duration; each 5 year increase of duration was associated with more than a 50% decrease in the odds of late onset asthma (OR:0.42, 95% CI: 0.30-0.59). Late onset subjects were also significantly less likely to be atopic (OR: 0.15, 95% CI: 0.04-0.58). Lastly, late onset subjects were more likely to have airway eosinophils than early onset subjects (OR: 3.96, 95% CI: 1.37-11.51). The effect of the source of the airway eosinophils (sputum or BAL) on this association was examined. Neither the source term nor the interaction between source and airway eosinophils was found to be statistically significant in the model ($p=0.43$, and $p=0.84$ respectively), thereby providing evidence that the two sources could be combined in this analysis. Also, no interactions of model

terms were found to be statistically significant. The effect of corticosteroids on eosinophils was evaluated by entering oral steroid and inhaled steroid use into the model separately. There was not an appreciable change in either the odds ratio or the p-values, suggesting that the relationship was not confounded by asthma severity or corticosteroid use (whether inhaled or oral).

The final multivariate logistic regression model was also applied to only the severe asthma subjects. The results were similar, but the odds ratios were strengthened while their confidence intervals were wider. These results are not surprising given the large number of severe asthmatics in the study, the non-significance of the univariate odds ratio between severity and age at onset, and the loss of precision resulting from exclusion of the non-severe subjects. The results are also summarized in Table 5.4. The final multivariate model could not be applied to the non-severe subjects due to the reduced sample size.

Discussion

This is the first study to evaluate the asthma phenotype based on age at onset utilizing multivariate analyses. The study served as a follow-up to the original report of the phenotype.[2] In this study, in addition to adding more asthma subjects, including both severe and non-severe asthma subjects, we have utilized a multivariate analysis. While we were able to replicate our previous univariate findings; including the association between age at asthma onset and family history of asthma, atopy, and airway eosinophils, we also found that these factors, as well as duration of disease, were important in independently explaining age at asthma onset.

Twelve years of age was selected as the cut-point between late and early onset of disease. Although this age has been previously used as the cut-point between early and late onset disease,[2,8] it was selected as a somewhat arbitrary age differentiating childhood from adolescence. In childhood, asthma prevalence is greater among males; during the teenage years, this trend reverses, and asthma is more prevalent among females.[19] Therefore, age twelve seemed a reasonable age to discriminate between early and late disease. In the present study, males were almost 42% more likely to have early onset disease ($p=0.049$). However, gender was not found to be a significant term in the multivariate analysis.

Family history of disease has been previously reported to be associated with early onset disease. London et al. also reported that early-onset asthma was more strongly associated with parental asthma than late onset.[7] Additionally, Liang et al. reported that having a sibling with either a history of asthma or uticaria was associated with early onset disease although parental history of allergy/asthma was not associated with early onset.[5] In the current study, parental history of asthma was associated with early onset disease. Information was collected on both maternal and paternal history. The respective variables were combined as the univariate odds ratios and p-values were not appreciably different for maternal and paternal history of asthma and for maternal and paternal history of allergy. The association between familial history of asthma and early age at asthma onset suggests that early onset disease is more likely to be genetically linked than asthma occurring later in life. This finding supports differentiating by age at asthma onset when examining possible genetic associations in severe asthma.

Atopy, as defined as one or more positive allergy skin tests, was also more common among subjects with early onset of asthma. Almost 87% of the early onset subjects were

atopic compared to 59% of late onset subjects. In our previous study, 98% of the early cohort and 76% of the late onset cohort were atopic.[2] The Liang et al. study also reported that food and milk allergen sensitization were more common in early-onset asthma as compared to later onset disease.[5] In The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study, adults with childhood-onset disease had higher IgE levels than subjects with adult onset disease,[6] also suggesting greater atopy among asthmatics with early onset disease. IgE levels were only available in a subset of the current cohort and were therefore not considered in the multivariate analysis. However, in the univariate analysis, increasing levels were marginally associated with early onset of disease. Subjects with early onset disease appear to have a more classic form of asthma accompanied by allergy. Although atopy is more widespread among early onset asthma, it does appear that a portion of the late onset subjects have a similar disease process. Further examination of these subjects should be undertaken as they may be more similar to early onset asthma.

Eosinophilic inflammation was strongly associated with late onset asthma. This was a finding in our previous report but has not been replicated. Liang et al. reported that subjects with early onset disease had higher blood eosinophil counts ($p=0.04$).[5] The inconsistency between these studies may be due to differing locations of eosinophils. Airway eosinophils are a better indication of pulmonary inflammation while blood eosinophils may be an indication of systemic inflammation. The relationship between age at asthma onset and eosinophils was not affected by corticosteroid used. Interestingly, the relationship between airway eosinophils and age at asthma onset is independent of both atopy and asthma duration. The effect of the location of eosinophil

(BAL vs. sputum) was also statistically evaluated. In this analysis it appears that the relationship between eosinophilic inflammation and age at asthma onset was not affected by the source of the eosinophilic inflammation. This result may not be surprising in that eosinophils from both sources are luminal; however, it is still possible that there are biologic differences between the two sources. In the previous study, we reported eosinophil status by a combination of tissue eosinophils and BAL, but the results were primarily based on tissue eosinophils.[2]

Perhaps not surprising, asthma duration was significantly associated with early age at asthma onset. As most subjects were enrolled as adults, we would expect duration to differ between the two groups. However, it is important to note that, even after adjusting for duration of asthma, the other factors remained statistically significant factors associated with the phenotype.

This report did not find an association between age at asthma onset and lung function parameters, as previously reported in other studies. In our previous report, subjects with late onset disease had lower lung function, measured by FEV_1 as an adult, than the early onset group, despite the fact that the late onset group had the disease for substantially fewer years.[2] Similar studies from Europe have supported the concept that adult onset asthma is associated with a more rapid decline in lung function.[20-22] Reasons for the discrepancy are not clear. It is possible that this decrease is occurring more rapidly in the late onset cohort, but this study is cross-sectional and therefore cannot account for changes over time.

Because asthma with a late age at onset appears to be different from early onset disease with respect to genetic linkage (based on familial history of asthma) and atopy, it is

possible that these subjects are more likely to suffer an insult to the airway which leads to disease. History of pneumonia may be one such insult; however, there was not a statistically significant difference between the two groups. In fact, the odds of having late onset disease was actually lower among subjects with a history of pneumonia. This relationship may be due to increasing odds of pneumonia with increasing duration of disease since it was not significant in the MLR model that adjusted for duration.

We hypothesized that sinusitis would be associated with late onset disease; however, the proportion of sinusitis between the two groups was nearly identical. Aspirin sensitivity, which has been reported to be more common among asthmatics with adult onset disease, was also not different between the two groups. It is possible that differences do exist but that the current study was not sufficiently powered to find an association. Additionally, both of these measures are self-reported by the subjects so that misclassification could attenuate the odds ratios.

There are several limitations to this study. Although we increased our sample size from our previous report, there were still only 124 subjects included in the multivariate analysis. The study may not have had the power to identify some associations between important covariables and age at asthma onset. Not all subjects completed all testing which further limited the study's power. Some of the variables were obtained via subject questionnaires, introducing the possibility of incorrect reporting and thus misclassification. As duration of disease was significantly higher among subjects with early onset of asthma, recall bias may be present. Subjects with a shorter duration of disease may recall events more accurately than those with a longer duration of asthma. However, duration was adjusted for in the MLR analysis, and relationships persisted

indicating that the associations are not likely to be merely a reflection of increased duration of asthma. Additionally, several variables, including eosinophilic status and atopy, were clinically measured and, therefore, would not be affected by recall bias. The study results may not be generalizable to the entire asthma population as these subjects were self selected to participate in clinical studies. Additionally, because this is a cross-sectional study, temporality cannot be assessed.

In spite of the limitations, the study was able to identify a group of variables in a multivariate model that illustrated the differences between late and early age at asthma onset subjects. The study utilized a well characterized cohort of asthma subjects of a wide range of asthma severity and completed an extensive battery of clinical testing. The clinical testing was carried out according to established protocols by trained coordinators. Much of the information was collected via such testing rather than through questionnaires.

These results provide further evidence that important differences exist between early and late onset asthma and that the difference is not just among severe asthma subjects. Our findings strengthen the case for further examination of this phenotype, including genetic study by phenotype which may lead to discoveries important to asthma treatment.

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Table 5.1. Descriptive statistics for continuous variables for all study subjects and by age at asthma onset

Variable	All			Early onset			Late onset			p-value*
	Mean	Standard Deviation	N	Mean	Standard Deviation	N	Mean	Standard Deviation	N	
Age	36.88	12.97	179	32.78	12.19	104	42.57	11.89	75	<0.0001
PC20	1.69	2.09	109	1.75	2.40	67	1.58	1.50	42	0.2884
IgE	203.23	326.26	66	256.53	387.08	38	130.89	203.73	28	0.0274
Duration of asthma	23.55	13.80	208	29.84	12.21	123	14.45	10.53	85	<0.0001
Age at asthma onset	14.09	14.86	223	3.42	0.25	131	29.27	11.38	92	<0.0001
BMI	29.05	7.17	157	29.00	0.78	86	29.11	7.16	71	0.6632
FeNO	62.60	56.09	67	60.90	8.72	40	65.13	58.40	27	0.8530
FEV1 percent predicted	63.42	22.81	201	63.49	2.11	114	63.33	23.27	87	0.9600
FVC percent B13predicted	81.04	21.44	199	81.50	19.72	113	80.43	23.61	86	0.5618
FEV1/FVC	0.64	0.13	203	0.64	0.13	116	0.65	0.13	87	0.4506
Neutrophils sputum	34.74	26.26	102	35.35	27.20	59	33.92	25.21	43	0.9973
Eosinophils sputum	8.10	16.82	102	6.95	16.02	90	9.67	17.92	43	0.6066
Eosinophils-BAL (SARP)	2.46	5.77	22	1.43	2.39	10	3.33	7.55	12	0.8423
Neutrophils-BAL (SARP)	6.65	10.55	22	3.13	2.65	10	9.58	13.64	12	0.5305
Eosinophils-BAL	1.47	4.06	101	0.76	1.36	63	2.65	6.26	38	0.1866
Neutrophils-BAL	2.97	6.84	101	3.62	8.37	63	1.88	2.69	38	0.0963
Eosinophils-biopsy tissue	38.75	72.44	135	24.75	47.55	83	61.11	96.56	52	0.0049
Neutrophils-biopsy tissue	91.24	131.64	138	111.88	154.53	85	58.13	72.59	53	0.0047

*Probability value (p-value) from Wilcoxon Rank Sum test

Table 5.2. Descriptive statistics for categorical variables for all study subjects and by age at asthma onset

Variable	All			Early Onset			Late Onset			p-value*
	Number	Percent	N	Number	Percent	N	Number	Percent	N	
Severe	143	64.71	221	82	63.08	130	61	67.03	91	0.5447
Male	95	42.60	223	63	48.09	131	32	34.78	92	0.0479
Overweight	102	64.97	157	51	59.30	86	51	71.83	71	0.1015
Obese	58	36.94	157	32	37.21	86	26	36.62	71	0.9393
Non-white	34	18.68	182	21	20.19	104	13	16.67	78	0.5459
BAL/sputum eosinophil status	58	34.52	168	27	26.47	102	31	46.97	66	0.0063
BAL/sputum neutrophil status	58	34.52	168	36	35.29	102	22	33.33	66	0.7940
Biopsy tissue eosinophil status	35	25.93	135	16	19.28	83	19	36.54	52	0.0259
Biopsy tissue neutrophil status	16	11.59	138	13	15.29	85	3	5.66	53	0.0856
BAL eosinophil status	18	15.65	115	5	7.35	68	13	27.66	47	0.0032
BAL neutrpohil status	23	20.00	115	14	20.59	68	9	19.15	47	0.8496
Sputum eosinophil status	47	46.08	102	23	38.98	59	24	55.81	43	0.0922
Sputum neutrophil status	38	37.25	102	24	40.68	59	14	32.56	43	0.4023
Inhaled corticosteroid use	118	67.82	174	69	69.70	99	49	65.33	75	0.5418
Oral corticosteroid use	80	46.51	172	43	43.43	99	37	50.68	73	0.3461
Sinusitis	104	61.90	168	60	61.22	98	44	62.86	70	0.8299
GERD	91	50.00	182	53	47.32	112	38	54.29	70	0.3606
History of pneumonia	100	65.79	152	67	72.83	92	33	55.00	60	0.0236
Aspirin sensitivity	29	20.00	145	14	16.87	83	15	24.19	62	0.2752
Father allergy	51	38.35	133	34	41.98	81	17	32.69	52	0.2826
Father asthma	31	20.00	155	23	25.00	92	8	12.70	63	0.0600
Mother allergy	62	42.76	145	41	47.13	87	21	36.21	58	0.1929
Mother asthma	35	21.60	162	26	26.53	98	9	14.06	64	0.0594
Parent allergy	88	56.77	155	55	59.14	93	33	53.23	62	0.4665
Parent asthma	59	35.54	166	42	42.00	100	17	25.76	66	0.0324
Atopy	153	76.12	201	105	87.50	120	48	59.26	81	<0.0001

*probability value (p-value) from chi-square tests of association

Table 5.3. Odds Ratios (OR), along with 95% confidence intervals (CI), from the univariate logistic regression analysis of age at asthma onset among clinically diagnosed asthma subjects (referent group early onset)

Variable	N	Coefficient	Standard Error	OR	95% CI	p-value*	Unit change
Duration	208	-0.120	0.018	0.887	0.857 - 0.919	<0.0001	5 year increase
Sex (referent=female)	223	-0.552	0.280	0.576	0.332 - 0.997	0.0487	1
PC20	122	-0.040	0.097	0.961	0.795 - 1.160	0.6765	1
IgE	66	-0.002	0.001	0.998	0.996 - 1.001	0.1531	1
FeNO	67	0.001	0.004	1.014	0.929 - 1.106	0.7605	1
GERD	112	0.279	0.306	1.322	0.726 - 2.406	0.3611	1
Age	179	0.064	0.014	1.904	1.460 - 2.483	<0.0001	10 year increase
Oral corticosteroid use	172	0.292	0.310	1.339	0.729 - 2.456	0.3465	1
Inhaled corticosteroid use	174	-0.199	0.327	0.819	0.432 - 1.554	0.5420	1
Sinusitis	168	0.069	0.323	1.072	0.569 - 2.017	0.8302	1
History of pneumonia	152	-0.785	0.350	0.456	0.230 - 0.905	0.0247	1
Atopy	201	-1.571	0.357	0.208	0.103 - 0.418	<0.0001	1
Asthma severity (referent=non-severe)	221	0.174	0.288	1.190	0.677 - 2.092	0.5449	1
FEV1 percent predicted	201	0.000	0.006	1.003	0.887 - 1.134	0.9608	10% decrease
FVC percent predicted	199	-0.002	0.007	1.024	0.898 - 1.167	0.7281	10% decrease
FEV1/FVC	203	0.677	1.040	0.935	0.755 - 1.156	0.5940	10% decrease
Sputum neutrophil status	102	-0.351	0.420	0.704	0.309 - 1.603	0.4031	1
Sputum eosinophil status	102	0.682	0.407	1.977	0.891 - 4.389	0.0939	1
BAL neutrophil status	115	-0.090	0.477	0.914	0.359 - 2.326	0.8496	1
BAL eosinophil status	115	1.572	0.568	4.817	1.583 - 14.653	0.0056	1
Biopsy tissue neutrophil status	138	-1.102	0.666	0.332	0.090 - 1.227	0.0983	1
Biopsy tissue eosinophil status	135	0.880	0.400	2.411	1.100 - 5.285	0.0280	1
Eosinophil status BAL and sputum	168	0.900	0.334	2.460	1.280 - 4.730	0.0069	1
Neutrophil status BAL and sputum	168	-0.087	0.333	0.917	0.477 - 1.762	0.7941	1
Aspirin sensitivity	145	0.453	0.417	1.573	0.695 - 3.562	0.2774	1
Parent allergy	155	-0.241	0.331	0.786	0.411 - 1.503	0.4668	1
Parent asthma	166	-0.736	0.347	0.479	0.243 - 0.946	0.0339	1
Father allergy	133	-0.398	0.372	0.671	0.324 - 1.391	0.2837	1
Father asthma	155	-0.829	0.449	0.436	0.181 - 1.051	0.0645	1
Mother asthma	162	-0.792	0.426	0.453	0.197 - 1.045	0.0633	1
Mother allergy	145	-0.451	0.348	0.637	0.322 - 1.258	0.1941	1

*probability value from maximum likelihood ratio

Table 5.4. Odds Ratios (OR), along with 95% confidence intervals (CI), from the multiple logistic regression analysis of age at asthma onset among 124 clinically diagnosed asthma subjects and among 86 severe asthma subjects

Variable	Coefficient	Standard Error	OR	95% CI	p-value
Among all subjects					
Parental history of asthma	-1.833	0.609	0.160	0.048 - 0.528	0.0026
Duration of asthma*	-0.175	0.035	0.418	0.298 - 0.587	<0.0001
Atopy	-1.934	0.707	0.145	0.036 - 0.578	0.0063
Eosiniophils in BAL/sputum	1.377	0.544	3.964	1.366 - 11.509	0.0113
Among only severe asthma subjects					
Parental history of asthma	-3.190	0.956	0.041	0.006 - 0.268	0.0008
Duration of asthma*	-0.217	0.055	0.338	0.200 - 0.580	<0.0001
Atopy	-2.335	0.870	0.097	0.018 - 0.532	0.0072
Eosiniophils in BAL/sputum	1.809	0.793	6.105	1.291 - 28.867	0.0225

*Odds ratio for duration of asthma for a 5 year increase in duration

Chapter 6

A multivariate analysis of risk factors for the air-trapping asthmatic phenotype as measured by quantitative CT analysis

Background: Severe asthma subjects have an increase in physiologically measured air trapping. However, a similar study using CT measures of air trapping has not been performed.

Rationale: The current study was designed to address two hypotheses: 1) air trapping, as measured by multi-detector CT quantitative methodology, would be a predictor of a more severe asthma phenotype; and 2) historical, clinical, allergic, or inflammatory risk factors could be identified via multivariate analysis to provide a model for this phenotype.

Methods: Multi-detector CT scanning of a subset (n= 60 severe, 34 mild moderate asthmatic subjects and 26 non-asthmatic subjects) of the Severe Asthma Research Program (SARP) subjects was performed using near isotropic reconstructions of scans performed at functional reserve capacity. The scan data were analyzed quantitatively to determine the amount of lung tissue less than -850 HU, -910 HU, -950HU. "Air trapper" was defined as those for whom 9.66% or more of the lung tissue was less than – 850 HU. CT determined air trapping were compared to select clinical and demographic data from SARP I participants. Univariate and multivariate statistical analyses were performed.

Results: "Air trappers" were more likely to have a history of asthma-related hospitalizations, intensive care unit visits and/or mechanical ventilation. Duration of asthma, history of pneumonia, high levels of neutrophils in the airway, air flow obstruction as measured by FEV_1/FVC and atopy, were identified as independent risk factors associated with the air trapping phenotype.

Conclusion: Quantitative CT determined air trapping in asthmatic subjects identifies a group of individuals with a high risk of severe disease, particularly those with high intensive health care utilization. In the 94 asthmatics studied, several independent risk factors for the presence of this phenotype were identified, perhaps most interestingly history of pneumonia, neutrophilic inflammation, and atopy.

Introduction

Physiologically defined air-trapping has been considered a risk factor for more severe forms of obstructive airways disease, including both asthma and COPD, for many years.[1,2] While air trapping is often defined physiologically by the increase in residual volume or by the relationship of residual volume to total lung capacity, air trapping can now also be defined and objectively quantified using high resolution multi-detector (MD) CT imaging and quantitative software analysis. Software programs have been utilized to quantify the amount of lung tissue that falls within a range of Hounsfield units (HU), producing a histogram curve of the lung voxels. Lower (negative) values represent the least dense (more air-like) areas, while higher numbers represent more dense areas, such as blood and bone.[3-19] Previous studies in emphysema patients have suggested that Hounsfield units <-950, -960, -970 HU are representative of emphysematous regions as identified on pathologic specimens.[4,8,15] On the other hand, the normal specific volume of the lung at total lung capacity (TLC) is 6.0 ml/gm, which corresponds to a CT density of - 856 HU.[6,7] The notion that at FRC the normal specific volume and hence CT density should normally be less than the TLC value suggests that -850 HU may also be a reasonable threshold for air trapping when scans are done at FRC. This CT density has been previously used to quantify air trapping in asthmatic children.[20]

Although severity of asthma has been associated with air trapping measured plethysmographically, little is understood regarding the factors which might predispose to this condition. In asthma, there is often a strong relationship between FEV_1 values and residual volume, suggesting that airway obstruction is strongly related to distal lung air trapping. However, no previous studies have integrated a range of possible risk factors, in-

cluding those related to allergy, past medical events, co-morbid conditions and inflammatory processes.

The current study was designed to address two hypotheses: 1) air trapping, as measured by MDCT quantitative methodology, would be a predictor of a more severe asthma phenotype; and 2) independent historical, clinical, allergic, or inflammatory risk factors could be identified in a multivariate analysis as a means of predicting this phenotype. In order to answer those questions, 120 well characterized severe and mild-moderate asthmatic and normal subjects enrolled in the NIH Severe Asthma Research Program (SARP) underwent MDCT scans at functional residual capacity (and total lung capacity) between October of 2002 and June of 2006. The CT images were compared across subject groups for the presence of air trapping, predefined as the percent of the lung <-850 HU. Following the identification of the air-trapping phenotype, a multivariate analysis was employed to identify the risk factors associated with this phenotype.

METHODS

Study design

As part of the NIH Severe Asthma Research Program (SARP), a cohort of subjects underwent a detailed history, physical examination, allergy skin testing, laboratory tests (including analysis of sputum and measurement of IgE levels), pulmonary function tests, and multi-detector CT of the chest prior to the performance of fiberoptic bronchoscopy – all using a standardized protocol developed by the SARP. Details regarding the procedures and a description of the entire SARP cohort have been previously described.[21] The study was approved by each SARP site's Institutional Review Board and monitored by an independent NIH Data and Safety Monitoring Board (DSMB).

Human subjects

Subjects were 13-60 years of age and were non-smokers with a smoking history of less than 5 pack-years and no smoking within the past year. The inclusion criteria for the three groups were as follows. Normal subjects were in good overall health with normal lung function and a negative methacholine bronchoprovocation (provocative concentration of methacholine causing a 20% decline in forced expiratory volume in one second (FEV_1) (PC_{20}) > 16 mg/ml). All asthma subjects had physician diagnosed asthma, no concurrent lung disease, and a positive methacholine bronchoprovocation ($PC_{20} \leq$ 8mg/ml) or $\geq$ 12% improvement in FEV_1 post-bronchodilator. In addition, asthma subjects were classified as severe or non-severe. Severe persistent asthma subjects met severe refractory asthma criteria from the ATS Workshop.[22] All subjects with asthma that did not meet the criteria for severe asthma were classified as non-severe asthma for this study.

Subjects underwent pulmonary function testing, methacholine challenge, allergy skin testing, CT scan, exhaled nitric oxide sampling, sputum induction, bronchoscopy and filled out questionnaires that asked about demographic factors, medication use, and medical history. In this report, subjects were considered atopic if they had one or more positive allergy skin tests.

CT technique

Subjects underwent MDCT spiral scans of the chest with 4, 16 or 64 detector rows (GE Light Speed Ultra, GE Lightspeed 16, Siemens Volume Zoom, Siemens Sensation 16, Siemens Sensation 64 multidetector CT scanners). Suspended expiratory measurements at FRC were obtained at the following settings: GE: 1.675-1.75 pitch, 0.6 sec rotation

time, 120 kV, 50-100 mAs, detector collimation 0.625 and 1.25 mm, 0.625-1.25 mm reconstructed slice thickness, medium smooth "standard" reconstruction algorithm; Siemens: 1.5 pitch, 0.5 sec rotation time, 120 kV, 50 mAs, detector collimation of 0.75 mm, 1mm reconstructed slice thickness, slice interval = field of view (mm)/512, and a medium smooth reconstruction algorithm (Siemens B31f) – effective mAs = 33 (low radiation dose). The radiation dose from the low dose CT scans (one at TLC and at FRC) ranged from 1.55 mSv effective dose to 1.70 mSv effective dose. The radiation dose from the higher dose CT scans ranged from 4.0 to 7.6 mSv effective dose. The higher effective doses occurred in larger female subjects. The total radiation dose (TLC and FRC combined) from the low dose CT scans ranged from 1.55 mSv effective dose to 1.70 mSv effective dose while the total radiation dose from the higher dose CT scans ranged from 4.0 to 7.6 mSv effective dose. The higher effective doses occurred in larger female subjects. The average dose per person from all sources of natural radiation is about 300 mrem or 3 mSv per year.[23] Thus a low dose volume MDCT scan (suitable for the measure of air trapping) as used in these analyses is equivalent to approximately 30% of the radiation an individual is naturally exposed to in a year, while the high dose is equivalent to 1.5 to 2 years of natural radiation exposure.

MDCT evaluation software

MDCT scans were obtained and analyzed using automated, lung parenchymal evaluation software. This software, using an approach built on the density mask technique, segments the lung from the rest of the thoracic anatomy and generates histogram curves of the lung voxels to analyze the percent of lung tissue between different MDCT voxel numbers, expressed in HUs (Pulmonary Profiler, VIDA Diagnostics, Iowa City, IA).[24] A

review of the software capabilities and a validation has been published elsewhere.[25] The specific MDCT measurements used in the data analysis included percent low attenuating area (%LAA) less than – 850 HU, %LAA – 900 HU, %LAA – 950 HU. The measurements were performed by a trained technician at the University of Iowa, Carver College of Medicine in a blinded manner. In Figure 1, a CT-derived image of the lung and airways (left column) for a severe (lower row) and a non-severe (upper row) asthmatic are illustrated. Trapped air defined as voxels within the lung field falling below -850 are highlighted in the right column. By clicking on any airway path, the software labels the bronchial segments along the path of interest. There is a marked increase in air trapping in the severe asthmatic.

Statistical analysis

Association with lung function

Initial correlations between lung function (specifically FEV_1/FVC as the most definitive parameter to measure airflow limitation) and the percent of lung at -850, -900 and -950 HU (at both FRC and TLC) were evaluated using Spearman's correlations in the asthma subjects (FRC: -850 HU:-0.583, -900 HU:-0.514, -950 HU: -0.403 $p<0.0001$ for each; TLC: -850 HU: -0.362 $p<0.001$, -900 HU:-0.318 $p=0.002$, -950 HU:-0.199 $p=0.06$). Correlations between lung function and percent of lung density were stronger at all densities in FRC scans (indicative of air trapping) as compared to TLC scans. Additionally, correlations at -850 HU were stronger than at -900 or -950 HU. Therefore, further studies were performed using -850 HU at FRC.

Subject classification

The percent of lung less than -850 HU units was then dichotomized using a median split of the full cohort (n=120, median = 9.66%). Because airways within the lung boundaries are included in the VIDA software version of the density mask[5,14,26-28] it is expected that all subjects will have some voxels falling within range of interest. Subjects above the median were considered to be air trappers and were compared to those below the median (non-trappers). Airway neutrophil and eosinophil variables were calculated based on sputum and bronchoalveolar lavage (BAL) data. Sixty six of the 90 asthma subjects had either sputum or BAL neutrophil and eosinophil levels. These subjects were classified as neutrophil positive if either sputum or BAL neutrophils were above the median among asthma subjects of either distribution (sputum or BAL). The median for BAL neutrophils was 1%, while the median for sputum neutrophils was 23.6%. If levels were below the median, subjects were considered neutrophil negative. Subjects were classified as eosinophil positive and negative in the same manner. The median for BAL eosinophils was 0%, while the median for sputum eosinophils was 1.7%.

Association with air trapping

The chi-square test for association was used to determine if air trapping was associated with severe asthma and its outcomes (such as intensive care unit admission). Logistic regression analysis was used to evaluate the univariate associations among variables for air trapping and then to determine a group of risk factors associated with air trapping in a population of asthmatic subjects. For the multivariate model, all covariates of interest that were associated with air trapping at the 0.20 significance level in univariate analyses were retained for possible inclusion in the final multivariable logistic regression model.

Variables were selected based on the purposeful selection method described by Hosmer and Lemeshow.[29] Continuous variables found significant in the final model (FEV1/FVC and asthma duration) were assessed for linearity in the logit graphically and using the fractional polynomials method. Both variables were found to be linear in the logit. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the relationships between air trapping and statistically significant covariates ($p<0.05$). Confounding was assessed by examining the change in magnitude of the estimates, with a change of greater than 10% considered to be confounding.[29] See Table 6.3 for a list of variables of interest by group and their univariate associations with air trapping. All analyses were conducted with SAS (SAS 9.1, SAS Institute Inc., Cary, NC).

RESULTS

A total of 120 of the SARP subjects underwent MDCT and were included in this study. There were 60 severe persistent asthma subjects, 34 mild-moderate persistent asthma subjects, and 26 normal controls. Subject demographics by asthma status are listed in Table 6.1 and by air trapping status in Table 6.2.

Air trapping and severity of illness

The relationship of severe asthma and its outcome with air trapping is illustrated in Figure 2. More subjects who were air trappers were severe asthmatics, although the relationship was not statistically significant ($p=0.058$). Air trappers were significantly more likely to have a history of asthma-related hospitalizations, intensive care unit visits, and/or mechanical ventilation compared to subjects classified as non-trappers. These differences suggested that air trapping as measured by CT, may identify a different and

more severe phenotype of asthma. We therefore sought to build an explanatory model of air trapping to identify a set of clinical variables that were risk factors for air trapping.

Univariate analysis of risk factors associated with air trapping

Subjects considered as air trappers were initially compared to the non-trapping referent group by determining univariate odds ratios (Table 6.3). Subjects who were considered air trappers had significantly greater air flow limitation as measured by FEV_1 and FVC percent predicted and FEV_1/FVC. Subjects with air trapping were also more likely to be male, be older, and have a longer duration of asthma than those who were not air trappers. Additionally, those who were air trappers were more likely to report a clinical history of pneumonia and be atopic than subjects not classified as air trappers. Presence of airway neutrophils was marginally associated with air trapping, while airway eosinophils were not associated. In a subset of subjects with exhaled nitric oxide measurements, the risk of air trapping was inversely associated with nitric oxide (OR= 0.85, 95% CI: 0.717-0.995 for each 10 unit increase in FE_{NO}). Because FE_{NO} values were available in only 62% of subjects, it was not considered when determining the final model. For a complete list of variables examined and their association with air trapping, see Table 6.3. The SARP testing is conducted at multiple locations with different study staff, so differences in locations may result. However, air trapping was not associated with the center location p=0.48, so it is not likely that the results differ by location, however, residual confounding by clinical location may still exist.

Multivariate logistic regression analysis

Imputation of cellular data and evaluation of the imputation model.

The odds of air trapping were almost twice as high in subjects who were classified as airway neutrophil-positive compared to neutrophil-negative subjects, but the association did not reach statistical significance. However, not all subjects had results for airway neutrophil status. In light of the possible association between asthma severity and neutrophilic inflammation, neutrophil status was imputed as follows. For those subjects with lung inflammatory cell data, logistic regression analysis was carried-out to identify variables that were significant predictors of neutrophilic inflammation. The model was then applied to the subjects missing neutrophil data (n=28), and the predicted probabilities were used to classify subjects who were missing lung inflammatory data as neutrophil positive or negative. Table 6.4 displays the predictor variables, their coefficients, standard errors, and probability values. The best main effects model included only history of pneumonia and overweight. Interactions were assessed, and the interaction between overweight and smoking history was significant at the 0.10 level. Therefore, the variables included in the final imputation model were: overweight (BMI>25), history of pneumonia, smoking history (ever, never), and the interaction between overweight and smoking. Figure 2 presents a plot of sensitivity and specificity of different cut-points of the predicted probabilities. Table 6.5 provides a summary of sensitivity, specificity, and 1-specificity based on the logistic regression model used for the imputation. The selection of the cut-point was made at the predicted probability (0.70) which maximized both sensitivity (75.6%) and specificity (68.4%). Figure 3 displays the receiver operating characteristic (ROC) curve for the imputation model. The ROC curve is a plot of sensi-

tivity versus one minus specificity over all possible cut points. The area under the ROC curve measures the model's ability to discriminate between the "diseased" (neutrophilic positive) and "non-diseased" (neutrophillic negative). The area under the ROC curve is 0.813, which is considered to be "acceptable to excellent" discrimination.[29]

Results from the multivariate logistic regression model are summarized in Table 6.6. Duration of asthma, history of pneumonia, high levels of neutrophils in the airway, air flow obstruction as measured by FEV_1/FVC and atopy were identified as risk factors associated with the air-trapping phenotype. The risk of air trapping was moderately increased with increasing duration of asthma. For every 5 year increase in asthma duration, there was a 42% increase in the odds of air trapping. A decreased FEV_1/FVC ratio was associated with increased risk of air trapping, with a 5% decrease corresponding to a 61% increase in the odds of air trapping. Subjects who reported a history of pneumonia were at a significantly increased risk of air trapping compared to those who reported no history of pneumonia. Having airway neutrophils above the median in either sputum or BAL was also associated with a significantly increased risk of air trapping. Subjects who were atopic were more likely to be air trappers than subjects who were non-atopic. Although the estimates were relatively large, the odds-ratio confidence intervals were wide for history of pneumonia, neutrophil status, and atopy, all of which are reflective of the relatively small sample size. The model building process was also completed using only those subjects with measured neutrophil data. The model terms were identical, and the coefficients were not substantially different (Table 6.6) from those in the model that included the imputed neutrophil status. After examination of the effect on the estimates, no variables were entered into the final model as confounders. The estimate for neutrophil

status did change more than 10% when oral steroids were entered into the model. However, oral steroids were not included as a confounder into the model due to model instability resulting from over-fitting the model.[29,30]

Air trapping in normal controls

As shown in Table 6.1, 9 of the 26 normal control subjects were classified as air trappers. Power limited the ability to analyze associations of clinical variables with air trapping among normal controls using multivariate models. However, the risk of air trapping increased significantly with increasing levels of airway obstruction (as measured by FEV_1/FVC) even though FEV_1/FVC values were within the normal range. Females were also less likely to be air trappers than males although the relationship did not reach statistical significance ($p=0.11$). None of the other variables that were associated with air trapping in asthmatics were associated with air trapping in the normal group.

DISCUSSION

This is the first large study of CT measured air-trapping in a range of extensively characterized asthmatic subjects to identify independent risk factors for the air trapping phenotype, a phenotype associated with the most severe form of asthma. This assessment of air trapping was quantitatively and objectively performed using a histogram based assessment of lung densities (VIDA Diagnostics, Coralville Iowa) based on the density mask, but which employs a more sophisticated method for identifying lung boundaries.[24] Muller et al.[28] developed the original concept for the density mask based on early observations which demonstrated that lung volume [31] and regional air content, or density,[32] could be accurately assessed via CT. This density mask method identified the lung field

and a density threshold within the lung field to count emphysema-like lung voxels. Since then, the histogram of voxel density within the lung field has been widely used to identify emphysema-like lung and fibrosis as reviewed in[26] and, in the case of this study, trapped air when the lung is imaged at low lung volumes.[19] The histogram-based assessment of the lung used here replaces the former "density mask" approach, but the essence of the measurement remains the same.

In this study, air trapping subjects were defined as individuals with ≥9.66% of their total lung volume at FRC <-850 HU. While this density is not as extreme as the -910 to -970 HU threshold applied to COPD/emphysema, previous reports suggest that this degree of hyperlucency (<-850 HU) should only be seen at TLC as this density is measuring a fully distended alveolus.[33] Additionally, higher correlations with lung function were seen at the -850 HU threshold than at either -900 HU or -950 HU, suggesting that -850 HU may be a more appropriate threshold for asthma. As asthma, even in severe cases, is not pathologically an "emphysematous" process involving alveolar septal destruction, the better discrimination of our data at this higher cut-off is not surprising.

This threshold applied to asthma, identified a marginally more severe cohort using the ATS Refractory Asthma definition, but who were much more likely to have had a history of a severe and/or near fatal asthma event, similar to previous reports for physiologic measures of air trapping.[1] A recent study, from this cohort, reported that severe asthmatics had a greater component of physiologically measured air trapping relative to airflow limitation than milder subjects and concluded that air trapping is broadly associated with severe asthma.[34] Further, Mitsunobu et al. assessed air trapping using MDCT and reported that the relative area of the lungs less than -950 HU correlated with air flow limita-

tion and with severity of asthma. Therefore, our findings are not completely unexpected.[35] Unlike previous studies, the SARP database contains a multitude of variables which were then utilized to determine risk factors for air trapping on MDCT scan using a multivariate modeling approach.

Based on first analyzing the data in a univariate manner to identify factors for consideration in the multivariate model, numerous factors were found to be associated with the air trapping phenotype. Not surprisingly, these included the degree of airway obstruction, as measured by FEV_1/FVC. We chose FEV_1/FVC for our analysis (among the multiple related spirometric values available) as the FEV_1% predicted can be low in restrictive, as well as obstructive disease, while the FEV_1/FVC decreases only with increasing airflow limitation (or obstruction). This relationship has been previously reported in air trapping measured physiologically [1] and in air trapping measured by CT at different attenuation rates – albeit, based on only univariate analysis.[35] In addition, a variety of other factors, including a longer duration of disease, male sex, and lower FE_{NO} were either marginally or significantly related. The association of air trapping with increased age and longer duration of disease suggests a contribution of remodeling over time, while the relationship with male sex could be explained by the greater likelihood of asthma arising in early childhood in boys than in girls or a greater susceptibility to elements of the remodeling process. Interestingly, when matched for severity, male asthmatics appear to have lower FEV_1 as percent predicted, as well as lower FEV_1/FVC, than females.[36] The relationship of air trapping with lower FE_{NO} is somewhat surprising but may suggest that, in this cohort, NO has a bronchodilating effect [37] that limits the degree of air trapping seen. However, because of its limited sample size, FE_{NO} was not considered in the multivariate

analysis. Further studies are needed to determine if it is in fact protective against air trapping. Despite the potential relationship with FE_{NO}, eosinophils were not associated with air trapping. The relationship of eosinophils to airway obstruction has been highly variable across studies.[1,38-41] This lack of a clear signal for eosinophils and airway obstruction in physiologic studies perhaps explains why no relationship was seen in these studies as well.

A multivariate analysis was undertaken selecting factors in the univariate analyses associated with air trapping ($p<0.20$). In the multivariate analysis, FEV_1/FVC, duration of disease, reported history of pneumonia, neutrophillic airway inflammation, and atopy were identified as independent risk factors. Some univariately associated variables were not significant in the multivariate model, likely due to the overlapping nature of these variables. Among the risk factors, perhaps the most interesting are history of pneumonia, neutrophilic inflammation, and atopy. Because this is a cross-sectional study, causal relationships can not be presumed, with the observed relationships as likely to be a consequence of air trapping as causes. Despite these uncertainties, the results remain provocative. Consistent with our finding of an association of the more severe air trapping phenotype with history of pneumonia, analysis of the entire SARP database (>400 subjects) determined pneumonia to be independently associated with asthma severity (OR=3.30 95%CI:1.92-5.69).[21] More severe disease may increase the risk for developing pneumonia due to poor secretion clearance and immunosuppression by corticosteroids (CS). An analysis of a large healthcare database found that asthmatics are at higher risk of development of pneumonia.[42] Inhaled CS (ICS) as a risk factor for pneumonia is also becoming increasingly identified. ICS use has been associated with an increased risk

for pneumonia in prospective studies of COPD.[43,44] All severe asthma subjects in this study were on high ICS doses which could have contributed to a higher pneumonia risk. Only longitudinal studies will confirm (or refute) that relationship.

Another interesting risk factor was airway neutrophilia. The observation that pneumonia and neutrophils are independent risk factors for air trapping suggests that historical pneumonia is not driving the neutrophilia, nor is the neutrophilia likely a residual of pneumonia. It is possible that neutrophlia is a by-product of high corticosteroid use in this population. Corticosteroids inhibit neutrophil apoptosis and enhance their activity and survival, which may explain their increase.[45,46] Unfortunately, despite greater than 100 patients in this trial, power limitations restricted our ability to adjust the model for corticosteroids. Whether caused by more severe disease or its treatment, higher levels of lung neutrophils could lead to air trapping. Neutrophils produce enzymes, including elastases and metalloproteinases which contribute to elastin (and other matrix elements) breakdown observed in fatal asthma and severe cases of asthma.[47-49] These airway and perhaps parenchymal changes could alter elastic recoil properties and lead to a more "emphysematous-like" pattern and increased air trapping. An emphysematous-like pattern seen on CT in chronic asthma subjects has been reported in other studies.[50]

The final risk factor of interest was atopy. Had the analysis included non-asthmatics, this association would not have been surprising, as atopy is strongly associated with asthma. However, the analysis was restricted to asthmatics, 82% of whom were atopic. Non-atopic asthmatics are a mix of individuals including aspirin sensitive to post-viral adult onset asthmatics.[39,51] Because the multivariate analysis is adjusted for asthma duration, the relationship cannot be attributed to non-atopic asthmatics having a shorter disease du-

ration. The relationship between atopy and air trapping has not been extensively evaluated. One study reported more extensive airway remodeling (assessed by high resolution CT) among non-atopic individuals than atopic individuals.[52] This study did not specifically assess air trapping, and only qualitative analyses were conducted. Further studies are needed to determine whether the remodeling process associated with non-atopic differs from atopic asthma, leading to differences in radiologic and physiologic changes.

Finally, a large percentage of normal controls met the threshold for air trapping. It is unclear whether these subjects are at increased risk for asthma, have genetic predisposition to air trapping, or had some past insult which induced these changes. Although these subjects had normal pulmonary function testing and negative methacholine challenges, they had a lower FEV_1/FVC and tended to be males, both seen in the asthmatics with air trapping. Studies of air trapping in normal subjects are needed to determine if air trapping is a risk factor for asthma development.

There are limitations to this report. Although this is one of the largest CT studies of asthma to date, a larger sample size would have provided increased power to identify independent risk factors, including the influence of corticosteroids. Additionally, airway neutrophil data were unavailable for 23% of the study subjects. We were able to impute the missing airway neutrophil status in order to consider this variable in the multivariate logistic regression model based on the entire study cohort. The imputation could have resulted in misclassification of neutrophil status; however, including the imputed values did not appreciably change the results obtained using only those subject with measured airway neutrophils. The study is a cross sectional study; therefore, conclusions about temporality of the association between air trapping and the risk factors cannot be made.

Further prospective studies should be carried out to determine if the risk factors precede the air trapping. Because this study made multiple statistical tests in an effort to find a set of risk factors associated with the air trapping phenotype, some of the results might be attributed to chance.

This study also has several strengths. The study population was well characterized and included a wide range of asthma severities, including normal control subjects. It also included lung inflammatory markers, which are difficult to obtain and therefore not widely used. We utilized a multivariate analysis that included a wide range of data and allowed for the examination of potential confounding. Additionally, air trapping was measured quantitatively rather than merely subjectively which should decrease the likelihood of measurement bias. Subjects were enrolled from multiple locations which increased the numbers of asthmatics thereby increasing the power. Confounding resulting from different locations may be present; however, air trapping did not differ by location of the clinic. Further studies in this population may want to include an analysis of the random effect of clinic location.

In conclusion, the data reported here support the use of CT scanning in asthmatic subjects to identify a group of individuals with a high risk of severe disease, particularly those with high and intensive health care utilization. In the 94 asthmatics studied, several independent risk factors for the presence of this phenotype were identified, perhaps most interestingly history of pneumonia, neutrophilic inflammation, and atopy. Further longitudinal and hypothesis-driven studies, which specifically evaluate the role of these factors in the development of this phenotype, are needed.

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Figure 1. CT-derived three-dimension display of the lungs, airways and regions of air trapping. Example comparison of two asthmatic subjects falling in the non-severe (upper row) or severe (lower row) categories. In the left column, the lung lobes and airway tree are shown from a ventral view. In the right column, the air trapping is is depicted, color coded by lobe and displayed from the dorsal aspect. Software allows one to click on an airway path of interest and airway segment labels are automatically generated. Trapped air defined as voxels within the lung field falling below -850 are highlighted and coded by lobe in the left column. The severe asthma subject has 21% of lung less than -850 HU as compared to the non-severe asthma subject with % of lung less than -850 HU. Images from Pulmonary Workstation 2.0 (Vida Diagnostics, Coralville, Iowa).

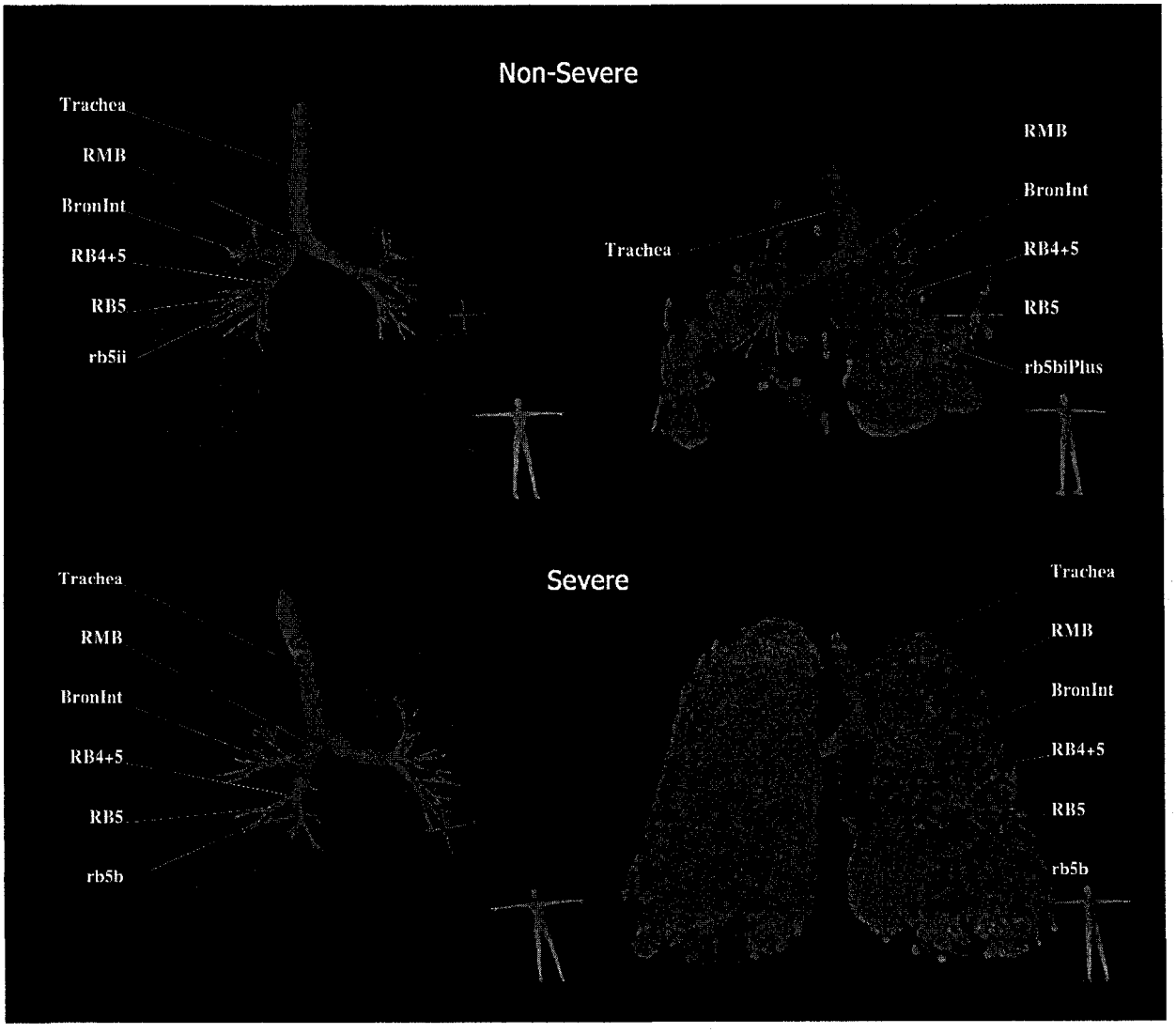

Figure 2. Association between air trapping and presence of severe asthma or severe asthma exacerbations.

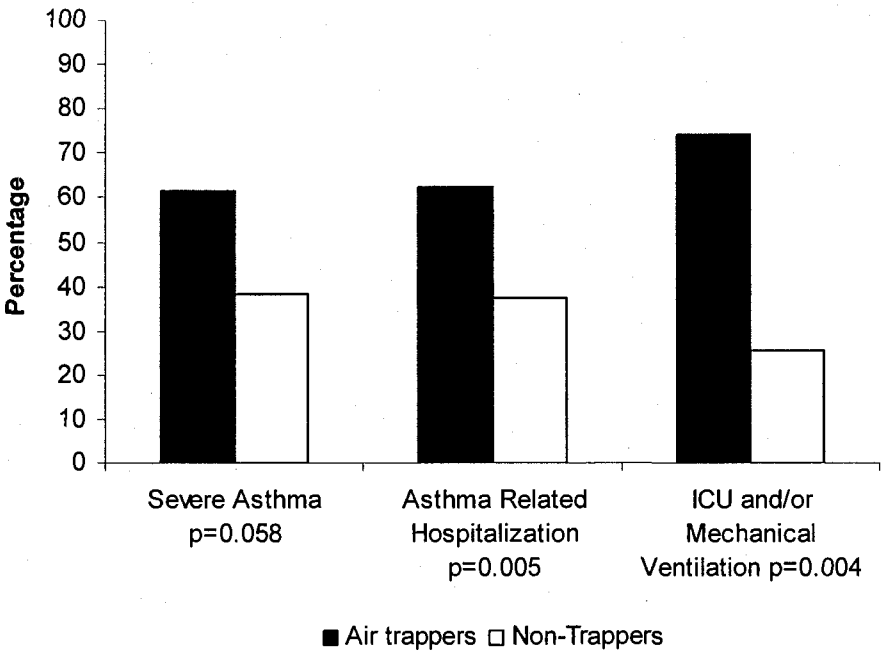

Figure 3. Sensitivity (Se) and specificity (Sp) plot of data imputation

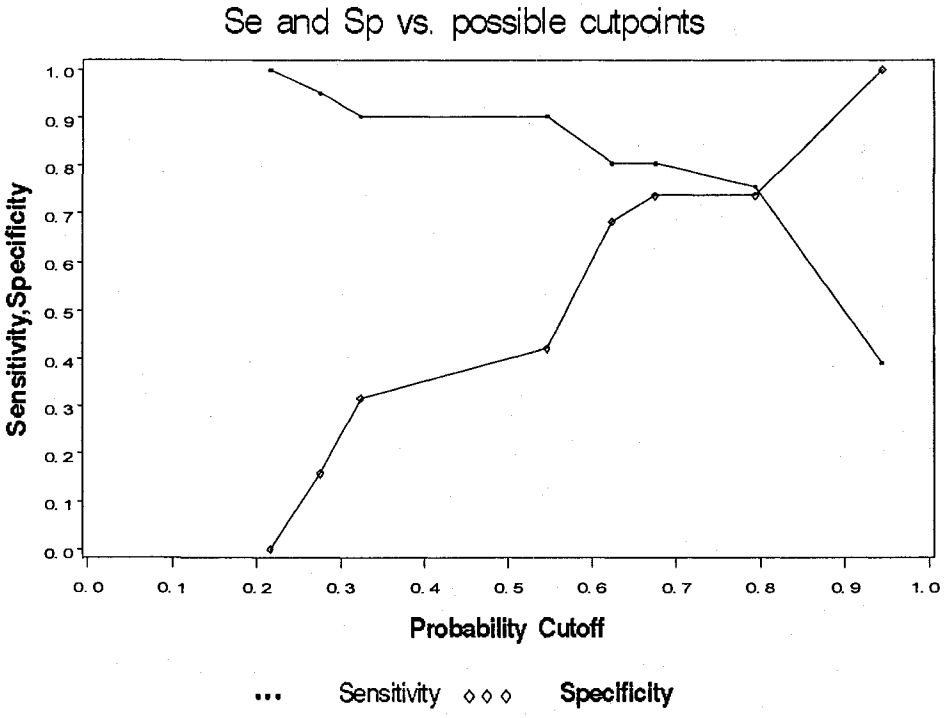

Figure 4. Receiver operating characteristic (ROC) curve of data imputation model

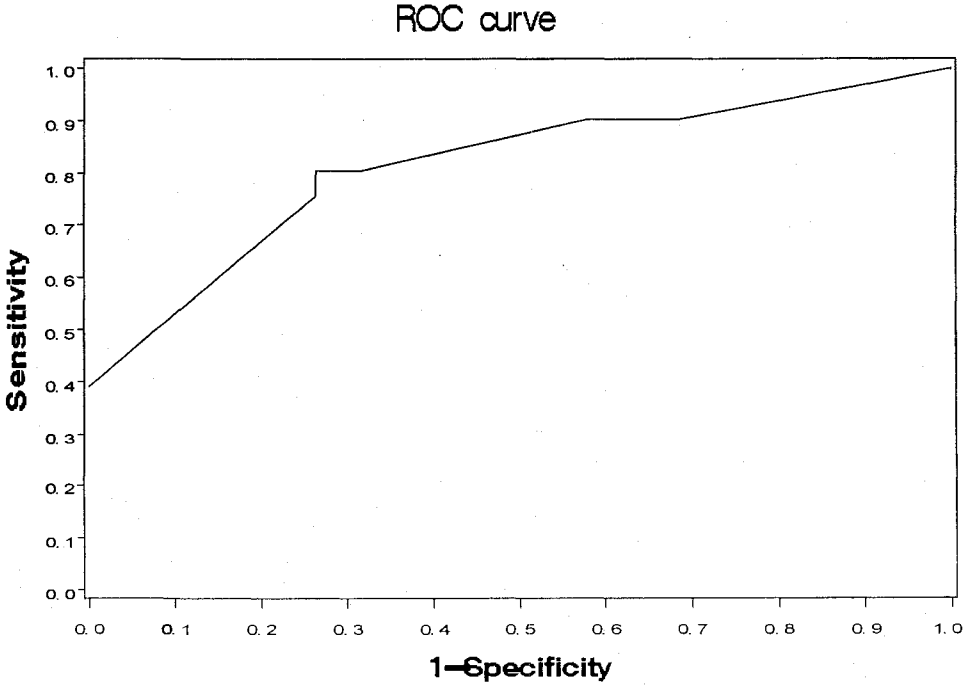

Table 6.1. Summary statistics of demographic and clinical variables for severe asthmatics, mild/moderate asthmatics, and normal controls

	Severe Asthmatics		Non-Severe Asthmatics		Normal Controls	
Number of study subjects	60		34		26	
Categorical Variables	Number	Percent	Number	Percent	Number	Percent
Female gender	33	55.00	20	58.82	17	65.38
Percent of lung less than -850 HU above median	37	61.67	14	41.18	9	34.62
Atopic	46	76.67	31	91.18	8	30.77
Current use of oral steroids	26	43.33	0	0.00	0	0.00
Current use of inhaled steroids	59	98.33	18	52.94	0	0.00
Continuous Variables	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Age	37.48	13.32	34.29	10.73	30.31	7.83
Percent less -850	20.15	16.70	12.07	12.03	12.30	16.74
FEV_1 percent predicted	62.68	22.07	79.74	16.57	99.65	9.97
FEV_1/FVC (x 100)	62.63	13.02	70.14	11.64	84.94	6.31
IgE level	441.57	694.77	229.65	295.04	93.52	171.46

Table 6.2. Summary statistics of demographic and clinical variables by air trapping status

	"Trappers"		"Non-trappers"	
Number of study subjects	51		43	
Categorical Variables	Number	Percent	Number	Percent
Female gender	24	47.06	29	67.44
Current use of oral steroids	17	33.33	9	20.93
Current use of inhaled steroids	41	87.23	36	76.60
High level of neutrophils	25	78.13	21	61.76
High level of eosinophils	9	28.13	10	29.41
Ever smoked	10	20.00	7	16.28
History of GERD	22	44.90	11	28.21
History of pneumonia	33	70.21	16	40.00
Severe asthma	37	72.55	23	53.49
Atopic	47	92.16	30	69.77
Continuous Variables	Mean	Standard Deviation	Mean	Standard Deviation
Age	39.76	12.13	32.26	11.76
Percent less -850	27.95	13.85	4.51	2.38
Duration of asthma	27.20	13.73	17.93	10.62
Age at onset of asthma	12.57	14.20	14.33	14.93
FEV1 percent predicted	59.41	21.03	80.05	16.94
FVC percent predicted	78.60	20.83	90.22	15.74
FEV1/FVC x 100	59.48	11.80	72.30	10.80
Positive skin reactions (number)	4.13	2.57	3.09	3.15
Percent eosinophils (sputum)	4.16	5.50	6.21	19.52
Percent eosinophils (BAL)	1.90	7.06	0.80	1.44
Percent neutrophils (sputum)	41.76	22.64	22.60	19.08
Percent neutrophils (BAL)	6.02	6.10	1.31	1.93
IgE level	257.80	327.51	450.46	735.57
FeNO	30.66	26.15	52.89	48.20

Table 6.3. Univariate odds ratios (OR), along with 95% confidence intervals (CI), from the univariate logistic regression analysis of air trapping clinically diagnosed asthma patients

Variable	N	Coefficient	Standard Error	OR	95% CI	p-value	Unit increase
FEV1/FVC	89	-0.099	0.023	1.638	1.307 - 2.055	<0.0001	-5
FEV1 percent predicted	94	-0.055	0.013	1.315	1.156 - 1.496	<0.0001	-5
FVC percent predicted	92	-0.036	0.013	1.197	1.052 - 1.362	0.0060	-5
Asthma duration	94	0.061	0.019	1.359	1.128 - 1.637	0.0012	5
Sex (female compared to male)	94	-0.846	0.430	0.429	0.185 - 0.996	0.0490	1
FeNO	94	0.002	0.428	0.845	0.717 - 0.995	0.0434	10
Paternal history of asthma	81	0.095	0.607	1.100	0.335 - 3.614	0.8754	1
Paternal history of allergies	79	-0.562	0.458	0.570	0.233 - 1.397	0.2192	1
Maternal history of asthma	89	-0.210	0.492	0.811	0.309 - 2.127	0.6700	1
Maternal history of allergies	82	-0.654	0.456	0.520	0.213 - 1.271	0.1515	1
History of pneumonia	87	1.263	0.454	3.535	1.453 - 8.603	0.0054	1
History of GERD	88	0.730	0.457	2.074	0.846 - 5.082	0.1107	1
Age	94	0.052	0.018	1.676	1.173 - 2.395	0.0045	10
the mean (no imputed data)	66	0.793	0.554	2.211	0.746 - 6.554	0.1525	1
the mean (no imputed data)	66	-0.063	0.544	0.940	0.323 - 2.729	0.9082	1
Oral steroid use	94	0.636	0.478	1.889	0.740 - 4.823	0.1836	1
Inhaled steroid use	93	-0.223	0.445	0.800	0.335 - 1.912	0.6157	1
BMI	93	-0.006	0.027	0.994	0.944 - 1.047	0.8277	1
Age of diagnosis	94	-0.008	0.014	0.959	0.833 - 1.104	0.5565	5
Race (white vs. non-white)	94	0.002	0.428	1.002	0.433 - 2.320	0.9960	1
PC20	60	-0.069	0.091	0.934	0.782 - 1.115	0.4482	1
Smoking history (yes/no)	93	0.251	0.544	1.286	0.443 - 3.732	0.6439	1
Nasal steriod use (yes/no)	93	0.300	0.417	1.350	0.596 - 3.058	0.4719	1
Eosinophil count from blood	90	0.473	0.807	1.605	0.330 - 7.809	0.5578	1
History of sinusitis	91	-0.329	0.423	0.720	0.314 - 1.649	0.4373	1
Severe asthma	94	-0.832	0.438	0.435	0.184 - 1.027	0.0575	1
Parental history of allergies	83	-0.550	0.516	0.577	0.210 - 1.587	0.2867	1
Atopy	94	1.628	0.618	5.092	1.517 - 17.086	0.0084	1
Overweight	94	0.549	0.450	1.732	0.717 - 4.187	0.2225	1

Table 6.4. Logistic regression models for imputation of neutrophilic data among 60 asthma subjects

Variable	Estimate	Standard Error	p-value
Main Effects Model			
History of Pneumonia	-1.567	0.729	0.0300
Overweight	2.061	0.732	0.0048
Model with Interactions			
History of Pneumonia	-1.473	0.768	0.0550
Overweight	2.641	0.853	0.0020
Smoking History	0.550	1.223	0.6528
Overweight*Smoking History	-2.872	1.596	0.0720

Table 6.5. Summary of sensitivity, specificity, and 1-specificity for classification tables using a cutpoint of 0.10 to 0.90 in increments of 0.10

Cutpoint	Sensitivity	Specificity	1-Specificity
0.1	100	0	100
0.2	90.2	0	100
0.3	90.2	15.8	84.2
0.4	90.2	31.6	68.4
0.5	90.2	42.1	57.9
0.6	75.6	42.1	57.9
0.7	75.6	68.4	31.6
0.8	39	68.4	31.6
0.9	39	100	0

Table 6.6. Odds ratios (OR), along with 95% confidence intervals (CI), from the multiple logistic regression analysis of air trapping among clinically diagnosed asthma patients with neutrophil status measured (n=60) and then with neutrophil status imputed and measured (n=87)

Variable	Coefficient	Standard Error	OR	95% CI	p-value
Neutrophil Status Measured					
Duration of asthma (5 year increase)	0.079	0.033	1.484	1.073 - 2.051	0.0262
FEV/FVC (5% decrease)	-0.073	0.033	1.438	1.044 - 1.980	0.0169
History of pneumonia	1.736	0.772	5.677	1.250 - 25.772	0.0245
Neutrophillic inflammation in airway above the median	1.965	0.819	7.137	1.435 - 35.507	0.0164
Atopy	2.912	1.306	18.384	1.422 - 237.748	0.0258
Neutrophil Status Imputed					
Duration of asthma (5 year increase)	0.070	0.028	1.420	1.081 - 1.866	0.0011
FEV_1/FVC (5% decrease)	-0.095	0.029	1.610	1.209 - 2.145	0.0118
History of pneumonia	2.146	0.723	8.547	2.072 - 35.260	0.0030
Neutrophillic inflammation in airway above the median	2.159	0.735	8.664	2.053 - 36.566	0.0033
Atopy	2.446	0.903	11.543	1.968 - 67.698	0.0067

Chapter 7

Association between aspirin intolerant asthma and C-reactive protein

Abstract

Background: C-reactive protein is an acute-phase plasma protein that is a marker of systemic inflammation. Non-allergic asthma has previously been associated with both aspirin intolerant asthma and elevated CRP levels, and aspirin intolerant asthma is a highly inflammatory phenotype of asthma.

Rationale: We hypothesized that CRP levels may be increased in aspirin sensitive asthma.

Methods: A retrospective electronic record review was conducted at National Jewish Medical and Research Center to collect various clinical data including aspirin tolerance and C-reactive protein levels. The relationship between aspirin intolerance and C-reactive protein was evaluated among 95 clinically diagnosed asthma subjects via multiple logistic regression analysis, adjusting for likely confounders.

Results: Increasing C-reactive protein levels were associated with increased odds of aspirin intolerant asthma. Additionally, forced vital capacity and blood eosinophils were found to be important variables in the relationship between C-reactive protein and aspirin intolerance.

Conclusions: This study provides evidence that C-reactive protein may be elevated in aspirin intolerant subjects and, consequently, that C-reactive protein deserves further study as a potential biomarker for the aspirin intolerant phenotype of asthma.

Introduction

C-reactive protein (CRP) is an acute-phase plasma protein that is a marker of systemic inflammation. It is mainly produced in the liver by hepatocytes in response to circulating inflammatory cytokines, namely interleukin-6 (IL-6), an important mediator of the acute phase response.[1] In the absence of infection, the stability of CRP has been shown to be similar to serum cholesterol.[2,3] CRP has been widely studied in cardiovascular disease, and more recently, CRP has been gaining attention in lung diseases, including COPD and asthma. CRP has been examined as a biomarker of systemic inflammation in airway disease, but not as a biomarker that is diagnostic for pulmonary disease. Increased CRP levels have been associated with a diagnosis of asthma,[4-6] bronchial hyperresponsiveness,[4] serum levels of eosinophil cationic protein,[6] and reduced lung function.[6-9] Olafsdottir et al. reported that non-allergic asthmatic subjects had significantly higher CRP levels than non-asthmatic subjects, while allergic asthmatic subjects had similar levels as non-asthmatic subjects,[10] although another recent study attributed the difference to confounding variables.[11] Additionally, a study examining data from the Third National Health and Nutrition Examination Survey found that body mass index accounted for a majority of the relationship between asthma and CRP.[12]

Biomarkers are objectively measured indicators of normal and abnormal biologic processes and may possibly modulate with therapeutic interventions. Additionally, they may be predictive of specific asthma phenotypes or outcomes. Several biomarkers, from different body compartments including bronchoaveolar lavage (BAL), bronchial tissue, induced sputum, exhaled breath, urine, blood and serum, have been examined in asthma, although there has not yet been a biomarker identified that is specific for this disease.

However, due to the heterogenic properties of asthma, biomarkers will likely differ between asthma phenotypes. Only a few biomarkers have been specifically identified with asthma phenotypes; two prominent examples are airway eosinophils and possibly exhaled nitric oxide in the eosinophilic asthma phenotype and IgE in the allergic or extrinsic asthma phenotype. Finding specific biomarkers that are associated with specific phenotypes will greatly aid in the identification and understanding of disease mechanisms, and eventually lead to a phenotypic specific approach to asthma treatment. Aspirin intolerant asthma (AIA) is a widely recognized, but poorly understood asthma phenotype with an often aggressive course and continuous inflammation of the airways.[13] One prominent feature is a respiratory reaction manifested by exacerbations of both asthma and rhinitis following ingestion of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs).[14] Asthma patients with aspirin sensitivity exhibit chronic, persistent inflammation, with elevated blood eosinophil counts[13] and up to a 4-fold increase in eosinophils on bronchial biopsy specimens.[14] Because non-allergic asthma has previously been associated with both aspirin intolerant asthma[15] and elevated CRP levels[10], and because AIA is a highly inflammatory phenotype of asthma; we hypothesized that CRP levels may be increased in aspirin sensitive asthma. As aspirin challenges to diagnose AIA are associated with significant morbidity, we further hypothesized that CRP could serve as a predictive biomarker for this phenotype of asthma.

Methods

Chart Review

A retrospective electronic record review was conducted at National Jewish Medical and Research Center (NJMRC). Electronic records of clinical asthma patients, who were under the care of two participating physicians (RK and SW), were reviewed. Only those asthma patients who had a CRP level drawn for clinical purposes were included in the review. Additional information included the complete blood count (CBC), age at onset of asthma, spirometry values (including FEV_1 liters and percent predicted, FVC liters and percent predicted, and FEV_1/FVC), atopic status (based on allergy skin-prick testing), chart-determined cardiac history (including hypertension, hypercholesterolemia, and diabetes), sex, race, height, weight, sedimentation rate, dose of corticosteroids (inhaled and oral) and aspirin intolerance (yes/no). Aspirin intolerance was ascertained from the medical history but was not confirmed by an aspirin challenge. Only those subjects for whom aspirin intolerance was listed as an allergy or noted in the chart were considered intolerant. No attempt was made to gauge the level of severity or the specific response reported. Subjects were considered to have early age of asthma onset if their disease occurred before age 12.[15] Subjects were considered to be atopic if they had at least one positive allergy skin test. Subjects who were current smokers, had evidence of autoimmune disease, or currently had cancer were excluded from the study. Not all subjects had data available on every variable of interest. All data was de-identified. The study was approved by the NJMRC Institutional Review Board (IRB) and the Colorado State University Human Research Committee (IRB).

Clinical Testing

All clinical testing was done as part of normal clinical examinations at NJMRC. Both high sensitivity CRP measurements and CBC measurements were performed according to hospital protocol by the NJMRC clinical laboratory on blood samples drawn at the clinical visit to NJMRC. CRP measurements were made using the IMMAGE CRPH (Beckman Coulter, Inc. Fullerton, CA) which utilizes a highly sensitive Near Infrared Particle Immunoassay rate methodology. Results are reported in mg/dL, and the lower detection limit of the assay is 0.02 mg/dL. CRP levels that were below the detection limit (n=2) were entered as half of the lower limit of quantification. Spirometry was performed at that (or an associated) clinical visit by a trained technician using the Jaeger spirometer (Würzburg, Germany). Forced vital capacity and forced expiratory volume in one second were presented as percent of predicted. Predicted values were calculated using Hankinson equations.[16] Height and weight measurements were obtained from the data collected at the time of the spirometric measurements.

Statistical Analysis

Descriptive statistics were calculated for the overall study population, as well as separately for both aspirin tolerant and aspirin intolerant subjects. Normality of continuous variables was assessed using the Shapiro-Wilk test. Mean differences in continuous variables between aspirin intolerant and tolerant asthma subjects were evaluated via either the two-sample t-test (variables that were normally distributed) or the Wilcoxon rank sum test (variables that were not normally distributed). Chi-square tests of association were used to evaluate differences between the two groups for categorical variables. All p-values presented are based on two-sided tests. The relationship between

aspirin intolerance and CRP was evaluated via multiple logistic regression (MLR) analysis. Linearity of CRP was examined using fractional polynomials;[17] and because the assumption was satisfied, CRP was kept in the model as a continuous variable. Variables known to have a biological effect on either CRP or aspirin intolerance were evaluated for confounding by examining the change in the odds ratio (OR) from the fitted MLR model.[17] Any variable for which its univariate association with aspirin sensitivity had a p-value less than 0.25 was evaluated for contribution to the multivariate model, as well as for potential confounding. Effect modification by allergy status and age of onset was also evaluated. All analyses were conducted with SAS (SAS 9.1, SAS Institute Inc., Cary, NC).

Results

A total of 95 subjects were included in the analysis. The mean and standard deviation of the continuous variables are presented in Table 7.1 both overall for the entire study population and by aspirin intolerance status. The mean CRP level was higher in aspirin intolerant asthma subjects than among tolerant subjects (p= 0.042). No other variables were significantly different at the 0.05 level; however, FVC (% predicted) was marginally higher among aspirin sensitive subjects (p=0.064) despite the lack of a statistically significant difference in FEV_1 (% predicted) (p=0.182). Age, smoking history (pack years), BMI, and erythrocyte sedimentation rate did not differ significantly between the two groups (p>0.23). Percent blood eosinophils were slightly higher among aspirin-sensitive subjects, although the difference was not statistically significant (p=0.109). The proportions for categorical variables among the entire study population and by aspirin intolerance status are presented in Table 7.2. The proportion of subjects who smoked

(ever/never), used corticosteroids (inhaled and oral), had cardiac risk factors, and were either overweight or obese did not differ significantly between the two groups ($p>0.26$). Age at asthma onset, atopy and sex also were also not significantly different between the two groups ($0>0.23$).

Results of the univariate logistic regression analyses of aspirin intolerance are summarized in Table 7.3. The odds ratio for CRP was the only variable to reach statistical significance at the 0.05 level. For each 0.5 mg/dL increase in CRP, the odds of AIA increased 44%. The odds ratio for FVC (% predicted) was marginally significant ($p=0.068$) with decreasing FVC (% predicted) being associated with decreased odds of AIA. Results were similar – although less significant – for FEV_1 (%predicted). The odds of AIA increased with increasing levels of blood eosinophils; however, the increase was not statistically significant ($p=0.175$). The only other odds ratio with a p-value less than 0.25 was sex, with females being more likely to have AIA ($p=0.234$).

The best MLR model explaining CRP association with aspirin sensitivity included FVC (% predicted) and percent blood eosinophils (Table 7.4). Neither variable influenced the CRP estimate and, therefore, did not meet the definition of confounding although both FVC (%predicted) and the percent of blood eosinophils were statistically significant predictors of AIA ($p=0.038$ and 0.049, respectively). In particular, the odds of AIA were significantly increased with increasing FVC (% predicted) and blood eosinophils. After adjusting for FVC (% predicted) and blood eosinophils, there was a 48% increase in the odds of aspirin sensitivity for each 0.5 mg/dL increase in CRP (OR=1.48, 95% CI=1.01-2.15, $p=0.042$). Variables known to influence CRP levels (smoking, BMI, age, cardiac disease, hypertension, hypercholesterolemia, corticosteroid use) were evaluated as

potential confounders, and none was found to appreciably influence the CRP estimate. Additionally, inhaled corticosteroids and oral corticosteroid use were also examined for confounding and did not influence the CRP estimate. Interactions between CRP and both atopy and age at onset were examined based on both a previously reported study[4] and the pilot data for the current report. Neither of the two interaction terms was statistically significant, indicating that in this population, the relationship between aspirin sensitivity and CRP did not depend on either atopy or age at onset of asthma.

Discussion

This is the first report of increased CRP levels in the phenotype of aspirin sensitive asthma. This relationship persisted even after evaluation of possible confounding variables, indicating that CRP should be prospectively examined as a potential biomarker for aspirin sensitive asthma. After adjusting for FVC (% predicted) and blood eosinophlis, an elevation in CRP to the very modest 0.5 mg/dl was associated with a 48% increased risk of AIA in known asthma patients. Given the morbidity associated with aspirin challenges in the diagnosis of AIA, an improved biomarker (or equation) for AIA would be highly desirable.

AIA subjects have been reported to exhibit chronic, persistent, generally eosinophilic inflammation, with a reported 4-fold increase in eosinophils on bronchial biopsy specimens[14] and raised blood eosinophil counts.[13] Based on these reports alone, AIA subjects appear to have more systemic inflammation; hence, the finding that increased CRP levels are associated with increased odds of aspirin sensitivity may not be unexpected as CRP is a non-specific marker of elevations in systemic inflammation. However, it is surprising that AIA subjects had increased CRP levels after adjusting for

blood eosinophils. This would seem to suggest that the inflammation in these subjects is not purely associated with eosinophils.

The biologic process associated with increased CRP levels among AIA subjects is not clear. IL-6 mRNA has been shown to be expressed in circulating eosinophils from both normal and hyereosinophillic subjects[18] where it is stored within the matrix of the specific granules.[19] Because CRP is induced principally by IL-6,[1] the increased eosinophils present in aspirin sensitive asthma are possibly producing IL-6 which is inducing CRP. However, the independence of the eosinophil numbers and CRP suggests either that only certain eosinophil phenotypes (i.e., "activated" eosinophls") are producing CRP independent of total numbers or that other cell types (macrophages/monocytes or epithelial cells/smooth muscle cells) are the source of the IL-6 or other CRP stimuli. Mast cell activation has also been reported in AIA.[20] IL-6 is the major cytokine produced by mast cells.[21] Therefore, increased levels of IL-6 leading to increased CRP can possibly be attributed to increased levels of mast cells among AIA subject. Finally, tumor necrosis factor alpha (TNF-ά) has been shown to indirectly regulate hepatic synthesis of CRP by stimulation of IL-6.[22] Although TNF-ά has yet to be specifically associated with AIA, subjects with AIA may well have higher levels of TNF-ά that are contributing to CRP synthesis.

Forced vital capacity was also found to be a significant contributing variable in the relationship between CRP and aspirin sensitivity. Subjects with higher FVC were found to have significantly higher odds of being aspirin sensitive. FVC is negatively correlated with CT-measured air trapping,[23] with lower values indicative of higher amounts of air trapping. Therefore, aspirin sensitive asthma subjects, with their greater degree of

systemic and pulmonary inflammation, may be less likely to trap air, which may indicate more large-airway involvement with less small-airway/parenchymal disease.

Interestingly, despite the age of these subjects, factors reported to affect CRP levels in the general population, including age, BMI, and corticosteroid use, did not influence CRP in this study. The definition of confounding requires that a confounder affect both the outcome and the predictor variable.[24] Neither age nor BMI were statistically different between aspirin sensitive asthma subjects and non-sensitive subjects. Additionally, these variables were not significantly correlated with CRP in this population. Fujita et al. reported that CRP was not correlated with age or BMI among their asthma subjects, in spite of being correlated to these variables within the non-asthma population.[6] The lack of an association between CRP and both age and BMI could suggest that asthma, particularly aspirin intolerant asthma, has a greater effect on CRP than effects reported in the cardiovascular literature.[9] However, sample size factors may also have limited the ability to detect such effects. Additionally, there may not have been a wide enough range of CRP levels to differentiate the effects of either age or BMI.

AIA represents a very severe phenotype of asthma. Up to 25% of hospital admissions for acute asthma that require mechanical ventilation in adults have been associated with NSAID ingestion, and AIA patients are more likely to have been intubated as compared to aspirin tolerant asthma subjects.[25,26] AIA is likely under-diagnosed in the asthmatic population both because asthmatics are often counseled to avoid NSAIDs and because some asthmatics may not recognize mild reactions.[14] Definitive diagnosis of AIA has traditionally required a provocation test using increasing doses of aspirin. These tests may illicit severe, life threatening reactions. Because of the severity of these reactions, a

biomarker to aid in diagnosis would be extremely helpful. Based on the findings from this study, further examination of CRP as a potential biomarker for aspirin sensitive asthma, both alone and in conjunction with blood eosinophils, lung function, and perhaps urinary leukotrine E_4 (LTE_4), is warranted.

Because of the study design, two major factors that have been previously associated with aspirin sensitive asthma were not examined. Nasal polyps have been associated with the disease.[27,28] Also, increased levels of urinary LTE_4 have also been associated with AIA. Basal concentrations are elevated in AIA compared to aspirin tolerant asthmatics, and concentrations increase after aspirin provocation among AIA subjects.[29-32] These two factors are important in relation to the aspirin sensitive asthma phenotype and should be considered in further studies examining the relationship between CRP and AIA. It is likely that the best predictive equation for AIA would include CRP, blood eosinophils, clinical history, and urinary leukotriene E_4.

There are several limitations to this study. All information was limited to that obtained from the electronic records available within the subjects' charts. Some data were missing or incomplete, which might have introduced bias. However, data are not likely to be missing at random, and therefore any bias is likely to be non-differential. Aspirin intolerance status was self-reported by subjects, which is not the ideal measurement of aspirin intolerance. While some subjects reported severe reactions, which were likely definitely due to aspirin sensitivity, other subjects may have been misclassified. Some subjects who did not report aspirin sensitivity may have had a reaction to aspirin which was sub-clinical and therefore not associated with aspirin or other NSAID use. Additionally, some subjects may have incorrectly associated an asthmatic reaction with

aspirin or other NSAID use. This misclassification is likely non-differential and would more than likely bias the estimate towards the null. The self-reported data would be more informative if there was a measure of the severity of the reaction; however, this was not feasible because of the study design. There may be residual confounding through misclassification of confounding variables, and unmeasured confounding may exist. Information about NSAID use was not recorded. It is possible that subjects who are not aspirin sensitive are taking NSAIDs more than aspirin sensitive subjects; that could contribute to the decrease in CRP level. Additionally, because this is a cross-sectional study, temporality cannot be assessed. It is not possible to determine if the increased CRP levels were present before the aspirin sensitivity developed or if the increases developed after the onset of disease. Although CRP measurement was not ordered in the presence of a clinical infection, it is possible that some subjects had a sub-clinical infection, such as *Chlamydia* or *Mycoplasma pneumoniae,* at the time CRP levels were measured. *Chlamydia pneumoniae*[33] infections are known to occur in the asthmatic population and have been reported to increase CRP levels.[5,34] The presence of such infection would have increased CRP levels; however, because it is not likely that sub-clinical infections would differ by AIA status, the resulting bias would most likely be non-differential. Although other unmeasured factors (such as air pollution or an unreported/undiagnosed co-morbidity) may affect CRP levels, such effects are likely to be non-differential between aspirin intolerant and intolerant asthmatics, thereby biasing any association towards the null. Lastly, this study was conducted in subjects who sought treatment through a pulmonary specialist. These subjects are likely to be moderate to

severe asthmatics who may be difficult to treat. Therefore, these results may not be generalizable to other asthma populations.

This study provides evidence that CRP may be elevated in AIA subjects and, consequently, that CRP deserves further study as a potential biomarker for the AIA phenotype of asthma. Specifically, the present study should be repeated under a prospective study design; confirming the asthmatic response to aspirin in a subset and measuring CRP levels in subjects with documented aspirin sensitivity would be necessary. However, a prospective study should be considered that then evaluates thresholds for identifying AIA, alone or in combination with urinary LTs, blood eosinophils, clinical onset of disease and nasal polyps/sinus disease.

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Table 7.1. Descriptive statistics for continuous variables among all subjects and by aspirin intolerance status

	All Subjects			Aspirin Intolerance Status						
				Positive			Negative			
Variable	Mean	Standard Deviation	N	Mean	Standard Deviation	N	Mean	Standard Deviation	N	p-value
CRP	0.61	0.76	95	1.02	1.08	19	0.51	0.63	76	0.0417*
BMI	30.77	8.76	87	31.20	12.92	18	30.66	7.43	69	0.5894*
Age	49.15	10.83	89	48.21	10.00	19	49.40	11.10	70	0.6736
FEV1 percent predicted	66.25	23.99	92	73.05	26.82	18	64.60	23.14	74	0.1816
FVC percent predicted	78.09	19.70	92	85.80	21.91	18	76.21	18.81	74	0.0637
FEV1/FVC	67.46	15.53	92	67.60	12.43	18	67.43	16.26	74	0.9657
Pack years	3.82	9.91	92	2.16	5.24	19	4.24	10.79	73	0.9434*
Percent eosinophils (CBC)	4.51	4.19	77	5.79	4.11	61	4.17	4.18	16	0.1093*
SED	13.28	11.76	73	15.39	10.03	60	12.93	12.21	13	0.2313*

*Probability-value is from the Wilcoxon rank sum test; all other probability-values are from the two-sample t-test

Table 7.2. Descriptive statistics for categorical variables for all study subjects and by aspirin tolerance status

	All Subjects		Aspirin Intolerance Status				
			Positive		Negative		
Variable	Percent	N	Percent among positive	N	Percent among negative	N	p-value*
Aspirin sensitive	20.00	95					
Female	67.40	92	78.95	19	63.38	73	0.2277
Clinical diagnosis of diabetes	9.78	92	10.53	19	9.59	73	0.9025
Clinical diagnosis of hypertension	26.60	94	26.32	19	21.51	74	0.9503
Clinical diagnosis of hypercholesterolemia	12.22	90	10.53	19	12.68	71	0.7994
Cardiac (any)	40.66	91	36.84	19	41.67	72	0.7033
Clinical diagnosis of coronary artery disease	4.40	91	0	19	5.56	72	0.2934
Overweight	71.26	87	73.68	19	63.16	76	0.3887
Obese	48.28	87	36.84	19	46.05	76	0.4697
Current regular use of oral steroids	71.59	88	27.78	18	30.99	71	0.7914
Current daily use of inhaled steroids	48.28	87	61.11	18	74.29	70	0.269
Adult onset of asthma	64.40	87	64.71	17	64.29	70	0.9741
Atopy	72.22	90	61.11	18	75	72	0.2393
Ever smoked	23.90	92	26.32	19	23.29	73	0.7828

*Probability-value based on chi square test for association

Table 7.3. Odds ratios (OR), along with 95% confidence intervals (CI), from the univariate logistic regression analysis of aspirin sensitivity among 95 clinically diagnosed asthma patients

Variable	N	Coefficient	Standard Error	OR	95% CI	p-value	Unit Change
CRP	95	0.730	0.304	1.440	1.069 - 1.940	0.0164	0.5 increase
FVC percent predicted	92	0.026	0.014	1.301	0.981 - 1.725	0.0680	10% increase
FEV1 percent predicted	92	0.016	0.012	1.167	0.930 - 1.465	0.1827	10% increase
FEV1/FVC (%)	92	0.001	0.017	1.007	0.722 - 1.406	0.9652	10% increase
Percent eosinophils (CBC)	77	0.085	0.063	1.088	0.963 - 1.23	0.1750	1
Erythrocyte sedimentation rate	73	0.017	0.025	1.017	0.968 - 1.069	0.4967	1
BMI	87	0.007	0.030	1.007	0.950 - 1.067	0.8150	1
Overweight (yes/no)	87	0.426	0.624	1.531	0.450 - 5.205	0.4952	1
Obese (yes/no)	87	-0.481	0.540	0.618	0.214 - 1.782	0.3733	1
Sex (female vs. male)	92	0.730	0.614	2.074	0.623 - 6.905	0.2343	1
Age	89	-0.010	0.024	0.990	0.944 - 1.038	0.6697	1
Oral steroid use (yes/no)	89	-0.155	0.586	0.857	0.272 - 2.699	0.7916	1
Inhaled steroid use (yes/no)	88	-0.609	0.556	0.544	0.183 - 1.616	0.2730	1
Atopy (yes/no)	90	-0.647	0.555	0.524	0.177 - 1.554	0.2438	1
Asthma onset (late/early)	87	0.018	0.566	1.018	0.336 - 3.085	0.9742	1
Any cardiac risk factor	91	-0.203	0.532	0.817	0.288 - 2.318	0.7036	1
Hypertension (yes/no)	93	-0.036	0.583	0.964	0.308 - 3.024	0.9505	1
High cholesterol (yes/no)	90	-0.210	0.828	0.810	0.160 - 4.109	0.7997	1
Diabetes (yes/no)	92	0.104	0.847	1.109	0.211 - 5.83	0.9025	1
Pack years	92	-0.028	0.035	0.758	0.383 - 1.499	0.4258	10 year increase

Table 7.4. Odds ratios (OR), along with 95% confidence intervals (CI), from the multiple logistic regression analysis of aspirin sensitivity among 74 clinically diagnosed asthma patients

Variable	Coefficient	Standard Error	OR	95% CI	p-value
CRP	0.778	0.3832	1.476*	1.014 - 2.148	0.0424
FVC %pp	0.039	0.0188	1.479**	1.023 - 2.139	0.0377
Percent eosinophils	0.1463	0.0743	1.158	1.001 - 1.339	0.0491

*Odds ratio represents a 0.5 increase in CRP

**Odds ratio represents 10% increase in FVC percent of predicted

Chapter 8

Discussion and Conclusions

Summary

This study used a well characterized cohort of asthma subjects with a wide range of asthma severity to examine clinical and demographic differences between several asthma phenotypes. The first analysis examined severe and non-severe asthma subjects. Based on multivariate logistic regression analysis, a model was developed that contained five variables to compare severe to non-severe disease: gastro-esophageal reflux disease (GERD), the ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC), history of pneumonia, history of sinusitis, and atopy. GERD is a treatable condition and, therefore, a modifiable risk factor for the disease. These results identified risk factors for severe asthma that deserve further investigation.

The second analysis focused on age of asthma onset. This phenotype has been previously examined via univariate analyses. A group of variables were identified using a multivariate model that illustrated the differences between late and early age at asthma onset subjects. The final multivariate model included parental history of asthma, duration of asthma, atopy, and airway eosinophils. These results give further evidence that important differences exist between early and late onset asthma and that the difference is not just among severe asthma subjects. Our findings strengthen the case for further examination of this phenotype, including a genetic study that would consider age of asthma onset, which may lead to discoveries important to asthma treatment.

A relatively new phenotype was also examined. Recent advances in radiology have allowed for the quantitative assessment of air trapping by multi-detector CT scans. This study utilized these methods to classify asthma study subjects based on air trapping. Subjects who exhibited air trapping were significantly more likely to have a history of asthma-related hospitalizations, ICU visits, or mechanical ventilation, supporting the idea that subjects with air trapping may be phenotypically different than subjects who do not trap air. Duration of asthma, history of pneumonia, high levels of neutrophils in the airway, air flow obstruction as measured by FEV_1/FVC, and atopy were identified as independent risk factors associated with the air trapping phenotype. Evaluation of the data reported supports the use of CT scanning in asthmatic subjects to identify a group of individuals with a high risk of severe disease, particularly those with high and intensive health care utilization. Further longitudinal and hypothesis-driven studies that specifically evaluate the role of these factors in the development of this phenotype are needed.

The final investigation aimed to determine if C-reactive protein (CRP) was elevated in aspirin intolerant asthma (AIA) subjects. In examining the relationship between CRP and AIA, two other variables, blood eosinophils and FVC, were found to be important to the relationship. This study provides evidence that CRP may be elevated in AIA subjects and, thus, deserves further study as a potential biomarker for the disease.

Asthma is a highly heterogeneous disease. There has been significant recognition that asthma likely exists in several different phenotypes. However, these phenotypes remain poorly characterized. This study added evidence that asthma can be divided into specific phenotypes. In spite of sharing some clinical variables, these phenotypes are unique.

Identification of variables that explain differences in a phenotype may eventually lead to a phenotype-specific approach to asthma treatment.

Limitations

There are several limitations to the current study. Selection bias, which occurs when there is a systematic error in the ascertainment of study subjects,[1] may result when selection of cases or controls varies according to exposure status. In an effort to limit this bias, subjects were selected based solely on disease status, not on exposure to any risk factor. As with many clinical observational studies, some subjects (predominately the mild/moderate asthma groups in this study) were self-selected; consequently, generalizability to the entire asthma population may be affected. Additionally, because severe asthma subjects are referred to the study from the clinic, results may be generalizable to only severe asthma subjects seeking medical treatment from a pulmonary specialist. However, because most severe asthmatics are not able to control their asthma without seeing a pulmonary specialist, their results would most likely be consistent with subjects not participating in clinical studies.

Misclassification of disease severity may have occurred and may have biased the results. However, asthma severity classification is based on a published, validated definition.[2, 3] The classification is made on the basis of objective tests performed by trained personnel using a strict definition. All study procedures were performed according to established, validated methods by trained personnel. Both of these are attempts to limit information bias. The possibility of recall bias is inherent in studies wherein potential exposures are examined retrospectively – in particular when those with a disease are more likely to remember past exposures than those without disease. The use of a referent group with

asthma should have decreased the probability of recall bias occurring. If recall bias is still present and is differential (for example, severe asthma subjects recall at a different level than non-severe asthma subjects), then the resulting bias would likely be away from the null but could be in either direction.

The cross-sectional design of the study may limit the conclusions. Day-to-day variation of the measurements is possible, although most of the clinical measurements in asthma subjects are thought to be relatively stable in the absence of a current exacerbation or infection. Subjects were not enrolled if they had an asthma exacerbation or infection in the weeks preceding study. This exclusion criterion should have ensured that the data gathered are reflective of the baseline status of the subjects. However, it is possible that subjects could have been enrolled with sub-clinical infections. The presence of such infections might have inflated inflammatory markers and decreased lung function values which could have biased the results in either direction.

Another drawback associated with cross-sectional studies is that cases with long duration of disease are over-represented while cases with a short duration of disease are under-represented.[4] Although this might have been a problem in the current study, many of the severe asthma subjects were newly diagnosed, thereby equalizing the above disparity and removing this bias. Additionally, data were collected on asthma duration, allowing for adjustment in the analysis for the effect of asthma duration. Survivor bias, or bias resulting when duration of disease after onset is different among exposed and unexposed subjects, might have occurred. This bias can occur in studies of chronic disease where disease duration is often related to survival.[1] However, severe asthma is not commonly

fatal; so, the likelihood of an effect is reduced. However, the results of these studies may not be generalizable to severe asthma subjects who die shortly after diagnosis.

This study used data that were collected in NIH sponsored studies. Because these studies focused on severe asthma, more severe asthma subjects were enrolled than non-severe asthma subjects. Therefore, the results may be driven by the higher percentage of severe asthma and, thus, may not be representative of the population of less severe asthmatics.

Missing data were present in this study and might have influenced the results. Subjects studied under specific aims 1 and 2 were subjected to a large number of clinical tests. Many subjects might not have had the time or the desire to complete all testing, which would likely result in data missing mostly at random. The severity of some subjects' asthma may limit their participation in certain testing (such as sputum induction and methacholine testing), which would result in data that are not missing at random. Bias may result from missing data. If those who are missing data are different than those who are not missing data, the resulting bias may be either away from or towards the null. Additionally, missing data presented problems in several of the statistical analyses. There are different methods available to deal with missing data in statistical analysis including: using only data from subjects with complete data sets; assuming that the missing data do not add predictive information (a normal value is inserted where the missing data exist); and data imputation.[5] Each of these approaches has the potential to introduce bias into the study results. For a majority of the analyses in this project, only data from subjects with full datasets were entered into the model. It has been documented that this approach can introduce bias into study results, especially when the data are not missing at random.[5] If data are not missing at random, a type of selection

bias may result. Subjects who were not included were less likely to have the testing. For example, very severe subjects might be less likely to have had a certain test, resulting in a study population wherein very severe subjects are under-represented.[5] The resulting bias might be in either direction depending on both the outcome and the variable with missing data. Other options, such as assuming a normal value or data imputation, have also been shown to introduce bias, with imputation producing the least biased results.[5] For this study a majority of the missing data are likely to be at random.

Excluding subjects with missing data affected the model building process in that the study population changed as subjects with missing data were excluded and then possibly added back into the population. The models were repeated using subsets of the study population to ensure that this process did not have a karge effect on the final model. In the investigation of the air trapping phenotype of asthma (Chapter 6), neutrophilic status was imputed to increase the number of subjects with complete data. A sensitivity analysis was conducted to ensure that data imputation did not substantially change the results. Results of the models should be interpreted with caution because of the large amounts of missing data and potential for bias. Further research into the effect of missing data in this study should be carried out to determine the optimum method for handling the missing data.

Power (i.e., the probability of rejecting a false null hypothesis) of a study may be reduced when variables are stratified. Reduced power limits the ability of the study to detect differences between the groups. Power is also limited by the sample size, the difference of effect, and the chosen level of significance. Missing data contributed to loss of power in this study. Additionally, some of the variables that were measured were rare in the

study population. For instance, there were 180 subjects who had a value for aspirin sensitivity. However, the outcome was rare among non-severe asthma subjects which limited the power to examine the association by severity.

This study also involved multiple statistical testing. In light of the multiple comparisons made, some of the results might be attributed to chance. The results of the investigations in this study will need to be replicated before they will be widely accepted.

Further Research

This study has generated several possibilities for further research. The relationships between asthma severity and phenotypes and some environmental exposures, such as tobacco smoke, were not examined in this project. Further research should consider the effect of these environmental exposures on asthma severity and phenotypes. Specifically, an analysis examining the contribution of these exposures to asthma severity may provide clues to the factors contributing to the development of severe asthma.

By design, a majority of the subjects included in this study were severe asthmatics; therefore, results from the phenotype analyses were driven by severe asthmatics. As more mild and moderate subjects are enrolled in the studies that provided the data for this study, the power will be increased allowing ordinal logistic regression analysis of asthma severity and risk factors. Such analyses will assist investigators in the evaluation of differences between mild and moderate disease in relation to severe asthma, thereby possibly providing either justification for combining of the non-severe subjects in analysis or evidence that the mild and moderate subjects should not be combined for analysis.

An in-depth analysis of the atopy variable should be carried out. The variable was defined in the current study as one or more positive allergy skin tests. Information is available in the subjects' charts about the response to each allergen, including size of each reaction (diameter of wheal and flare). This information could be analyzed to determine if a certain allergen is more strongly associated with differing severities or even different asthma phenotypes and if there is a more appropriate way to combine the information to classify atopy. Additionally, subjects responded to questions about asthma symptoms in relation to allergic triggers. The scores for these symptom scores could be combined with the allergy testing results to classify subjects more thoroughly. The symptom scores could also be considered to determine if there is a difference between symptomatic and asymptomatic subjects.

More studies examining the association between GERD and severe asthma are needed. GERD can be effectively controlled with proton pump inhibitors. The effect of complete control of GERD on asthma severity should be examined over time in a clinical trial setting.

An analysis of the allergic asthma phenotype and the age of onset phenotype should be carried out in light of the strong relationship between early age of onset and atopy. A similar approach could be taken to build a multivariable logistic regression model for the allergic phenotype, the results of which could be compared to the age at asthma onset analysis.

Further analysis of the air trapping phenotype should also be undertaken. Normal controls who exhibit air trapping on MDCT scan should be identified and followed. It is possible that air trapping is a precursor to asthma. As subjects continue to be enrolled,

more subjects of varying asthma severities will be available for analysis. The increased number of study subjects will likely allow for the analysis of interactions in the previously reported model. Additionally, the association between asthma severity and air trapping should be prospectively evaluated. Following mild/moderate asthma subjects over time may help to determine if air trapping increases over time and results in increasing asthma severity.

The relationship between aspirin intolerant asthma and C-reactive protein should be further examined with either a case-control study or, ideally, a prospective study to determine if the relationship between AIA and CRP can be confirmed. The study should include detailed questionnaires about reactions to aspirin/NSAID use, cardiac history, and onset of asthma. Further, participants should be challenged with aspirin to ascertain AIA. Additionally, urinary LTE_4 should be measured, and the presence/absence of nasal polyps should be ascertained. With this information, it may be possible to build a predictive model for aspirin sensitivity that could be used by clinicians in place of aspirin challenges.

Missing data is a reality in clinical epidemiologic studies. The effects of missing data on asthma studies, especially in severe asthma, should be further examined. The Severe Asthma Research Program has a growing data base. This database may be large enough to conduct simulation studies that could be used to evaluate different methods of handling missing data in severe asthma, including multiple imputations.

One of the most valuable contributions to defining asthma phenotypes may be in the genetic analysis of the phenotypes. For example, the association of the early age of onset phenotype with a family history of asthma strongly suggests that a genetic component is

associated with the early onset phenotype. Consideration of this association may help in the search for genetic markers of asthma. Other phenotypes should be investigated in genetic analysis.

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Appendix A

Clinical Methods

Lung Function Testing

Spirometry. Spirometry is a time based physiologic measurement of how an individual inhales or exhales volumes of air.[1] The most important measurements from spirometry are forced vital capacity (FVC), the volume of air after exhaled a complete and forceful expiration after maximal inhalation, and forced expiratory volume in one second (FEV_1), the volume of air exhaled in the first second of a forced expiratory volume maneuver.[1] FEV_1 and FVC values will be presented as percent predicted values. Hankinson prediction equations will be used to calculate the values.[2] Spirometers are calibrated daily per American Thoracic Society guidelines.[3] All subjects will undergo spirometry testing per ATS guidelines, withholding inhaled medications as recommended by ATS.[3] Subjects held short acting bronchodilators for four hours and long acting bronchodilators for 12 hours if their asthma permitted. Values from an appropriate medication withhold day are used as baseline values. Subjects must have three acceptable, reproducible maneuvers. An acceptable maneuver has a rapid onset of maximum flow, extrapolated volume of less than 5% of FVC, is smooth without hesitation or cough, continues until the flow rate is zero and has a minimum expiratory time of six seconds. A reproducible effort FEV_1 or FVC must be within 5%. No more than eight attempts may be made in a single session. The effort with the highest FEV_1 and FVC will be used in analysis. To measure reversibility, spirometery will be performed on subjects after withholding medications. A short acting bronchodilator will be administered and fifteen minutes later spriometery will be repeated. The level of reversibility will be calculated as a percent change in FEV_1 from the initial measurement. Spirometry will also be performed as part

of methacholine challenge testing, sputum induction and maximum bronchodilation testing. All staff is certified in spirometry.

Methacholine Challenge. At the initial visit, all subjects undergo methacholine challenge. Methacholine challenge testing is done to measure the severity of airway reactivity. Most individuals without pulmonary disease show no change in lung function when they inhale low concentrations methacholine. Conversely, nearly all asthmatic patients with active disease exhibit narrowing of their airways when they inhale low concentrations of methacholine. All methacholine challenges will be performed according to American Thoracic Society guidelines by the following procedures.[4] Baseline spirometry will be performed. Subjects will be asked to withhold all bronchodilators for the recommended period of time. The subject will perform 5 inspiratory capacity inhalations of methacholine slowly and deeply from the nebulizer in a 2 minute time period. An acceptable-quality FEV_1 will be measured, at about 30 and 90 seconds after the fifth inhalation from the nebulizer, at each time point. To keep the cumulative effect of methacholine relatively constant, the time interval between the commencement of two subsequent concentrations will be kept to 5 min. At each dose the highest FEV_1 will be reported. If the FEV_1, falls less than 20%, the next higher concentration will be administered, repeating previous steps. If the FEV_1, falls more than 20% from baseline (or the highest concentration has been given), signs and symptoms will be noted, administer inhaled albuterol, wait 10 min, and repeat the spirometry. Provocative concentration causing a 20% fall in FEV_1 (PC_{20}) is calculated and presented as mg/ml. Subjects will be classified as mild asthmatic if they have a PC20 $\leq$ 8 mg/ml. Subjects with a previous asthma diagnosis who do not respond to methocholine challenge

or reversibility testing will be excluded from participation in the studies. Additionally, normal controls who do respond to methacholine will be excluded from participation in the studies.

Allergy Testing

Allergy Skin Testing. All subjects will undergo allergy skin testing to determine their atopic status. Skin testing will be performed on the subjects' back or forearm. The Duo-tip device will be used to puncture and apply up 14 skin tests to one area of the back or one forearm. Exactly 15 minutes after application of allergen, each wheal and flare with will be measured. Wheals larger than 3x3 mm indicate a positive skin test. Subjects will be classified as atopic if they have at least 1 positive reaction out of 12 common aeroallergens. Allergens include: Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat, dog, American Cockroach, Alernaria, Cladosporium, Aspergillus mix, Timothy grass (or grass mix), short ragweed, common weed mix, and Eastern 7 tree mix. Histamine is used for a positive control and diluent (50% glycerin and 50% saline) is used for a negative control. All extracts are obtained from Greer Laboratories. All staff is certified in allergy skin testing. Anti-histamines are withheld for 3 days prior to allergy skin testing.

Radiology

Multi-detector CT scan. A multi-detector spiral CT (MDCT) of the chest with 16 or 64 detector rows (GE Light Speed Ultra 16 or Siemens Volume Zoom, Sensation 16 or 64) which was calibrated daily was performed on some subjects. Suspended expiratory measurements at FRC were obtained at the following settings: GE: 1.675-1.75 pitch, 0.6 sec rotation time, 120 kV, 50-100 mAs, detector collimation 0.625 and 1.25 mm, 0.625-

1.25 mm reconstructed slice thickness, medium smooth "standard" reconstruction algorithm; Siemens: 1.5 pitch, 0.5 sec rotation time, 120 kV, 50 mAs, detector collimation of 0.75 mm, 1mm reconstructed slice thickness, slice interval=field of view (mm)/512 to produce isotropic voxels, and a medium smooth reconstruction algorithm (Siemens B30f) – effective mAs=33 (low radiation dose). The MDCT scans were performed after bronchodilation with albuterol (540-720 mcg) to minimize the potential effect of bronchoconstriction on airway wall measurements. The radiation dose from the low dose CT scans (two CT scans for each subject, one at TLC and one at FRC) ranged from 1.55 mSv effective dose to 1.70 mSv effective dose. The radiation dose from the higher dose CT scans (two CT scans for each subject, one at TLC and one at FRC) ranged from 4.0 to 7.6 mSv effective dose. The higher effective doses occurred in larger subjects, especially larger female subjects.

MDCT airway evaluation software

MDCT scans were analyzed using automated, lung parenchymal evaluation software designed to segment the lung from the rest of the thoracic anatomy and to generate histogram curves of the lung voxels which can be further analyzed to determine the percent of lung tissue that lies between different CT voxel numbers expressed in Hounsfield Units (Pulmonary Profiler, VIDA Diagnostics, Iowa City, IA). MDCT images from various types of scans (low and regular radiation dose, normal and diseased subjects) can be used without the need for the user to manually adjust any parameters. The specific MDCT measurements used in the data analysis included percent low attenuating area (%LAA) less than – 850 HU, %LAA – 900 HU, %LAA – 950 HU. The

CT Pulmonary Workstation measurements were performed by a trained technician at the University of Iowa, Carver College of Medicine.

Assessment of Pulmonary Inflammation

Exhaled Nitric Oxide. Fractional concentration of exhaled nitric oxide (Fe_{NO}) was measured online by chemiluminescence at a constant expiratory flow (50 mL/s), consistent with published guidelines.[5] Briefly subjects inhaled to capacity and then exhaled into the flow controlled analyzer for 10 seconds.

Bronchoaveolar Lavage (BAL) and Endobronchial Biopsy. If the patient is within 10% of their screening FEV_1, the subject will be given 2 puffs (180μg) of albuterol and the spirometry repeated within 15-30 minutes. The subject will be taken to the research laboratory or bronchoscopy suite where bronchoscopy with endobronchial biopsy (x8) and bronchoalveolar lavage (BAL) will be performed, following the recommended guidelines. The subject will be pre-medicated with codeine and atropine. Oximetry testing, oxygen therapy and IV access will be maintained throughout the procedure. Topical anesthesia with lidocaine (4%) will be delivered to the nares and oropharynx. Midazolam (2-7 mg) IV and Fentanyl (25-100 μg) will be given prior to the procedure. The bronchoscope will be passed nasally or orally and local anesthesia (lidocaine 2%) delivered at the vocal cords, carina, mainstem bronchi and just prior to the endobronchial biopsies or wedge. The bronchoscope will be positioned over the subcarinae of the right or left lower lobe and 8 endobronchial biopsies obtained using an alligator forceps. The bronchoscope will then be repositions and wedged on the opposite side from where the biopsies were taken and 4-(60 ml) aliquots of warm, sterile saline instilled and sequentially removed under gentle manual aspiration. The subjects will be monitored

until their respiratory function has returned to 10% of baseline and effects of the anesthesia have diminished. Nebulized albuterol will be available to reverse any bronchoconstriction, which may develop during or after the procedure. Standards are in place to determine appropriate aftercare.

To undergo endobronchical biopsies, subjects must be 18-60 years of age, have a pre-bronchodilator FEV1 of $\geq$ 35% of predicted and a post-bronchodilator FEV1 of $\geq$40% of predicted, be clinically stable (per bronchoscopist), have no history of asthma related hospitalization within the past 6 weeks and have no history of intubation within the past 6 months.

Tissue processing.

Lavage Fluid Processing

BAL samples were collected, placed on ice and then spun at 4 degrees Celsius (600x *g*) for 10 min to separate fluid from cells. Cell counts were performed using a hemocytometer and trypan blue exclusion testing. Differentials were obtained on cytospin preparations, using a Diff-Quik™ (Scientific Products, McGraw Park, IL) stain, counting 300 cells. Fluid was processed for eicosanoids using a Sep-pak purification system, stored at -70 degrees Celsius until analysis using enzyme immunoassays.

The fluid was immediately placed on ice and centrifuged to separate fluid from cells. The fluid was aliquoted and frozen at -70 degrees C for histamine and tryptase analysis.[6]

Tissue Processing

Endobronchial tissue was fixed overnight at -20 degrees Celsius in acetone and embedded in glycol methacrylate resin. Tissue blocks were stored at -20 degrees Celsius until 2-mm sections were cut using a Reichert Ultracut E ultramicrotome (Leica Inc.,

Deerfield, IL). Tissue sections were stained with antibodies against cell markers: eosinophils (eosinophil major basic protein, clone BMK-13; Accurate Chemical & Scientific Corp, Westbury, NY), neutrophils (neutrophil elastase; DAKO, Carpinteria, CA), lymphocytes (CD3 1, CD41[both from Beckton-Dickinson,Bedford, MA] and CD8 1[DAKO]), mast cells–AA1, macrophages–CD68 (both from DAKO), and transforming growth factorb 1,2,3 (TGFb) (Genzyme, Cambridge, MA). Sections were treated with 0.3% H_2O_2 in 0.05 M TRIS-buffered saline (TBS, pH 7.6) for 30 minutes to inhibit endogenous peroxidase, and incubated with 1% normal horse or goat serum for 30 minutes to block potential nonspecific binding sites. The slides were then incubated with the primary antibodies mentioned previously for 2 hours at room temperature, followed by incubation with biotinylated horse antimouse IgG or goat anti-rabbit IgG for 1 h at room temperature. After rinsing the slides in TBS, 0.03% aminoethylcarbazole (AEC) in 0.03%

H_2O_2 was used as substrate to develop a peroxide-dependent red color reaction. Slides were counterstained with Mayer's hematoxylin and covered with Crystalmount (Biomeda Corp., Foster City, CA). Appropriate control slides were similarly treated but with primary antibodies replaced by nonimmune serum or TBS. Positive cells were counted blindly in the submucosa of the biopsy slices and expressed as number of cells/mm2.[7] The cell counts were normalized per tissue area and expressed as cells per square millimeter of airway submucosa. Tissue analysis was performed by a single observer who was blinded to the subjects' identity and status. The coefficient of variation in cell counts for the same section was less than 7%.[8]

Sputum Induction.

Sputum induction is a non-invasive method of collecting airway cells and fluids. An average of $2x10^6$ inflammatory cells are collected during the induction which is conducted by a trained individual. Subjects are pre-treated with four puffs of bronchodilator and lung function is monitored throughout the procedure due to the risk of bronchoconstriction. After receiving the bronchodilator, the subject performs post-bronchodilator spirometry and peak flows according to ATS guidelines.[3] The induction is stopped if the FEV_1 falls below 20% of baseline the post-bronchodilator value or if the subject reports bothersome symptoms. The subject inhales 3% buffered saline solution mist from an ultrasonic nebulizer for four minutes. After the four minute inhalation, the subject stops breathing the solution and blows his/her nose, rinses out the mouth and then takes one large breath of the saline solution and produces a deep cough. Any sputum that is brought up is then expectorated into a sterile collection cup. This sequence is repeated three times. The subject performs peak flows after each sequence. If the peak flow value is 20% from the post-bronchodilator value, the induction is stopped and spirometry is preformed. If the subject's FEV_1 falls 20% from the post-bronchodilator value, the induction is stopped and the subject is treated with bronchodilator. Spirometry is repeated 15 minutes after administration of the bronchodilator. Spirometry and bronchodilator administration is repeated until the subject is within 10% of their original post-bronchodilator FEV_1.

Sputum Processing. Once received in the laboratory, the induced sputum sample is weighed (weight (grams) is assumed equal to volume (milliliters)). Sputum is diluted to 50% with a solution of 0.1% dithiothreitol so that the sputum contains no more than a

50% dilution of any sputum mediator and a final dithiotheritol concentration of 0.05%. The mixture is aspirated several times with a sterile transfer pipette and placed in a shaking water bath at 37 degrees Celsius for fifteen minutes. Intermittent aspiration is performed every five minutes. Using an aliquot of the resulting cell suspension, a total cell count is performed using a hemacytometer and cytospins are made for differential cell counts, which are performed by two separate counters and recorded as white blood cell percentages. The cell suspension is then centrifuged to separate the cells from the supernatant. The supernatant is divided into 1 ml aliquots for storage and later analysis of mediators along with the cellular pellet.

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Appendix B

Questionnaire from Severe Asthma Research Program (SARP)

2549

Severe Asthma Research Program

Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

Screening Questionnaire for Severe Asthma

1. Did you ever smoke? O Yes O No
2. Are you still smoking? O Yes O No

   If **Yes**, then **STOP** unless this subject also has COPD. If **No**, then proceed.

3. How many years did you smoke? ☐☐
4. What was the usual number of cigarettes you smoked a day while you were an active smoker?

   ☐☐☐ cigarettes a day

   To get number of packs/day, divide usual number of cigarettes a day by 20.
   If **[(packs/day) x years] > 5**, then ***STOP the questionnaire***.

5. Have you been told by a physician that you have cystic fibrosis?

   O Yes O No O Uncertain

   If **Yes**, then **STOP**.

6. Have you been told by a physician that you have chronic obstructive pulmonary disease, chronic bronchitis, or emphysema?

   O Yes O No O Uncertain

   If **Yes**, then **STOP**.

7. Have you been told by a physician that you have vocal cord dysfunction?

   O Yes O No O Uncertain

   If **Yes**, then **CONTINUE** if your FEV_1 is less than 80% of predicted ***and*** if a physician has told you your vocal cord dysfunction is ***not*** the main cause of your respiratory illness.

8. Have you been told by a physician that you have asthma?

   O Yes O No O Uncertain

9. If **Yes**, how many years have you been known to have asthma?

   ☐☐ years

2549

Severe Asthma Research Program

Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

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8. Have you been told by a physician that you have asthma?

   O Yes O No O Uncertain

9. If **Yes**, how many years have you been known to have asthma?

   ☐☐ years

2549

Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

Severe Asthma Research Program

Screening Questionnaire for Severe Asthma

1. Did you ever smoke? ○ Yes ○ No

2. Are you still smoking? ○ Yes ○ No

   If **Yes**, then **STOP** unless this subject also has COPD. If **No**, then proceed.

3. How many years did you smoke? ☐☐

4. What was the usual number of cigarettes you smoked a day while you were an active smoker?

   ☐☐☐ cigarettes a day

   To get number of packs/day, divide usual number of cigarettes a day by 20.
   If [**(packs/day) x years**] **> 5**, then ***STOP the questionnaire.***

5. Have you been told by a physician that you have cystic fibrosis?

   ○ Yes ○ No ○ Uncertain

   If **Yes**, then **STOP**.

6. Have you been told by a physician that you have chronic obstructive pulmonary disease, chronic bronchitis, or emphysema?

   ○ Yes ○ No ○ Uncertain

   If **Yes**, then **STOP**.

7. Have you been told by a physician that you have vocal cord dysfunction?

   ○ Yes ○ No ○ Uncertain

   If **Yes**, then **CONTINUE** if your FEV_1 is less than 80% of predicted ***and*** if a physician has told you your vocal cord dysfunction is ***not*** the main cause of your respiratory illness.

8. Have you been told by a physician that you have asthma?

   ○ Yes ○ No ○ Uncertain

9. If **Yes**, how many years have you been known to have asthma?

   ☐☐ years

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SARP ID

Major Characteristics of Severe Asthma: ***1 or 2 criteria required***

Treatment with continuous or near-continuous oral corticosteroids

10. Do you take systemic corticosteroids (pills or shots but not bursts) on a regular basis (more than 6 of the last 12 months)?

    ○ Yes ○ No

    If **Yes**, provide dose [ ][ ][ ] mg and frequency [ ][ ] months in the last year

Treatment with high-dose inhaled corticosteroids (fluticasone propionate >880 mcg/day or equivalent; see equivalency chart from NHLBI Guidelines on next page)

See next page.

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Treatment with high-dose inhaled corticosteroids (fluticasone propionate >880 mcg/day or equivalent; see below equivalency chart from NHLBI Guidelines)

11. Do you use high-dose inhaled corticosteroids on a regular basis (more than 10 of the last 12 months)?

O Yes O No

If **Yes**, then mark all that apply.

Adults	*Min mcg/day*	*Min puffs/day*
O Advair: 500/50 mcg/inhalation	1000	1 inhalation BID
O Aerospan	800	10 puffs: 80 mcg/puff
O Asmanex	880	4 puffs: 220 mcg/puff
O Qvar: 40 mcg/puff	640	16 puffs: 40 mcg/puff
O Qvar: 80 mcg/puff	640	8 puffs: 80 mcg/puff
O Budesonide: Pulmicort DPI	1600	8 inh@200 mcg/inh or 4@400
O Flunisolide: Aerobid	2500	10 puffs: 250 mcg/inhalation
O Fluticasone propionate: Flovent	880	8 puffs: 110 mcg/puff
O Fluticasone propionate	880	4 puffs: 220 mcg/puff
O Symbicort: 160/4.5 mcg/inhalation	640	2 inhalations BID
O Symbicort: 80/4.5 mcg/inhalation	640	4 inhalations BID
O Triamcinolone acetonide: Azmacort	2500	25 puffs: 100 mcg/inhalation

Children: less than 12 years of age		*Min mcg/day*	*Min puffs/day*
Advair	O DPI: 500/50 mcg/inhalation	500	1 inhalation
	O HFA: 115/21 mcg/inhalation	460	2 inhalations BID
	O HFA: 230/21 mcg/inhalation	460	1 inhalation BID
Beclomethasone	O CFC: 42 or 84 mcg/puff	672	16 puffs: 42 mcg/puff
	O HFA: 40 or 80 mcg/puff	320	8 puffs: 40 mcg/puff
	O Qvar MDI: 40 or 80 mcg/inhalation	160	4 inh@40 mcg/inh or 2@80
Budesonide	O DPI: 200 mcg/inhalation	800	4 inhalations/200 mcg
	O Nebulizer suspension	2000	2 mg solution
Flunisolide	O MDI: 250 mcg/puff	1250	5 puffs/250 mcg
Fluticasone	O MDI: 44, 110, 200 mcg/puff	440	4 puffs /110 mcg
	O DPI: 100, 250, 500 mcg/inhalation	400	4 inhalations/100 mcg
Pulmicort	O Flexhaler: 90 mcg/inhalation	450	5 inhalations
	O Flexhaler: 180 mcg/inhalation	540	3 inhalations
	O Turbuhaler: 200 mcg/inhalation	600	3 inhalations
Symbicort	O 80/4.5 mcg/inhalation	480	6 puffs /80 mcg
	O 160/4.5 mcg/inhalation	480	3 puffs /160 mcg
Triamcinolone	O MDI: 100 mcg/puff	1200	12 puffs /100 mcg

Minor Characterisitics: *2 of 7 criteria required*

Require daily treatment with controller medication in addition to inhaled corticosteroids (e.g., long-acting inhaled beta-agonist, theophylline, or leukotriene antagonist)

12. On a daily basis, do you use a controller medication in addition to an inhaled corticosteroid? [Answer this question ***only*** if subject takes corticosteroids. Answer ***yes*** if subject takes Advair® or Symbicort®.]

O Yes O No

If **Yes**, do you use

O salmeterol	O zarfirlukast
O formoterol	O zileuton
O theophylline	O Advair®
O montelukast	O Symbicort®

Asthma symptoms requiring short acting beta-agonist use on a daily or near-daily basis

13. Do you use a beta-agonist inhaler on a daily or near-daily basis (at least 5 of 7 days)?

O Yes O No

If **Yes**, do you use

O albuterol O maxair O terbutaline O primatine inhaler

Persistent airway obstruction

14. Do you have persistent airway obstruction?

O Yes O No

If **Yes**, pick one or both criteria:

O FEV_1 <80% predicted (assess from spirometry on this day)

O diurnal PEF variability >20% (assess from diary data collected over the next 2 weeks)

Daily variability : [(PM PEF - AM PEF) / Mean PEF] x 100
Example : [(410 L/min - 320 L/min) / 365 L/min] x 100 = 24.7% variability

Diary Card variability : sum of daily variability values divided by number of days

One or more urgent care visits for asthma per year

15. Over the last year, have you had any emergency room visits, urgent care visits, or unscheduled emergency visits to your doctor for your asthma?

O Yes O No

If **Yes**,

O No more than once a year

O At least twice a year but no more than 4 times a year

O More than 4 times a year but less than once a month

O More than once a month

Three or more oral corticosteroid bursts in the last 12 months

16. In the last 12 months, have you had 3 or more oral corticosteroid bursts?

O Yes O No

Deterioration with reduction in oral or inhaled corticosteroid dose

17. Do your asthma symptoms return or worsen when you decrease your oral corticosteroids or inhaled corticosteroids? [Answer this question ***only*** if subject takes corticosteroids.]

O Yes O No

If **Yes**, how many days did it take? ☐☐ days

Near-fatal asthma event in the past

18. Have you ever required intubation or assisted ventilation for a severe asthma attack?

O Yes O No

If **Yes**, when was the most recent date this happened? Month ☐☐ / Day ☐☐ / Year ☐☐☐☐

Subject meets criteria for severe asthma with at least 1 major *and* at least 2 minor criteria

***Major Criteria*: at least 1**

O continuous or near-continuous oral corticosteroids

O high-dose inhaled corticosteroids

***Minor Criteria*: at least 2**

O daily controller medication in addition to inhaled corticosteroids

O beta-agonist required daily or near daily

O persistent airway obstruction

O one or more urgent care visits per year

O 3 or more oral corticosteroid bursts in the last 12 months

O deterioration with reduction in corticosteroid dose

O near-fatal asthma event in the past

	Normal	COPD	Not Severe	Severe
At this point, subject is classified as	O	O	O	O

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Interviewer (initials) | SARP ID

Month / Day / Year | Center (eg, WFU)

Severe Asthma Research Program

Atopic Diseases

1. Have you *ever* had allergies (for example, hay fever) or a runny, stuffy nose accompanied by sneezing and itching when you *did not* have a cold or flu?

   O Yes O No O Uncertain

   If **No**, then go to **Question 6**.

2. If **Yes**, how old were you when these allergies first started? ☐☐ years old

3. Are you still having symptoms?

   O Yes O No O Uncertain

4. Were the above allergies diagnosed by a doctor?

   O Yes O No O Uncertain

5. Do your allergies make your breathing worse?

   O Yes O No O Uncertain

6. Have you ever had skin testing or blood testing to determine whether you have allergies?

   O Yes O No O Uncertain

7. Do you take nasal steroids: for example, beclomethosone (Beconase®, Vancenase AQ®), fluticasone (Flonase®), budesonide (Rhinocort®)?

   O Yes O No O Uncertain

   If **Yes**,

   8. Which one(s) do you take? ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

      ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

   9. How often do you take them?

      O Never
      O Weekly but less than or equal to twice a week
      O More than twice a week but less than once a day
      O Daily
      O More than twice a day

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SARP ID

10. For each of the last 4 seasons and for the entire last year, please mark the word that best describes your allergic symptoms for that period.

	None	*Mild*	*Moderate*	*Severe*
Spring	O	O	O	O
Summer	O	O	O	O
Fall	O	O	O	O
Winter	O	O	O	O
Entire year	O	O	O	O

11. Have you ever had a prolonged, itchy, scaly, or weepy skin rash such as eczema? (Do not include hives.)

O Yes O No O Uncertain

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Interviewer (initials) SARP ID

Severe Asthma Research Program

Month Day Year Center (eg, WFU)

Demographic Information

Read the following information to each participant.

The answers to the questions on this form will help in the diagnosis and classification of asthma severity. The information that is provided will be used by this National Institutes of Health sponsored study on severe asthma. Please answer each question as carefully as possible.

All information you give will be kept strictly confidential. Only study staff who are working on this study will be able to identify you with the specific information you give. Eventually, the data will be published, but you or anyone about whom you give information will be unable to be identified.

1. What is the relationship of the person being interviewed to the subject?

   O Self O Natural Mother O Natural Father O Other

2. If other, please specify.

Note: If the subject is not being interviewed directly, please obtain responses appropriate to the subject and not the individual being interviewed for the entire questionnaire. For example, the answer to *Question 3* should be the age of the subject, not the person being interviewed.

3. What is your birth date? Month Day Year

4. What is your gender? O Male O Female

5. What is your current residence?

City State Zip Code Country

6. What is your current occupation?

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7. What is your highest level of education? (If subject is a child, then use parents' highest level.)

- O Did not complete high school
- O High school or GED degree
- O Some college
- O College degree
- O Postcollege coursework
- O Graduate or professional degree
- O Other

Read the following information to each participant.

For many diseases there are known connections between genetic background, racial characteristics or common customs, and ethnic characteristics. These two aspects of your heritage may be difficult to separate and you may consider them to be one and the same thing. To help us better understand your disease, we would like for you to identify yourself using the following categories.

Please select a number to identify the subject from the list below.

1 White	**4** Asian	**7** Uncertain
2 Black or African American	**5** Native Hawaiian	**8** Refused
3 American Indian or Alaska Native	**6** Other	**9** Multiple Races

8. What is your racial background? ☐
9. What is your father's racial background? ☐
10. What is your mother's racial background? ☐

If an answer to *Questions 8, 9,* or *10* was **6** Other, have the interviewee specify.

11. Yourself ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
12. Father ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
13. Mother ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

NIH Ethnic Categories

14. In terms of these ethnic categories, how do you identify yourself? ☐

1 Hispanic or Latino	**3** Unknown
2 Not Hispanic or Latino	**4** Refused

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Severe Asthma Research Program

Environmental Factors

Interviewer (initials) | SARP ID

Month / Day / Year | Center (e.g. NJC)

1. Do you currently have any of the following animals or pets *inside your home*?

Animal or Pet	*Yes*	*No*	*Uncertain*
Cat	O	O	O
Dog	O	O	O
Bird	O	O	O
Rodent: hamster, gerbil, mouse, etc.	O	O	O
Other	O	O	O

If other, please specify:

Notes

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Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

Severe Asthma Research Program

Family History

1. How many siblings did you grow up with? ☐☐
2. How many children do you have? ☐☐
3. Tell me if any of your biologic family members have any of the following problems:

		Father	*Mother*	*Any Sibling*	*Any Child*
Asthma	Yes	O	O	O	O
	No	O	O	O	O
	Uncertain	O	O	O	O
Hay fever or Allergies	Yes	O	O	O	O
	No	O	O	O	O
	Uncertain	O	O	O	O
Eczema	Yes	O	O	O	O
	No	O	O	O	O
	Uncertain	O	O	O	O
Cystic Fibrosis	Yes	O	O	O	O
	No	O	O	O	O
	Uncertain	O	O	O	O
COPD, Emphysema or Chronic Bronchitis	Yes	O	O	O	O
	No	O	O	O	O
	Uncertain	O	O	O	O

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Interviewer (initials) SARP ID

Month Day Year Center (e.g. NJC)

Severe Asthma Research Program

General Symptoms of Lung Disease

In general, over the last 3 months, how often do you have the following symptoms?

	Never	*Once a month*	*Weekly but < twice a wk*	*More than twice a wk but < once a day*	*Daily*	*At least twice a day*
1. *Cough*: deep, chest, chronic	O	O	O	O	O	O
2. *Sputum:* phlegm or mucus while coughing	O	O	O	O	O	O
3. *Chest tightness*: difficulty taking a deep breath or pressure in the chest	O	O	O	O	O	O
4. *Wheezy, whistling, or musical sound* in the chest	O	O	O	O	O	O
5. *Shortness of breath*	O	O	O	O	O	O
6. *Nighttime symptoms*: includes waking from sleep, nighttime use of albuterol, early morning chest tightness	O	O	O	O	O	O

Notes

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Severe Asthma Research Program

Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

Medical History

1. How old were you when you were first diagnosed with breathing problems?

   ☐☐ years old O Not applicable

	Yes	*No*	*Uncertain*
2. In the last 12 months, have you been seen by a doctor because of breathing problems?	O	O	O
3. Have you *ever* visited a hospital emergency room because of breathing problems?	O	O	O
4. If **Yes**, have you visited a hospital emergency room because of breathing problems in the last 12 months?	O	O	O
5. Have you *ever* spent a night in the hospital because of breathing problems?	O	O	O
6. If **Yes**, have you spent a night in the hospital because of breathing problems in the last 12 months?	O	O	O

7. How often do you need to increase your rescue inhaler use?
   - O Never
   - O Once a month
   - O Weekly but less than or equal to twice a week
   - O More than twice a week but less than once a day

8. How long do your attacks of asthma last?

   O <1 hour O 1-6 hours O 6-48 hours O >2 days O Not applicable

	Yes	*No*	*Uncertain*
9. Have you *ever* had an ICU admission because of an asthma attack?	O	O	O
If **Yes**, O 1 time O 2-5 times O >5 times			
10. In the last year, have you had an ICU admission because of an asthma attack?	O	O	O
11. Have you *ever* needed intubation and assisted ventilation because of an asthma attack?	O	O	O
If **Yes**, O 1 time O 2-5 times O >5 times			
12. Have you *ever* had pneumonia or bronchopneumonia?	O	O	O

If **Yes**, mark all that apply.

O Diagnosed by physician O Seen on x-ray O Took antibiotics O Hospitalized

SARP ID

	Yes	No	Uncertain
13. Have you had bronchitis more than once a year treated with antibiotics?	O	O	O
14. Has a doctor *ever* told you you have chronic bronchitis, a productive cough for more than 3 months of each of two years in a row?	O	O	O
15. Has a doctor *ever* told you have emphysema or COPD (chronic obstructive pulmonary disease)?	O	O	O
16. Currently, do you use supplemental oxygen at home?	O	O	O
17. Do you use CPAP or BIPAP?	O	O	O
18. If **Yes**, how many times a week?			
19. Have you *ever* had acute or recurrent sinusitis treated with antibiotics?	O	O	O
20. Have you *ever* had sinus surgery?	O	O	O
21. Do you have nasal polyps?	O	O	O
22. Have you *ever* had a nasal polyp removed?	O	O	O
23. Do you have gastroesophageal reflux disease (GERD)?	O	O	O

24. Are you currently receiving treatment for any of the following conditions?

	Been Diagnosed			*Age of Onset*		*Currently Treated*		
	Yes	*No*	*Uncertain*			*Yes*	*No*	*Uncertain*
Hypertension	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O
Coronary artery disease	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O
Congestive heart failure	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O
Osteoporosis	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O
Diabetes	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O
Vocal chord dysfunction	O	O	O	O <= 2 yrs old O 2 < <= 18 yrs old	O >18 yrs old O Unknown	O	O	O

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Severe Asthma Research Program

Interviewer (initials) | SARP ID

Month / Day / Year | Center (eg, WFU)

Medication History

1. Have you used medication to treat your breathing problems in the last 3 months?

O Yes O No O Uncertain

If **Yes**, then mark all that apply.

	Never	*Once a month*	*Weekly but < twice a wk*	*More than twice a wk but < once a day*	*Daily*	*At least twice a day*
2. *Inhaler beta-agonist* ProAir®, Proventil®, Ventolin®, Xopenex®	O	O	O	O	O	O
3. *Nebulized beta-agonist* Alupent® soln, Proventil® soln, Xopenex®	O	O	O	O	O	O
4. *Oral beta-agonist* Volmax®, Repetab®	O	O	O	O	O	O
5. *Long-acting beta-agonist* arformoterol: Brovana®, formoterol: Foradil®, salmeterol: Serevent®	O	O	O	O	O	O
6. *Theophylline* Slo-bid Theodur®, Theobid®, Uniphyl®	O	O	O	O	O	O
7. *Leukotriene modifiers* montelukast: Singulair®, zafirlukast: Accolate®, zileuton: Zyflo®	O	O	O	O	O	O
8. *Cromones* cromolyn sodium: Intal® nedocromil sodium: Tilade®	O	O	O	O	O	O
9. *Ipratroprium bromide* Atrovent®	O	O	O	O	O	O
10. *Tiotroprium bromide* Spiriva®	O	O	O	O	O	O
11. *Combination therapy* albuterol and ipratropium bromide: Combivent®	O	O	O	O	O	O

Corticosteroid Use: *for all subjects on corticosteroids*

For each category of corticiosteroids, select frequency, dose, and total puffs per day. Mark all that apply.

12. *Inhaled corticosteroids* ○ Never

		Total Puffs or Ampules per Day									
	Dose	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Aerobid®	○ 250 mcg/puff	○	○	○	○	○	○	○	○	○	○
Aerospan® HFA	○ 80 mcg/puff	○	○	○	○	○	○	○	○	○	○
Asmanex®	○ 220 mcg/inh	○	○	○	○	○	○	○	○	○	○
Azmacort®	○ 100 mcg/puff	○	○	○	○	○	○	○	○	○	○
Flovent®	○ 44 mcg/puff	○	○	○	○	○	○	○	○	○	○
	○ 110 mcg/puff	○	○	○	○	○	○	○	○	○	○
	○ 220 mcg/puff	○	○	○	○	○	○	○	○	○	○
Pulmicort® Turbuhaler	○ 200 mcg/inh	○	○	○	○	○	○	○	○	○	○
Pulmicort® Flexhaler	○ 90 mcg/inh	○	○	○	○	○	○	○	○	○	○
	○ 180 mcg/inh	○	○	○	○	○	○	○	○	○	○
Pulmicort® Respules	○ 250 mcg amp	○	○	○	○	○	○	○	○	○	○
	○ 500 mcg amp	○	○	○	○	○	○	○	○	○	○
Qvar® HFA	○ 40 mcg/puff	○	○	○	○	○	○	○	○	○	○
	○ 80 mcg/puff	○	○	○	○	○	○	○	○	○	○

13. *Combination inhaled corticosteroid + long-acting beta-agonist* ○ Never

		Total Puffs per Day									
	Dose	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Advair® Diskus	○ 100/50 mcg/inh	○	○	○	○	○	○	○	○	○	○
	○ 250/50 mch/inh	○	○	○	○	○	○	○	○	○	○
	○ 500/50 mcg/inh	○	○	○	○	○	○	○	○	○	○
Advair® HFA	○ 45/21 mcg/puff	○	○	○	○	○	○	○	○	○	○
	○ 115/21 mcg/puff	○	○	○	○	○	○	○	○	○	○
	○ 230/21 mcg/puff	○	○	○	○	○	○	○	○	○	○
Symbicort®	○ 80/4.5 mcg/inh	○	○	○	○	○	○	○	○	○	○
	○ 160/4.5 mcg/inh	○	○	○	○	○	○	○	○	○	○

14. *Oral corticosteroids* ○ Never
methylprednisolone: Medrol®
prednisone: Deltasone®

Total daily dose, mg ○ 1-10 ○ 11-20 ○ 21-30 ○ 31-40 ○ 41-50 ○ 51-60 ○ 61+

	Never	*Once a month*	*Weekly but < twice a wk*	*More than twice a wk but < once a day*	*Daily*	*At least twice a day*
15. *Injectable corticosteroids* triamcinolone: Kenalog®, dexamethasone: Decadron®, methylprednisolone: Depomedrol®, Solumedrol®	○	○	○	○	○	○

Medical History continued: *for all subjects*

	Never	*Once a month*	*Weekly but < twice a wk*	*More than twice a wk but < once a day*	*Daily*	*At least twice a day*
16. *Immunotherapy* allergy shots	O	O	O	O	O	O
17. *Anti-IgE therapy* Xolair®	O	O	O	O	O	O
18. *Alternative therapies*	O	O	O	O	O	O

If you have used alternative therapies to treat your breathing problems, which ones have you used?

	Yes	*No*	*Uncertain*
19. *Herbal or natural treatments, vitamins,* etc.	O	O	O
20. *Acupuncture*	O	O	O
21. *Chiropractic treatments*	O	O	O

22. Other

23. Have you ever received an investigational asthma therapy: e.g., monoclonal antibodies?

O Yes O No O Uncertain

If **Yes**, which one?

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Interviewer (initials) SARP ID

Severe Asthma Research Program

Month Day Year Center (e.g. NJC)

Provoking Factors of Asthma and Lung Disease

Have any of the following caused you to have asthma symptoms such as coughing, wheezing, or shortness of breath, or made your symptoms worse?

	Yes	*No*	*Uncertain*
1. Respiratory infections: colds	O	O	O
2. Pets or animals	O	O	O
3. Routine physical activities: walking or climbing stairs	O	O	O
4. Physical exercise: sports	O	O	O
5. Aspirin or aspirin-based products: Aleve, Motrin, etc.	O	O	O

Notes

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Severe Asthma Research Program

Interviewer (initials) ☐☐☐ SARP ID ☐☐☐☐☐

Month ☐☐ / Day ☐☐ / Year ☐☐☐☐ Center (eg, WFU) ☐☐☐

Smoking History

1. Are you exposed to second-hand smoke during your day?

   ○ Yes ○ No ○ Uncertain

   If *Yes* ... *Where* are you exposed to second-hand smoke? ○ Home ○ Work ○ Other

   How many *days* a week are you exposed to second-hand smoke? ☐ days

   How many *hours* a day are you exposed to second-hand smoke? ☐☐ hours

2. Would you classify yourself as

   ○ Never smoked ○ Former smoker ○ Current smoker

   Never means less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than 1 cigarette a day for 1 year.

   If *Never*, then **STOP** completing this form.

3. Which of the following do you currently smoke or have smoked?

   ○ Cigarettes ○ Cigar or Pipe

4. At what age did you start smoking? ☐☐ years old

5. How many years have you smoked? ☐☐ years

6. If you are a former smoker, how many years ago did you stop smoking? ☐☐ years

7. During the years you have smoked, on average, how many cigarettes and cigars or pipes did you smoke every day?

   ☐☐☐ cigarettes a day

   ☐☐☐ cigars or pipes a day

40503

Interviewer (initials) | SARP ID

Month / Day / Year | Center (eg, WFU)

Severe Asthma Research Program

For Women Only

1. Have you ever noticed that you have respiratory symptoms (such as wheeze, tightness in your chest, or shortness of breath) at a particular time of your monthly menstrual cycle?

   O Yes O No O Uncertain

   If **Yes**, have you ever increased your use of oral steroids or have you ever been hospitalized because of those respiratory symptoms?

   O Yes O No O Uncertain

2. Do you take oral contraceptives, or do you use other medical contraceptives (eg, implants)?

   O Yes O No O Uncertain

3. When was *day 1* of your last menstrual period? Month / Day / Year

4. Have you had surgery to remove your uterus (womb)?

   O Yes O No O Uncertain

   If **Yes**, on what date did you have this surgery? Month / Day / Year

5. Have you had surgery to remove one or both ovaries?

   O Yes O No O Uncertain

   If **Yes**, on what date did you have this surgery? Month / Day / Year

6. Are you receiving hormone replacement therapy?

   O Yes O No O Uncertain

7. Are you postmenopausal?

   O Yes O No O Uncertain