Defining the relationship between clinical and immunological features of asthma

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Abstract

Background: Asthma is a chronic inflammatory disease in children and adults that is caused by many factors. The most common respiratory symptoms associated with asthma are wheezing, shortness of breath, chest tightness, and coughing, which alter with the duration and severity of the disease. Furthermore, other comorbidities are associated with severe asthmatics patients, such as allergic rhinitis, hay fever, and non-allergic rhinitis. Asthma is increasingly prevalent and affects around 300 million people worldwide. Approximately 5-10% of the asthma population suffers from a more severe form of the disease and has frequent exacerbations. There is a need for better understanding of how asthma presents in different patients, which will allow for stratified medicine approaches.

Objective: To define the relationship between the clinical and immunological features of asthma, I establish and perform a quality control (QC) on a well-characterized asthma cohort from multiple centres. In this cohort, I examine the relationship between blood eosinophils and clinical features of asthma, the impact of smoking on asthma presentation, and clinical and immunological features of asthma subjects that are prone to exacerbation.

Method: In this thesis, the Genetics of Asthma Severity and Phenotypes (GASP) recruited 8534 subjects. The GASP has four different cohorts, which are classified as control (n=1913), asthma (n=5713), chronic obstructive pulmonary disease (COPD) (n=678), and asthma-COPD overlap (n=230). Statistical analysis was performed on the GASP using the software package SPSS and graphs presented with the use of GraphPad Prism software.

Findings: This study included 3804 non-related asthma subjects, Majority are females (66%) with mean age of 40.48 years old. I identified a relationship between blood eosinophils and IgE utilizing data from 635 patients presented with r= 0.232 and p=value of <0.0001. Moderate-severe asthma population identified a relationship between blood eosinophil counts and atopy with p-value of 0.004. On the other hand, a total of 307 subjects reported with current smoking, and they are significantly associated with a lower lung function. I identified a relationship between lung function and ever vs never smoking groups, it has been showed that FEV_1 % predicted presented with median value of 76.56% in ever group. While a reduction in median (72.72%) among never group has been showed. Females were over-represented in those patients that were prone to exacerbation, and a higher symptoms score in subjects experienced exacerbation as reported with p value 0.007

Conclusion: I developed a comprehensive database of clinical and immunological features of asthma. Using this database identified that blood eosinophil counts associated with atopic subjects. Also, it has been identified that smoking were more likely associated with reduction of lung function. In addition, females were more associated with recurrent exacerbation and presented with higher asthma symptoms. Future work will include examined the role of genetics in driving these phenotypes/characteristics.

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Contents

Chapter 1 General introduction

1.1 Definition of asthma

Asthma is a chronic inflammatory disease that occurs depending on many factors. Causes in children and adults are identified based on different respiratory symptoms and variable airflow obstructions related to a chronic bronchial inflammation response (Horak et al. 2016). Asthma is considered one of the most common non-communicable diseases which is not caused by infectious factors and lasts for a long period of time, while progressing slowly (Papi et al. 2017). Moreover, asthma is the result of a mixture of interacting gene–environment agents, with heterogeneity in phenotype. In addition, asthma is affected by a change in the severity of airflow inflammation (Papi et al. 2017).

The most common respiratory symptoms associated with asthma are wheezing, shortness of breath, chest tightness and cough, which change with time and severity and are linked to variable expiratory airflow control. Therefore, asthma is largely defined by the presentation of respiratory symptoms (Reddel et al. 2015). Furthermore, there are comorbidities associated with severe asthma, as allergic rhinitis, hay fever and non-allergic rhinitis (Shaw et al. 2015). Also, patients with severe asthma are well documented to have gastro-oesophageal disease, as firstly explored by Osler (1912). The severity of these symptoms can affect the daily life activities of the patient. Therefore, the patient's quality of life may be decreased, and there may also be a decrease in productivity, because of absence from work or school (Meltzer et al. 2011, Jaén and Dalton 2014).

1.2 **Asthma as a global disease**

Most research on asthma has emphasised an increase in prevalence, and that it affects around 300 million people worldwide (Meltzer et al. 2011), which can be viewed as an economic threat to various nations (Gonzalez-Garcia et al. 2015). There is a large volume of published studies describing the increase in prevalence of asthma around the world, and asthma is reported as a global health problem, affecting people of all ages, from children 6–7 years old up to and through adulthood (Lai et al. 2009). On the one hand, it is claimed that 1–21% of adults, both male and female, experience asthma and associated severe wheezing (To et al. 2012), and that up to 20% of children are similarly affected (Lai et al. 2009). On the other hand, it seems that in recent years there has been a reduction in asthma prevalence amongst children (Backman et al. 2017). Approximately 5–10% of asthma patients are likely to have severe asthma, which is characterised by frequent exacerbations (Chen et al. 2016).

In 2017, McCracken et al. described the prevalence of asthma in the United States, finding that 7.5% of people affected by asthma are adults, regardless of ethnicity. The results of a population-based study of adults from 40 to 93 years of age in Colombia show that 9% of individuals have asthma compared to 11.9% who experience wheezing, which is linked to asthma (Gonzalez-Garcia et al. 2015). The prevalence of asthma varies over countries and differs among various groups. A recent cross-sectional survey was carried out across 70 countries, which found a variation in the prevalence of asthma from 1% in Vietnam to 21.5% in Australia. In line with previous results, an estimated increase in the prevalence of asthma stands at 4.5% of the global population

(To et al. 2012). Therefore, asthma is considered a chronic and non-transmittable illness that affects around 334 million people (Papi et al. 2017). Lastly, studies have shown that there may be an increase in the global prevalence of asthma by 100 million before 2025 (Masoli et al. 2004).

1.3 **Diagnosis of asthma**

Presently, there is no specific standard to diagnose asthma; it is diagnosed based on symptoms and lung function tests. Because the heterogeneity of asthma is significant to its clinical presentation, pathophysiology and clinical error can occur, especially without measuring patients' lung function (Aaron et al. 2017). Therefore, discussing a patient's medical history and conducting a full patient assessment followed by a lung function test are important. Recently, the Global Initiative for Asthma (GINA) strongly recommended conducting a lung function test on patients suspected to have asthma. It is known that asthma includes airway obstruction and hyperresponsiveness, and if the patient is characterised by $FEV_1/FVC < 0.7$ L and improves on the FEV_1 by 12% or 200 mL after being administered short-acting beta-2 agonist (SABA), this indicates a diagnosis of asthma, as shown in Figure 1.1 (So, Mamary and Shenoy 2018).

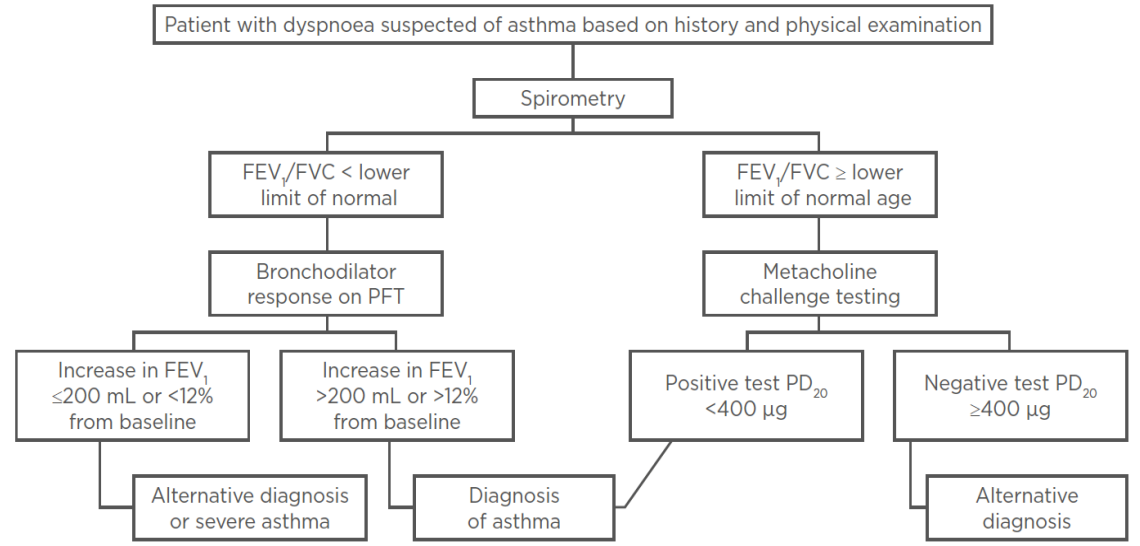

Figure 1.1 Asthma diagnosis algorithm

FEV_1: forced expiratory volume in 1 second; FVC: functional vital capacity; PC20: provocative concentration causing a 20% decline in FEV1; PD20: provocation dose causing a 20% decline in FEV1; PFT: pulmonary function testing. Adapted from (So, Mamary and Shenoy 2018).

1.4 **Morbidity in asthma**

Over the past century, there has been a dramatic increase in morbidity of asthma patients. Traditionally, asthma is tracked by physician visits or when an exacerbation attack sends a patient to an emergency department for medical intervention and hospital admission. The major factors correlated with morbidity of asthma are likely to be linked directly in both genders: obesity, smoking history, life activities and decreasing levels of lung function (Kankaanranta et al. 2016). Moreover, researchers find that some of the most frequent conditions related to asthma are gastro-oesophageal reflux disease (GERD), rhinosinusitis, psychological instabilities, respiratory infections and obstructive sleep apnoea (OSA) (Boulet 2009).

Several researchers have reported that half of asthmatic patients claim that asthma is connected to other respiratory conditions, for instance upper respiratory tract infection, pneumonia and allergic rhinitis (Kankaanranta et al. 2016). Moreover, obesity is another condition associated with asthma, especially in female patients. It seems that increases in neutrophilic sputum and a decline in the range of eosinophilic sputum lead to recurrent and frequent asthma attacks (Papi et al. 2017). Therefore, as has been demonstrated through much research, a decrease in weight and body mass index (BMI) results in improvements in both clinical and physiological asthma factors (Taylor et al. 2008). Several studies have reported a profound link between asthma and obesity, and its effects on asthma parameters, specifically in adults (Kankaanranta et al. 2016). Table 1.1 presents different risk factors for asthma.

Risk factors of asthma	
Personal factors	**Environmental factors**
Genetic predisposition	Indoor/allergens
Atopy	Socioeconomic factors
Airway hyper-responsiveness	Family factors
Gender	Smoking history
Race/ethnicity	Air pollution
Poor lung growth and development	Obesity
Exercise	Respiratory infections
Stress	Pets, pollen and dust

Table 1. 1: **Personal and environmental risk factors for asthma.**

Rhinitis is one of the most common comorbidities affecting asthma parameters. Studies have reported a significant relationship between allergic rhinitis and asthma, and researchers have suggested that rhinitis comes before asthma in the atopic march (Leynaert et al. 2004). This effect can occur via different mechanisms, including relief of the mediators to the airways or peripheral circulation, neural impulses, improvements in bone marrow creation, high exposure of lower airways to infections because of mouth breathing, and, eventually, an increase in a patient's demand for moist air (Boulet 2009). In terms of metabolic mechanisms, asthma may be affected by many of them, especially with adult onset, including diabetes mellitus type 2 (DM2), cardiovascular disease and psychiatric diseases (Kankaanranta et al. 2016).

Another very common comorbidity in the asthmatic patient is cardiovascular disease (CVD), and its role in affecting the inflammatory process in asthma patients. Chronic heart disease (CHD), which is in the CVD family, is the most common morbidity related to asthma, because of its inflammatory marker influence on asthma's biomarker nature. Moreover, studies have shown that women with CHD are highly likely to have asthma and more likely to experience frequent asthma attacks. Adult-onset asthma is highly associated with an increased risk of developing CHD and suffering stroke, especially in women (Lee, Truong and Wong 2012).

In conclusion, comorbidities of asthma have a significant influence on health conditions, health care utilisation and hospitalisation. In addition, there is some

genetic evidence that allergic conditions, including allergic rhinitis, atopic dermatitis and asthma, share risk factors and hence co-occur. Therefore, it seems these comorbidities may affect health costs and mortality, and should be considered and supported by ongoing research.

1.5 **Lung function in patients with asthma**

Traditionally, lung function was measured by spirometry and was considered one of the confirmation procedures to diagnose airway obstruction diseases, especially asthma and chronic obstructive pulmonary disease (COPD). In addition, many reports have observed that reversibility in response to bronchodilation contributes to asthma diagnosis (Macy et al. 2005). Lung function is an increasingly important area in the respiratory field, and it is a good indicator of disease progression as it is always available and easily accessible. GINA has classified asthma severity (mild, moderate or severe) based on medication use (Horak et al. 2016). Furthermore, terms such as ‘uncontrolled’ or ‘difficult to treat’ asthma have changed the concept of asthma severity levels and stated clearly as severe asthma (Horak et al. 2016).

In terms of monitoring asthma progression, spirometry findings using FEV1 in line with the ratio of FEV1 over FVC after using a particular bronchodilator (Horak et al. 2016). Moreover, reversibility has been reported by the American Thoracic Society (ATS) as a significant improvement if FEV1 increases by 200 mL or more or improves by 12% after bronchodilator administration (Ye, Liao and D’Urzo 2018). Different studies over the years have reported that asthmatic patients show evidence of lung function impairment because of the fast

deterioration in FEV1 (Coumou et al. 2018). This decline in lung function can be accelerated by the influence of various factors, including smoking status and repeated asthma attacks, and via the low FEV1 baseline (Bai et al. 2007). Moreover, it is important to uncover these early stages for any asthmatic patient with a high risk of accelerated reduction in lung function, due to the increase of both morbidity and mortality (Coumou et al. 2018).

1.6 **Current asthma medications**

To date, no drug exists that will cure asthma; however, there are medications to control and relieve asthma symptoms. For example, SABA and long-acting bronchodilator (LABA) are most commonly recommended to treat asthma symptoms and bronchoconstriction (Svensson et al. 2011, O'Byrne et al. 2018). However, these medications may not be beneficial with severe asthma, and more control of patients' symptoms is needed to avoid deterioration and eventually exacerbation. Therefore, inhaled corticosteroids are used to treat severe asthma, especially with type 2 inflammation (Dunican and Fahy 2017). Furthermore, studies have shown that the use of a LABA/ICS combination for mild to moderate asthma is associated with improved lung function and a decrease in the frequency of asthma exacerbations (Svensson et al. 2011, Mahr and Eppley 2017).

1.7 **New biological asthma medication**

Recently, newly developed biological medications, which target type 2 cytokines, have been launched by scientists. Table 1.2 presents the target, indication and clinical effects of each of these biological medications. These

biological medications works as an inhibitor for some relevant asthma pathway. Type 2 cytokines play a key role in asthma pathology and genetics. It has been reported as a potential treatments on targeted antibodies, such as IL-4, IL- 5, and IL-13 pathways (Robinson et al. 2017).

Biological medication	Target	indication	Evidence	Reference
Omalizumab	**IgE**	Poor control on ICS or LABA Total serum IgE level ≥30 IU/mL	25% reduction in all exacerbation 50% reduction in severe exacerbation	Humbert et al. 2005
Mepolizumab	**IL-5**	Poor control on ICS or LABA More than 2 exacerbation attack per year Eosinophil counts > 150 cells/μL	> 50% reduced in all exacerbation > 60% reduced in hospital admission and emergency visits	Bel et al. 2014
Dupilumab	**IL-4**	Eosinophil counts > 300 cells/μL FeNO ≥ 25 ppb	47% improvement in severe exacerbation 320 mL improvement in FEV_1	Castro et al. 2018
Tezepelumab	**TSLP**	Poor control on ICS or LABA More than 2 exacerbation attacks per year	Reduction in exacerbation by > 60% Improvement in FEV_1 by > 110 mL	Corren et al. 2017

Table 1. 1: New biological medications for asthma

1.8 **Asthma heterogeneity**

Asthma is a complex disease, most commonly an immunological disease, that affects children and adults. It is thought that asthma is a heterogenous disease in terms of phenotype, endotype, response to treatment, and/or long-term clinical outcomes. In severe asthma, more heterogeneity has been discovered by molecular phenotyping of blood, induced sputum and bronchial epithelial brushings. Studies have reported that asthma clusters may differ as a reflection of genetic differences, due to environmental effects, or because of a combination of genetic and environmental factors (Fahy 2015, Loxham and Davies 2017).

During the past two decades, because of this heterogeneity, many researchers have sought to determine the relationship between asthma and atopy in both children and adults. Studies show atopy is found in 50–60% of asthmatic adults and children (Papi et al. 2017). In fact, atopy has increased in prevalence and affects more than 30% of the UK population (Joe 2011). According to the GINA guidelines, atopy has been classified at the same level as other allergic conditions such as eczema and rhinitis. At certain times of the year, asthma is prevalent among children and adults, and sometimes is increased, a factor which is associated directly with atopic sensitisation (Masoli et al. 2004).

Traditionally, atopy is affected by continuous exposure to allergens and different environmental factors, leading to clinical asthma. This exposure to different proteins tends to reduce airway inflammation and hyper-responsivity of

the bronchi, and eventually leads to variable airflow obstruction. Previously, atopy was focused on the specific factors that may affect asthma biomarkers, seemingly collected from family histories, including allergic rhinitis (hay fever), eczema, and some other non-specific allergic conditions. However, atopy is now recognised by a reaction to common environmental allergens, and even by the skin prick test in association with an increased production of specific IgE serum (Pearce, Pekkanen and Beasley 1999).

Many studies have reported that asthma is a heterogeneous disease, which has many subgroups linked directly with various disease causes, clinical findings and genetic bases. Cluster analysis has identified several phenotypic clusters, which show many differences in sex, age of onset, lung function, atopy, asthma control and exacerbation frequency (see Figure 1.2). Using cluster analysis, researchers recognise similarities among subjects within a population in specific variables after grouping them into clusters. These groups are classified into different clusters according to lung function (clinical), IgE and blood inflammatory cells (immunological), and gender and age of onset (Haldar et al. 2008, Jarjour et al. 2012). The UK Leicester study's adult cohort (Haldar et al. 2008) and the Severe Asthma Research Program (SARP)'s cohort (Moore et al. 2010) demonstrate many similarities between their clusters. Patients having atopy, associated with allergy, were correlated with early-onset disease, while in late-onset disease patients displayed obesity and an absence of eosinophilic inflammatory profile.

Moreover, in the UK Leicester adult cohort, subjects were classified into three

clusters in terms of refractory asthma and based on physician diagnoses. The first cluster comprised the subgroup with early-onset atopic asthma. The finding from that cluster is that there is evidence of clinical deterioration including symptoms, eosinophilic level and impairment in lung function. A further finding from this cluster was a significant association between patient visits to hospital and asthma exacerbation that needed to be resolved by oral corticosteroids. In contrast, cluster 2 included obese female subjects and demonstrated that patients' eosinophilic airway inflammation more likely disappeared. In this subgroup study, early-onset atopic asthma and obesity together with eosinophilic asthma were common within clusters (Haldar et al. 2008).

Another example of cluster analysis is the SARP adult cluster, which was analysed in terms of clinical characteristics. SARP identified five different clusters of asthma in comparing adults with mild, moderate and severe asthma. The clustering was based on age of onset, lung function, sex, atopy, the use of medication and health care utility (Moore et al. 2013). Furthermore, SARP reported that eosinophilic phenotype presented in late-onset disease subjects with nasal polyps and exacerbation (Wu et al. 2014).

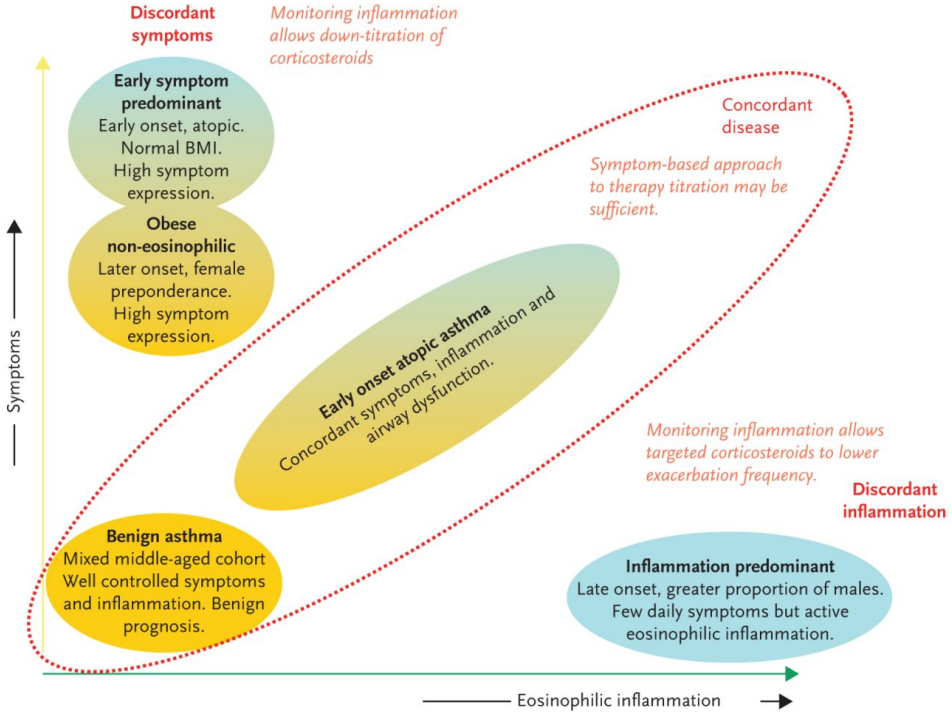

Figure 1. 2: Cluster plot identifying several phenotypic clusters.

A summary of asthma phenotypes identified using cluster analysis, based on symptoms and inflammation (Haldar et al. 2008).

1.9 The correlation of clinical and phenotypic measurements in patients with asthma

One of the most significant current discussions regards identifying subsets of asthma based on clinical and immunological markers. The characteristics of asthma are similar in all patients; they have largely the same symptoms and abnormalities that are diagnosed by similar techniques, including spirometry and with a peak expiratory flowmeter. Despite these common characteristics, a strong relationship was noticed among the severity of airway obstruction, clinical phenotypes, and the development of medication responsiveness (Nirav and Prescott, 2011). Nirav and Prescott (2011) examined two different groups to investigate the correlation of clinical and phenotypic findings associated with

asthma. The results of their study were interesting; they classified the subjects with asthma as both Th2 high and Th2 low. However, there was a clear decrease in FEV1, slow or decreased bronchodilator responsiveness, and positive results of the skin prick test.

One study reported a significant increase in total serum IgE in a group of patients with shortness of breath (SOB). However, the peripheral eosinophilic percentage was significantly increased in association with a cough (Zedan et al. 2013). Other studies examined the relationship of asthma with lung function. Lung function shows impairment in up to 30–45% of patients with asthma and induced to high doses of ICS (Nirav and Prescott , 2011). On the other hand, the significance of airflow limitation prevalence is remarkable, especially with age, even when linked to the highest bronchodilator (BD) reactions. Further, allergen sensitisation and blood eosinophilia, which are known to be essential markers of Th2 inflammation, were significantly reduced after childhood (Teagut et al. 2017).

1.10 **Predictors of asthma exacerbation**

Recent literature shows different results related to the importance of knowing predictors of asthma exacerbation. The recurrence of increased symptoms in the asthmatic patient is called exacerbation or asthma attack. Exacerbation is further defined as a sharp increase in breathlessness, wheezing and chest pain and a decrease in lung volume and capacity. Exacerbation is rapid in children, but can appear in adults over a week or longer. The state of exacerbation is severe and can be fatal, and affects daily life activities and quality of life (Papi

et al. 2017). In addition, SARP data show that patients with a high level of inflammatory Th2 are at high risk of an exacerbation. Further, it is stated that patients experiencing exacerbation are inversely associated with IgE (Denlinger et al. 2017).

1.11 **The genetics of asthma**

The primary cause of asthma is presently unknown; however, the risk factors related to family history or genetics have been known for a long time. Genetics plays a vital role in asthma disease understanding and control. This importance associated with asthma biomarkers in terms of heritability varies between 35% and 95% (Mims 2015). In some studies, allergic rhinitis (hay fever) has been reported to range from 33% to 91%. In others, the ratio of atopic dermatitis and the total serum of IgE is presented at the 84% level. Therefore, the genetic variation is significant and contributes to an increased risk to the asthmatic (Figure 1.3) (Ober and Yao 2011).

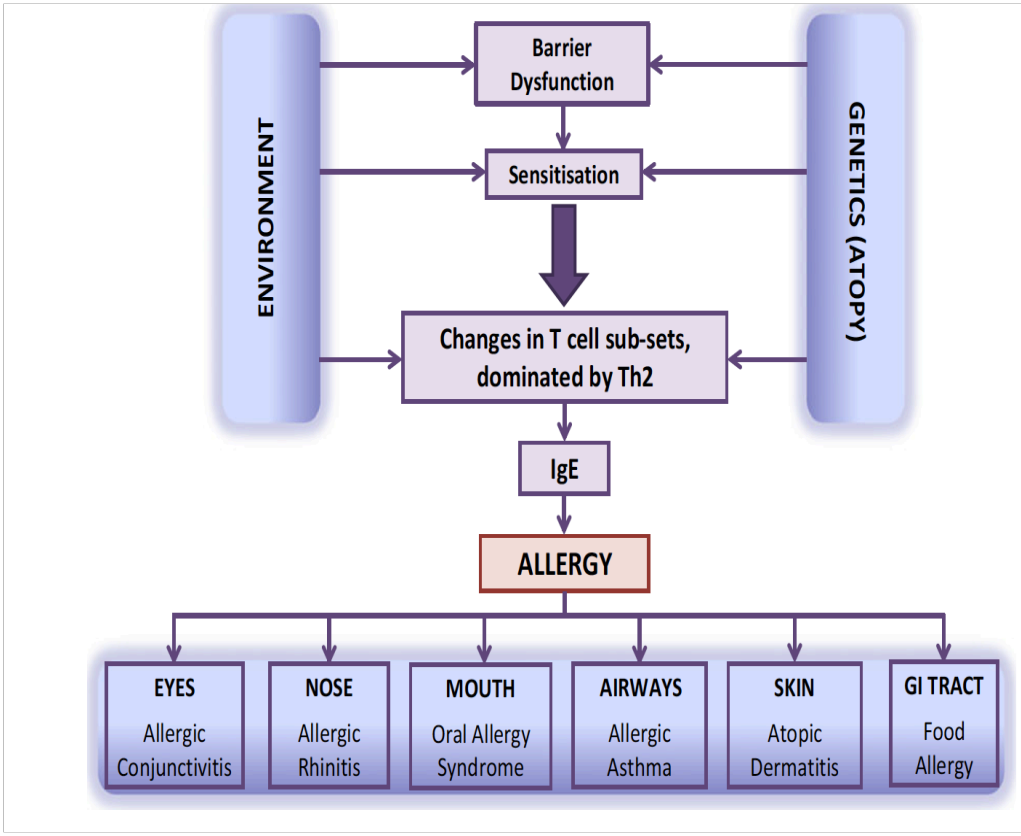

Figure 1. 3: The influence of both environmental and genetic factors, affected by allergens, on different organs.

Genome-wide association studies (GWAS) is a recent method utilised by scientists to identify genes involved in complex diseases such as asthma. GWAS was pioneered by Moffatt et al. (2007), in their study of 1243 non-asthma controls and 994 subjects with childhood-onset asthma that identified single-nucleotide polymorphisms (SNPs). Interestingly, using more than 317,000 SNPs, they found a highly significant relationship between multiple genes associated with loci on chromosome 17q21 (Moffatt et al. 2007). The 17q21 loci have been explored in other large-scale studies carried out by the European GABRIEL and have shown a significant association with asthma biomarkers (Moffatt et al. 2010). Similarly, studies of different cohorts have reported the same association of loci on 17q21 with severe asthma (Wan et al. 2012). Since

then, more evidence of this association has emerged (Bønnelykke et al. 2014). Interestingly, across independent cohorts, the importance of the locus as defined by the first GWAS has come through associations with several clinical findings linked to asthma, such as lung function (FEV1), bronchial hyper-responsiveness (BHR) and disease severity (Tulah, Holloway and Sayers 2013). In addition, more than 50 studies have demonstrated a significant association between the variant genes and asthma, using between 300 and 2000 subjects diagnosed with asthma; they are therefore estimated to have identified around 50% of the association of common variants (Welter et al. 2014, Hakonarson, March and Sleiman 2015). Overall, it is important to mention that around 49% of the lifetime risk of asthma could be clarified by the loci identified in the study reported by the GABRIEL consortium (Moffatt et al. 2010).

While scientists continue to examine the association between asthma and phenotypes, other studies explore the association of asthma with total serum IgE to understand the genetic nature of allergic diseases. Weidinger et al. (2008) published the first GWAS for IgE, using a cohort of asthma patients with replication in different population study samples (n = 9769). This first GWAS found that total serum IgE was significantly associated with loci on chromosomes 1q23, 5q31 and 12q13 (Weidinger et al. 2008). The second GWAS reported by the GABRIEL consortium confirmed the association between serum IgE levels and chromosomes and introduced two other relevant loci on chromosomes 6p21.3 and 16p12 (Granada et al. 2013).

1.12 **Translating genetics**

Using GWAS, more than a million DNA polymorphisms in the genome have been examined for a relationship with asthma. According to GWAS, the genes most commonly linked with asthma are IL33 and IL-1 receptor-like 1 (IL1RL1). Additionally, eight IL33 SNPs are reported by many studies to be related to asthma phenotypes. However, IL1RL1 has been found with 15 different SNPs (Grotenboer et al. 2013). Further, there is a need to identify the genetic contribution to the development of specific features of asthma, with the potential to identify genetically determined subgroups of asthma.

Aims of this MSc thesis

This MSc thesis comprises composed of four themed chapters. The main aim of the thesis is to establish a large database of clinical and immunological features for asthma patients recruited as part of the GASP initiative. The GASP database includes a large population; therefore, I will present basic demographics for each cohort in the second chapter. The third chapter will concentrate specifically on the subjects with moderate to severe asthma. The fourth chapter will study the subjects to identify different clinical and immunological features of asthma patients. These questions are formulated to examine the following relationships:

- Blood eosinophil counts and clinical features of asthma;
- The impact of smoking on asthma presentation;
- Clinical and immunological features of asthma subjects that are prone to exacerbation.

Chapter 2: Demographics and clinical features of the Genetics of Asthma Severity and Phenotypes (GASP) cohort

2 Introduction

Cohort studies of respiratory disease patients and control subjects have led to a new understanding of the clinical, immunological and molecular features and heterogeneity of asthma and COPD. The SARP and U-BIOPRED cluster analyses are examples of these studies. As mentioned in Chapter one, a considerable amount of literature has been published on asthma and COPD. These studies recognized asthma and COPD based on various diagnoses and different clinical management, and they classified them as the most distinguished obstructive pulmonary diseases (GINA + GOLD COPD). In this chapter, clinical and immunological features of asthma and COPD cohorts will be shown, and these features will be compared with the literature to obtain a good understanding of asthma and COPD features. There have been many longitudinal studies involving asthma subjects that have reported different clinical and immunological findings, as will be described in the next chapter.

A previous study by Teague et al. (2018) demonstrated a compromise between children and adult asthma subjects. In that study, they clarified the differences with age among children and adults having asthma in several clinical variables, including: body weight, lung function and inflammatory biomarkers after being studied by their association with age. Their research showed that out of 188 children there was an increase in the exacerbation-proneness to asthma but no effects on body weight or lung function after bronchodilator administration. In parallel to the previous studies, Denlinger et al. published a

paper in 2017 that described the clinical and inflammatory factors in severely asthmatic patients and associated with many exacerbations. Out of 709 subjects involved in this study, approximately 34% had several exacerbations; however, around 24% were exposed to recurrent exacerbations. Patients with recurrent exacerbation episodes are most likely to present with direct association with the severity of asthma. Moreover, this study confirmed what was mentioned before about the association of asthma severity and elevation of eosinophil counts, and it reported that with more exacerbation episodes there is a positive association with eosinophilia and body mass index (BMI) (rate ratio of 1.3 [1.1–1.4]). Similarly, the same study found that patients with exacerbation appeared more likely to have gastroesophageal reflux (Denlinger et al. 2017).

To understand better the mechanisms of COPD and its effects, Kania et al. analysed the clinical outcomes and treatment of patients who were included in a Polish cohort of the phenotypes of COPD. In a different classification of diseases, 430 Poles aged more than 40 years old were recruited with COPD diagnosis based on doctor assessment. They found statistically significant changes concerning disease pathology, lung function and treatment. This study reported that the lowest lung function recorded, especially with COPD patients having exacerbations, was linked with chronic bronchitis. Besides, it appeared that with their decline in lung function, they seemed to have frequent episodes of depression and anxiety. In addition, they reported that subjects with asthma–COPD overlap are more likely to be found atopic and their obesity level is high (Kania et al. 2018).

Together, the studies in this section provide important insights into the importance of understanding the role of asthma and COPD in the respiratory medicine field and give an understanding of their pathologies and treatments across different published cohorts.

Aims of the chapter:

- To summarize clinical and immunological findings of GASP cohort that consists of control, asthma, COPD and asthma/COPD subjects.
- To describe the different basic demographics in:
    1. Asthma patients with doctor diagnosed.
    2. COPD patients with doctor diagnosed.
    3. Patients with a combined doctor diagnosis of asthma and COPD.
- To identify differences in clinical features associated with asthma and COPD.
- To present the allergic findings among asthma and COPD subjects across GASP cohort.

2.1 **Genetics of Asthma Severity and Phenotypes (GASP) cohort**

The Genetics of Asthma Severity and Phenotypes (GASP) cohort was developed as part of an Asthma UK-funded programme to establish a cohort of asthma patients enriched in moderate-severe disease. This initiative had two main phases, i) the consolidation of existing small cohorts from Nottingham and collaborators of the Sayers research group and ii) prospective recruitment of new subjects from centres across the UK (Figure 2.1). As part of the consolidation process, cohorts containing control subjects and/or patients with COPD were included to provide a usable respiratory database with controls, asthma, COPD patients and patients with a doctor's diagnosis of both asthma and COPD. Furthermore, prospective recruitment has been ongoing since 2000 but this cohort was expanded dramatically across the UK in 2014. This database was collected under ethical approval (GM129901) that was obtained from the multicentre research ethics committee, and samples were transferred to the University of Nottingham with a particular agreement. These samples are collected in the GASP based on the patient's clinical condition, and they are defined into groups based on updated GINA 2018 and GOLD COPD strategies. Moreover, all of the subjects are accepted after a written participation consent to be legally part of this study. As shown in Table 2.1, subjects were recruited from 20 UK centres and, for completeness, an Italian cohort was included in GASP during the consolidation of the cohort resources process outlined. It can be seen that control subjects on the GASP presented with small numbers with a total of 1913 subjects recruited compared with asthma and COPD. However, the largest population recruited was asthma with a total of 5713, until the date I

started this project. In addition, COPD recruited a total of 678 subjects. Moreover, asthma–COPD overlap syndrome has been involved in this cohort with 230 subjects.

Centre	Status				Total
	Control	Asthma	COPD	Asthma & COPD	
BELFAST	0	105	0	0	105
BIRMINGHAM	0	185	0	0	185
CHESTERFIELD	0	4	0	0	4
CUMBRIA	0	193	0	0	193
DERBY	0	52	0	0	52
GLASGOW	0	820	0	0	820
ITALIAN	42	367	39	3	451
LEICESTER	0	378	410	227	1015
LINCOLNSHIRE	0	14	0	0	14
LIVERPOOL	0	23	0	0	23
MANCHESTER	0	925	0	0	925
NOTTINGHAM	1284	864	229	0	2377
NORTHUMBRIA	0	46	0	0	46
PORTSMOUTH	0	307	0	0	307
SHEFFIELD	0	61	0	0	61
SOUTHAMPTON	587	1263	0	0	1850
STOKE	0	102	0	0	102
WEST MIDDLESEX	0	3	0	0	3
Total	1913	5713	678	230	8533

Table 2.1: Samples collected from various centres, and characterized based on their status (control, asthma, COPD and asthma–COPD overlap). The table lists each centre with the number of subjects recruited for each population.

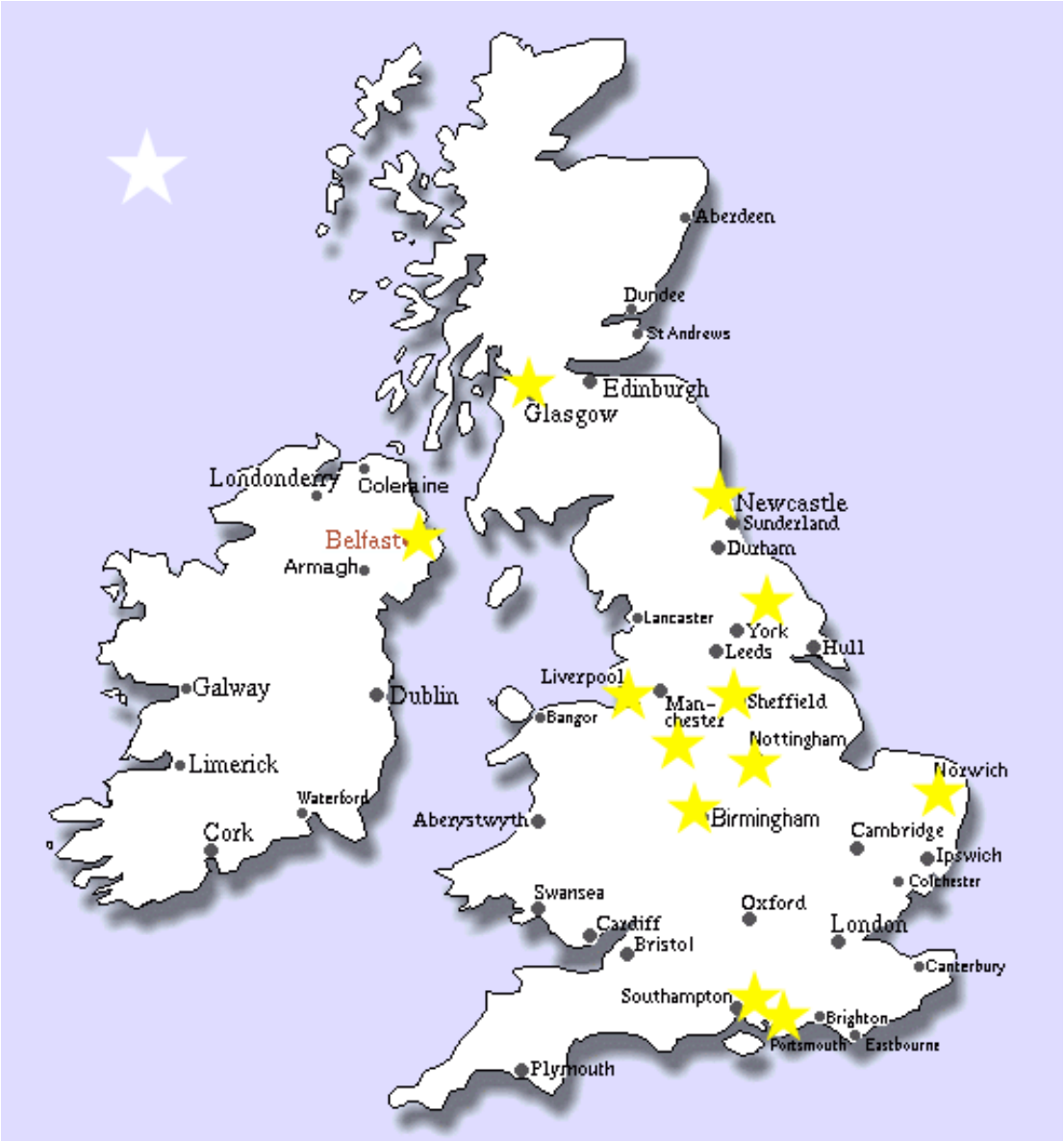

Figure 2. 1: **United Kingdom map demonstrating the GASP subject centres**. Stars in the map represent the cities that have been participating by recruiting subjects in the GASP.

Methods

2.2 For consolidation of existing cohorts

Data for this study were collected depending on selected clinical findings values as appeared in GASP cohort, which included age, date of birth, age of onset, gender, lung function measurements (pre- and post-bronchodilator FEV_1, pre- and post-bronchodilator FVC, FEV_1 % predicted and FEV_1/FVC), blood eosinophils, septum neutrophil, serum IgE, presence of allergic rhinitis (AR) and atopic dermatitis (AD).

Besides, the importance of this study on asthma and COPD patients led to recording their smoking status and how many packs in a year were smoked. Furthermore, the Asthma Control Questionnaire (ACQ) was completed by patients themselves based on a formal questionnaire with seven questions. Subject recruitment relied on children and adults either from a family or individually. Further, age and gender classified from the questionnaire were checked by the Respiratory Medicine Division of the University of Nottingham and obtained after they had filled out the participation consent. In addition, all lung function measurements were collected from their spirometry test, and the rest of the blood values, e.g. eosinophil and serum IgE, added to the database from their blood tests.

2.3 Quality Control (QC) on the GASP

Because the cohort was established using a combination of digital and hard copy phenotype data that had to be manually curated, extensive QC measures were applied to ensure consistency between researchers entering these data and to establish the error rate in the entire database prior to commencing

analyses to obtain clear and reasonable findings. The QC was checked on each variable by organising them ascendingly or discerningly. For continuous variables, checking was to find any missing data or typing error. The checks were applied on the date of birth, age and lung functions (FEV_1/FVC and FEV_1 % predicted).

The spirometry test is essential to diagnose and manage patients with asthma and COPD. Regarding spirometry quality control, it is essential to make sure of QC before starting the test. The accuracy of the test and assuring performing the procedure in very great technique will avoid misdiagnosed patients in the early stage of COPD and will help individuals managing their condition. In the GASP, subjects are examined in the clinics under the supervision of pulmonary function technicians or trained nurses. The pulmonary function test provider is required to make a routine check to the spirometry. Spirometry was performed before and after bronchodilator. Furthermore, FEV1 % predicted was calculated based on the last ERS update (NHANES 1990). There is no particular reason for choosing the NHANES III, except the spirometry test is performed on the clinical sites where this equation is still used. Besides, the GASP started to recruited patients since 2000. Therefore, for those reasons normative equation was used rather than the GLI 12 equation.

2.4 **Handling missing data**

As mentioned previously, the GASP information was collected from 20 different centres in the UK and inserted manually to create the GASP dataset. After the QC was done, a check was performed over all the dataset to find any

missing data. The reason for checking the missing data is to avoid ending up with inaccurate inference data or wrong predictions. While checking the dataset, any data found to be missing were rechecked in two ways, first by reviewing the written clinical form/source in archived files. Second, emailing the centre from where the data were provided for confirmation. These missing values can be attributed to the clinical centres not recording a specific variable. There was less than 3% missing values in certain variables of the entire dataset. Missing data were not processed and excluded from this study.

2.5 **Statistical analysis**

This chapter describes the GASP population generally, as mentioned at the beginning of this chapter. Therefore, basic statistical analyses have been used. There was no significant To describe the continuous data, means and ranges have been presented. To describe the categorical data, the percentages of presence among each group ($100 \times n/N$) with subjects involved were reported. Normality was checked all over the database to find out any non-normal distribution on continuous variables. Finally, there were some uncorrected cases either missing or typed errors, so these cases were deleted because of their inadequacy. An important issue found during analysis was the non-normal distribution shape in both blood counts and the total IgE serum levels. Therefore, a normality test using the Kolmogorov–Smirnov (K–S) test was used to confirm the lack of normality. Transformation to log 10 was performed, but still, the K–S test showed results as statistically not normally distributed (Figure 2.2).

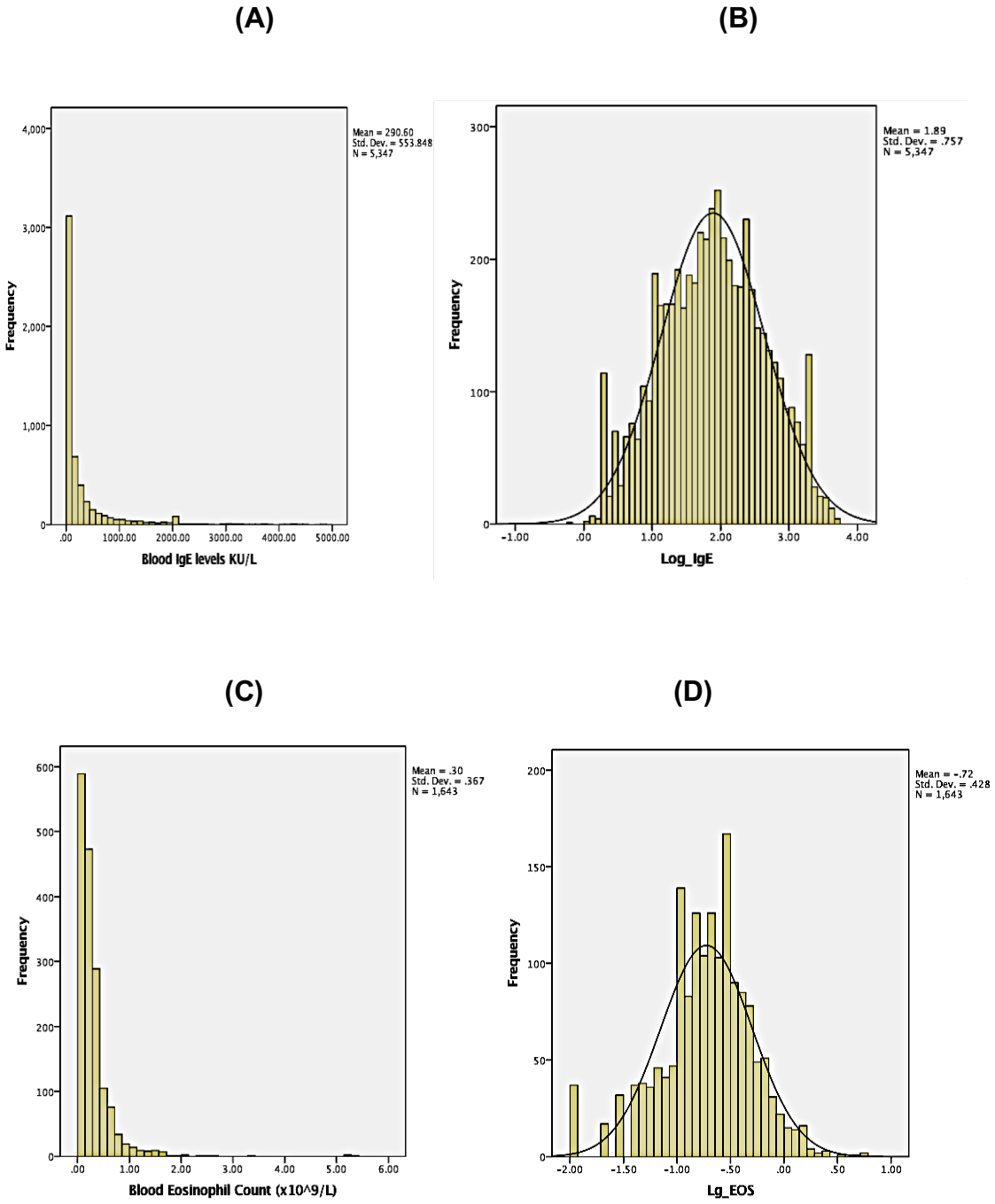

Figure 2. 2: (A) Non-normal distribution histogram for blood IgE levels. (B) Normal distribution histogram of blood IgE levels after log 10 transformation applied. (C) Non-normal distribution histogram of blood eosinophil counts. (D) Normal distribution histogram after log 10 transformation applied. However, the K–S test distributed data as statistically non-normal.

Result

2.6 Basic demographics of the GASP population:

This chapter summarises the basic, clinical and immunological features of subjects in the GASP cohort of the GASP population to clarify the contents of this database and the different cohorts included in this study. The basic demographics of this study population are presented in Table 2.2. The GASP study recruited 8534 patients to this study, they are categorized into four different categories. These categories are control 1913 patients, asthma 5713 patients, COPD 678 and asthma–COPD overlap syndrome 230 subjects. Overall, in this study, female subjects were the majority of the GASP subjects. Furthermore, the highest number of females were recruited in the asthma population (71.2%). In addition, the mean ages among the GASP was between 40.48 (in asthma) and 66.95 years old (in asthma–COPD). Because this study contained asthma and COPD subjects, it is essential to clarify the smoking status of subjects to get a better understanding while analysing different associations. Active or current smoking have been noticed to be higher in the asthma population with a total of 560 subjects, while in COPD the mean of packs/year was higher than other groups, as expected with COPD's heavy smoking habit.

	Control N= 1913	Asthma N= 5713	COPD N= 678	Asthma & COPD N= 230	Total N= 8534
Gender	Female (21.0%) N= 943	Female (71.2%) N= 3198	Female (5.3%) N= 236	Female (2.6%) N= 117	(55.8%) N= 4494
Age	48.72 (5-80) N= 1892	40.48 (3-89) N= 4964	66.78 (40-93) N= 558	66.95 (46-89) N= 230	45.23 (3-93) N= 7644
Height (m)	1.68 N= 1812	1.63 N= 4556	1.67 N= 538	1.65 N= 229	1.64 N= 7135
Smoking status	**N= 1834**	**N= 4620**	**N= 546**	**N= 230**	**N= 7230**
Never	1021 (24.7%)	3108 (75.2%)	2 (0.0%)	0 (0.0%)	4131
Ex-smoker	477 (23.9%)	952 (47.8%)	391 (19.6%)	173 (8.7%)	1993
Current smoker	336 (30.4%)	560 (50.6%)	153 (13.8%)	57 (5.2%)	1106
Smoking Pack/Yrs	18.42 N= 758 (0.05-217.50)	13.79 N= 1180 (0.03-161)	48.98 N= 542 (6.66-255)	47.77 N= 222 (0.45-240)	24.94 N= 2702 (0.03-255)

Table 2. 2: Basic demographics of clinical features of the GASP. Continuous variables are presented with mean, ranges and the number of subjects involved (N). Categorical variables are presented with the percentage of presence among each group (100 × n/N) with subjects involved. Each variable has been stratified based on the GASP population to demonstrate the available clinical relationship.

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2.7 **Blood IgE level and blood Eosinophil counts**

There was a thought that quantitative measures would usefully supplement both blood IgE level and blood eosinophil counts for the purpose of allergic reactions or infections. Blood counts and atopy were stratified among all populations (Table 2.3). This stratification presented that blood IgE extracted from subjects was higher in asthma patients with a total of 3154 samples and reported to be elevated in asthma (mean 412.39) more than the rest of the populations. A total of 1134 subjects with blood eosinophil in asthma, and they have shown elevated ranges (0–5.24 $\times$ 10^9/L). However, there were certain drawbacks associated with the IgE levels because out of range were found (more than 5000 KU/L) in this database. In addition, hay fever was reported on the GASP with 22.9% of control subjects and appeared with asthma as 77%. Atopy is an important finding reported in the GASP with 81.4% in asthma, 17.6% in control and only 1.1% found in COPD subjects..

	Control	Asthma	COPD	Asthma & COPD	Total
Blood IgE levels KU/L	108.27	412.39	132.08	146.10	290.54
	N= 1674	N= 3154	N= 292	N= 228	N= 5348
Blood Eosinophil Count (x10^9/L)	0.12	0.31	0.24	0.28	0.29
	N= 16 (0.04-0.28)	N= 1134 (0-5.42)	N= 278 (0.01-3.32)	N= 221 (0.01-2.03)	N= 1649 (0-5.42)
Hay Fever (yes)	N= 532 (23.0%)	N= 1786 (77.0%)	N= 0 (0%)	N= 1 (0%)	N= 2319
Atopy (Yes)	N= 365 (17.6%)	N= 1691 (81.4%)	N= 22 (1.1%)	N=0 (0%)	N= 2078 (55.9%)

Table 2. 3: Blood counts and atopy stratified among the GASP.

Continuous variables are presented with mean, ranges and the number of subjects involved (N). Categorical variables are presented with the percentage of presence among each group (100 × n/N) with subjects involved

2.8 Lung function stratification among the GASP

As shown in Table 2.4, potentially as anticipated, the control subjects had a normal lung function, e.g. FEV_1 % predicted mean values (93.64%) compared with asthma and COPD, both of which showed evidence of airway obstruction, 76.62% and 41.37%, respectively. Furthermore, FEV_1/FVC mean values reported in COPD and asthma values were reduced as evidence of airway limitation or/and obstruction, such as 0.70 with asthmatics and 0.48 with COPDs compared with control subjects.

Lung Function	Control	Asthma	COPD	Asthma & COPD
FEV1 (L) brpd	2.91 (0.58-5.81) N= 1242	2.43 (0.32-6.06) N= 3353	1.14 (0.25-3.06) N= 552	0.98 (0.35-3.22) N= 227
FVC (L) prbd	3.72 (0.80-7.13) N= 1242	3.41 (0.43-7.60) N= 3353	2.36 (0.35-4.77) N= 552	2.17 (0.55-5.66) N= 227
FEV1/FVC prbd	0.78 (0.35-100) N= 1242	0.70 (0.23-100) N= 3353	0.48 (0.19-0.93) N= 552	0.46 (0.23-0.87) N= 227
FEV1 (% predicted)	93.64 (24-153) N= 1226	76.62 (7-160) N= 3236	41.37 (13-91) N= 536	38.13 (13-100) N= 227

Table 2.4: Pre-bronchodilator lung function demonstration for the entire group

FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity exhaled in one breath. FEV1/FVC = the ratio of the forced expiratory volume in the first one second to the FVC of the lungs. Values presented with means, ranges and number of subjects. Prbd = pre-bronchodilator.

2.9 Subjects with complete PRE and POST bronchodilator lung function data

Interestingly, the classification of lung function on pre-/post-bronchodilator showed more realistic results. As shown in Table 2.5, subjects are more likely to improve their lung function after a bronchodilator has been administered. This is evidence of the effectiveness of the bronchodilator, and that is more confirmation of obstructions among subjects.

Lung Function	Asthma	COPD	Asthma & COPD
FEV1 (L) brpd	2.35 (0.55-5.36) N= 524/1270	1.14 (0.25-3.06) N= 536/1270	0.97 (0.35-3.22) N= 210/1270
FVC (L) prbd	3.45 N= 524 (0.95-7.18)	2.36 N= 536 (0.35-4.77)	2.14 N= 210 (0.55-5.66)
FEV1/FVC prbd	0.68 N= 524 (0.31-0.98)	0.48 N= 536 (0.19-0.93)	0.46 N= 210 (0.26-0.87)
FEV1 (% predicted)	72.28 N= 513 (21-125)	41.51 N= 520 (13-91)	38.04 N= 210 (13-100)
FEV1 (L) pobd	2.61 N= 524 (0.60-5.96)	1.26 N= 536 (0.29-3.19)	1.08 N= 210 (0.34-3.15)
FVC (L) pobd	3.67 N= 524 (1.25-7.27)	2.61 N= 536 (0.50-4.93)	2.39 N= 210 (0.74-5.76)
FEV1/FVC pobd	0.71 N= 524 (0.32-0.98)	0.48 N= 536 (0.23-1.00)	0.47 N= 210 (0.23-0.90)

Table 2.5: Pre-bronchodilator lung function demonstration for the entire cohort.
FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity exhaled in one breath. FEV1/FVC = the ratio of the forced expiratory volume in the first one second to the FVC of the lungs. Values presented with means, ranges and number of subjects. Prbd = pre-bronchodilator and pobd = post-bronchodilator.

2.10 **Discussion**

In the project, we set out to consolidate existing cohorts and combine with the ongoing recruitment of asthma patients to establish a working cohort of control, asthma, COPD and asthma–COPD subjects for epidemiology and genetic studies. The current chapter aimed to provide an overview of this process and summarise the cohort with respect to the number of individuals available across subject groups and differences in basic, clinical and immunological features across groups. We established a cohort comprised of 1913 control, 5713 asthma, 678 COPD and 230 asthma–COPD overlap subjects and identified significant differences across asthma and COPD basic, clinical and immunological features. The establishing of this research cohort now paves the way for a more in-depth study of asthma's clinical and immunological features and will be the focus of Chapters 3 and 4.

In an investigation into gender/sex over many years of follow-up, just over half of the GASP population is female (55.8%), while only 44.2% are male. Specifically, in asthma, females were higher in prevalence, they presented with 71.2% compared with COPD which showed in around 5%. In addition, females in the control group formed about 21%. These findings give a hint of who is higher in the prevalence of many diseases in GASP. As mentioned in the literature review, asthma develops more in females and becomes higher in its severity too (Zein and Erzurum 2015A). Moreover, it tends to appear that high incidences occurred in females, especially after puberty (Tantisira et al. 2008). This finding was been confirmed by Hansen et al. in 2015, as the report provided a population-based cohort by physician diagnosis, females (7.5%) were found

slightly higher than males (5.1%), with a long period of follow-up. Another multicentre study reported that exacerbations had been recorded highly in association with women (Patel et al. 2014A).

The most important clinically relevant finding was the smoking status among asthma and COPD patients. Of the study population, smoking status of 7230 patients was recorded, which is considered a good number for a large cohort compared with previous studies. This parameter was divided into three groups, namely, non-smoker, ex-smoker or current smoker. As is well known, smoking plays an important role in airway obstruction diseases. Interestingly, the majority of GASP results are non-smoker or ex-smoker, but still, there is a large number of smokers to analyse and find the relationship with asthma or COPD. As shown in Table 2.1, the asthma population is more likely to contain heavy smokers, and that is related to the number of subjects recruited with asthma. However, COPDs presented with a total of 546 with a smoking history, and only 153 subjects were active smokers. Furthermore, it was shown that the range (6.66–255) of packs/year smoked by COPDs was the highest in the cohort.

Smoking or tobacco use is a remarkable element in deteriorating COPDs, and it is reported to be higher in women than in men (Liu et al. 2015). In 2018, a Swedish multicentre study reported that between 2005 and 2015, the prevalence of smoking had been reduced from 11% to 6% with asthma patients; in contrast, there was no significant change in COPD (Stegberg et al. 2018). Therefore, the GASP will examine the smoking effects, especially on asthma, in the next chapter to investigate more and compare the results with the literature review findings.

Another important finding in the GASP is lung function, which has been measured for all subjects with asthma, COPD and asthma–COPD overlap syndrome. Lung functions were applied for subjects to measure FEV1, FVC and their ratio FEV1/FVC. Among the database, 5374 tests were calculated with completed lung function results (Table 6). For asthma subjects, 3353 tests were applied. For example, the FEV1 was measured at baseline and after a bronchodilator (salbutamol) was administered. The mean of FEV1 was 2.42 L and seems to decrease gradually with severe cases. However, only 552 lung function tests were calculated for COPD with FEV1 mean of 1.13 L, as this study is mainly the moderate-severe asthma cohort. This drop in means is generally because of the nature of airflow obstruction in COPD as is quite general in disease pathology. The effectiveness of the lung function technique has been exemplified in the literature by many studies. Scientists recommended that lung function test spirometry must be measured initially, then completed again after administration of the bronchodilator; moreover, it should be followed up within 1 to 2 years after airflow resistance is revealed (Celli, 2000). Furthermore, FEV1 % predicted was calculated in the GASP depending on the updated equation with NHANES III (1999) as similar to the reported literature.

Recently, a longitudinal study was published with 4983 subjects. It has three subgroups; 668 young adults, 3147 middle-aged adults and 1168 older adults. As a result of this study, there was a reduction of FEV1 % for all subgroups. However, the older age group had a more significant reduction than others (Porsbjerg, Lange and Ulrik 2015). In addition, one more study measured the lung function between 1993 and 2013, within this period 262 patients had

declined in mean FEV1 by 23.1 and FVC by 22.9 (Abramson et al. 2016). These studies confirm that lung function reduced in asthma with increasing age.

Blood eosinophil count is one of the remarkable clinical factors affecting the heterogeneity of asthma and plays a role in allergy reactions. 1634 patients in the GASP had blood eosinophil counts measured with a maximum of 5.42×10^9 cells/L. However, eosinophil counts showed more subjects were recruited with asthma, a total of 1134. There were fewer COPDs who presented with a mean value of 0.24×10^9 cells/L. Because there was a non-normal distribution for blood eosinophil counts, the log 10 transformation was applied to the entire eosinophil counts. Because eosinophil count is considered an airway inflammation marker, we decided to investigate the literature reports, and later in another chapter will compare our findings with previous research. One study on asthmatic patients proved the eosinophil relationship with lung function and atopy. Out of 961, there were 193 patients with high eosinophils ($>0.4 \times 10^9$ cells/L). Similarly, there was a correlation of eosinophil with decreased values of FEV1 and the ratio FEV1/FVC.

To conclude, the GASP cohorts presented different clinical and immunological features that were stratified on control, asthma, COPD and asthma–COPD subjects. The summary of clinical and immunological features among populations in the GASP will facilitate examining different relationships in the next chapters.

Chapter3: Non-Related Asthma population in the GASP

3 Introduction

Chapter 2, the clinical and immunological features of asthma and COPD cohorts were described and compared to other cohorts in the literature. Although the GASP includes asthma and COPD cohorts, only asthma subjects were included in the non-related cohort. This chapter focuses on a specific cohort within the GASP that included only subjects diagnosed as having moderate to severe asthma; this cohort was termed the non-related asthma (NRA) cohort, and it comprised subjects recruited from several centres in the UK. The criteria for selection will be discussed in the methods section. This chapter and the next (Chapter 4) will discuss the NRA cohort.

Asthma, a major area of interest in the field of respiratory disease, is considered a chronic disease in adults, but it is more common in children (Gibson et al. 2013). In this study, the relationship between the clinical and immunological features of asthma will be investigated in subjects who identified as belonging to the moderate to severe asthma population. In past decades, many researchers have sought to determine the worldwide prevalence of asthma, but NRA subjects have rarely been investigated as a cohort in a study. Thus, the NRA cohort may be one of the largest cohorts suitable for investigating various phenotypic and genetic relationships, being a unique cohort comprising many unrelated people with moderate to severe asthma.

As discussed in Chapters 1 and 2, a considerable body of literature has been published on the asthma population and the relationships of clinical and molecular features in it. This chapter will present various findings in the GASP, and the comparison of these findings will be scan over the literature. One large, multicentre cohort study published by the U-BIOPRED study group in 2015 addressed asthma heterogeneity and its phenotypic variability problems (Shaw et al. 2015). The SARP study used a well-organised cohort of asthma patients and aimed to define the relationships of the clinical features of asthma. Moreover, it assessed the factors that determine whether the severity and age of onset of asthma will influence these phenotypic measurements. It reported on 610 adults recruited and followed up in an 18-month period, divided into four distinct groups (severe asthma among non-smokers, smokers and ex-smokers with severe asthma, mild/moderate non-smoking asthmatics and healthy non-smoking controls). The study found valuable evidence that the severe asthmatics group experienced more exacerbation events linked with remarkable signs and symptoms of asthma than did subjects with mild/moderate asthma. Furthermore, the same study conducted lung-function tests in both groups and found that the severe asthmatics group tended to have a greater incidence of reduced lung function and had a lower FEV1 and FVC than the other groups, especially when compared to the mild/moderate asthma group (Shaw et al. 2015).

Relatively few studies on moderate to severe asthma pay special attention to the age at which asthma starts, but it is essential to identify the age of asthma onset as it may affect later asthma-related findings (Mirabelli et al. 2013).

Researchers report that early childhood is the time of asthma onset in most populations (de Nijs, Venekamp and Bel 2013). Additionally, clinicians note that asthma is consistently more prevalent among young males than among young females (Almqvist, Worm and Leynaert 2008). Moreover, Mirabelli et al.'s 2013 study of 12,216 adults with asthma confirmed previous findings in the literature, showing that, among adults aged 18–99 years, an estimated 42% experienced asthma onset before the age of 16, with a mean age of 7 years. Late-onset asthma occurred at a mean age of 38 years (Mirabelli et al. 2013). Research in genetics suggests that, while adult- and childhood-onset asthma overlap, they are characterised by distinct genetic susceptibilities and potentially have different mechanisms.

Late-onset asthma describes cases in which the patient's symptoms appear first in adulthood. Studies have shown that, in contrast to childhood-onset asthma, it mainly effects females, most often without a family history of asthma, and it is less often associated with atopic and allergy diseases (Wenzel 2012, Amelink et al. 2013). The Tasmanian Longitudinal Health Study published in 2016 reported on a study that differentiated between early-onset and late-onset asthma. It reported that, in the early-onset asthma group, a family history of asthma and atopy were more common than in the other group. In late-onset asthma, female subjects and current smokers were more common. Also, lung function is more significantly affected in early-onset asthma than in late-onset asthma (Tan et al. 2016).

Aims of this chapter:

In this chapter, I will:

1. Provide demographics of the NRA population in GASP that will be used to address specific research questions.
2. Stratify these data to investigate the relationship between disease severity and clinical and immunological parameters.
3. Investigate the characteristics of asthma patients with adult and childhood onset to provide further understanding of these two populations.

3.1 **Method:**

Characterization and assessments of Non-Related Asthma (NRA) cohort

The population of moderate to severe asthma was identified by Sayers's research group from the GASP database and was based on the selection criteria described in Chapter 2. In the GASP, there were 5713 asthma patients. The exclusion of related individuals by selecting only one family member led to a group of 3841 individuals ranging from 16 to 60 years old. This selection created the NRA cohort. The subjects were selected based on smoking status (never, ex-smoker and currently smoking) and by excluding related families from the Southampton family cohort and Nottingham asthma family (NAF) cohort. The cohort included only one member, aged 16–60 years old, from each family. Also, the GASP database included a non-UK cohort, which was excluded because of its potentially different genetic heterogeneity; ultimately, research into genetics is planned for that cohort.

Following GINA guidelines, the asthma control questionnaire (ACQ) was conducted for most of the asthma subjects in the NRA cohort. The ACQ aims to identify how asthmatic patients control their condition and also describes symptoms, relief medication and lung function. The questionnaire was distributed to patients at each clinical site. It included seven questions on the basis of which the mean of the final scores will be identified. Patients will be considered controlled if they scored ≤0.75 while a score of ≥1.5 indicates that patients are uncontrolled. Moreover, the severity of asthma was reported based on GINA scores (Fig. 3.1). GINA included five steps and aimed to measure

symptoms, FEV_1 and the number of exacerbation events. The subjects were classified based on their medication use. Mostly in GINA 3 and GINA 4, there was a insufficient number of subjects with ICS dosage records, so GINA 3 and GINA 4 subjects were combined to identify the moderate to severe group.

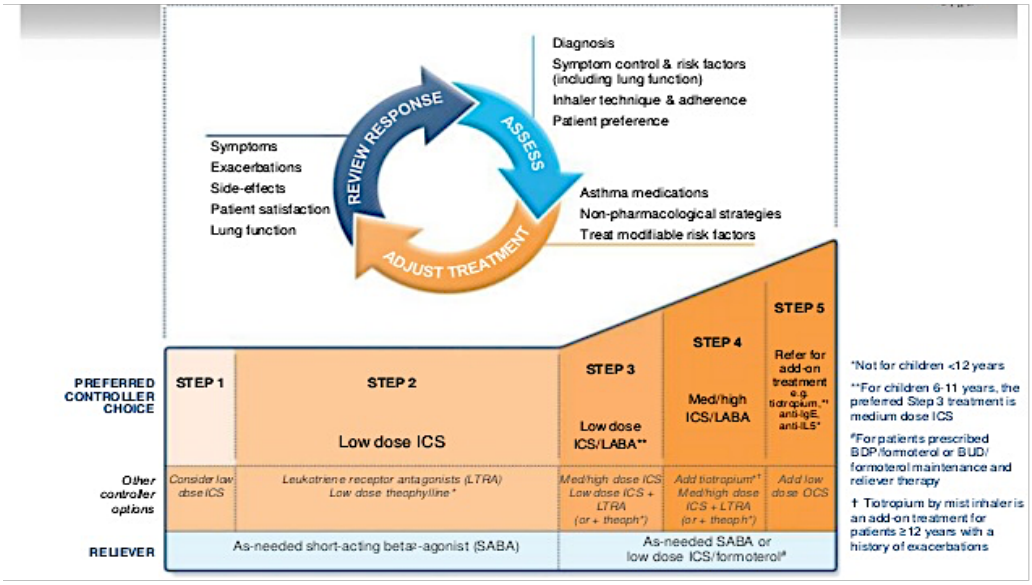

Figure 3.1: New GINA 2018 scoring based on patient's medication (GINA 2018)

3.2 **Statistical analysis**

In this study, statistical analyses were performed using the SPSS premium version 24 software package, and the P-value was considered statistically significant at $p < 0.05$. Graphs were displayed using GraphPad Prism 7. Continuous variables were calculated using means and ranges while categorical variables were presented using percentages. Because of the abnormality distribution of most variables, non-parametric distribution was used. The Kruskal-Wallis test was used to compare groups of more than two for continual variables while the chi-squared test was performed for categorical variables. For calculating lung function stratified by age of onset, the Mann-Whitney U test was employed.

3.3 **Results**

3.4 **NRA basic demographics**

At the time of the analysis, the GASP database included a total of 3841 subjects with NRA disease. The main demographics of this cohort are shown in Table 3.1. There were more females (66%) than males in the asthma cohort, and the ages of the subjects in the cohort ranged from 16 to 60 years old, with a mean age of 42.36. Regarding smoking status, 63.8% had never smoked while 13.6% were current smokers. The smoking history in pack/years in the asthma cohort resulted in a mean of 12.47 and a range of 0.03–120. Regarding immunology, blood IgE levels were determined for 1730 subjects and showed a mean value of 385.93 KU/L. Blood eosinophil counts were slightly high, with a mean of 0.33 x10^9/L. Allergy and skin prick tests were performed and showed that 73% of the 1574 subjects in the asthma cohort were diagnosed with atopy (Table 3.1).

	Non-related Asthma (NRA) **N= 3841**
Gender	Female N= 2280/3452 (66.0%)
Age	42.36 (16-60) N= 3104
Smoking status	**N= 2878**
Never	1835 (63.8%)
Ex-smoker	652 (22.7%)
Current smoker	391 (13.6%)
Smoking Pack/Yrs	12.47 (0.03-120) N= 816
Blood IgE levels KU/L	385.93 (0.60-4900) N= 1730
Blood Eosinophil Count (x10^9/L)	0.33 (0.01-5.42) N= 791
Hay Fever **(yes)**	(59.6%) N= 1068/1791
Skin Prick Test **(Yes)**	(73.0%) N= 1149/1574

Table 3.1: Basic clinical and immunological demographics of non-related asthma cohort. Continuous variables presented by mean and ranges. Categorical variables presented with percentages. N = number of subjects with data field available.

3.5 **Distribution of data and data transformation**

The predicted FEV1 percentage displayed a normal distribution, while the FEV1/FVC pre-bronchodilator presented a non-normal distribution after the K-S test was performed. Neither the blood IgE level nor the blood eosinophil counts were normally distributed as shown by the GraphPad Prism software. Therefore, I transformed them using the log 10 formula (Fig. 3.2).

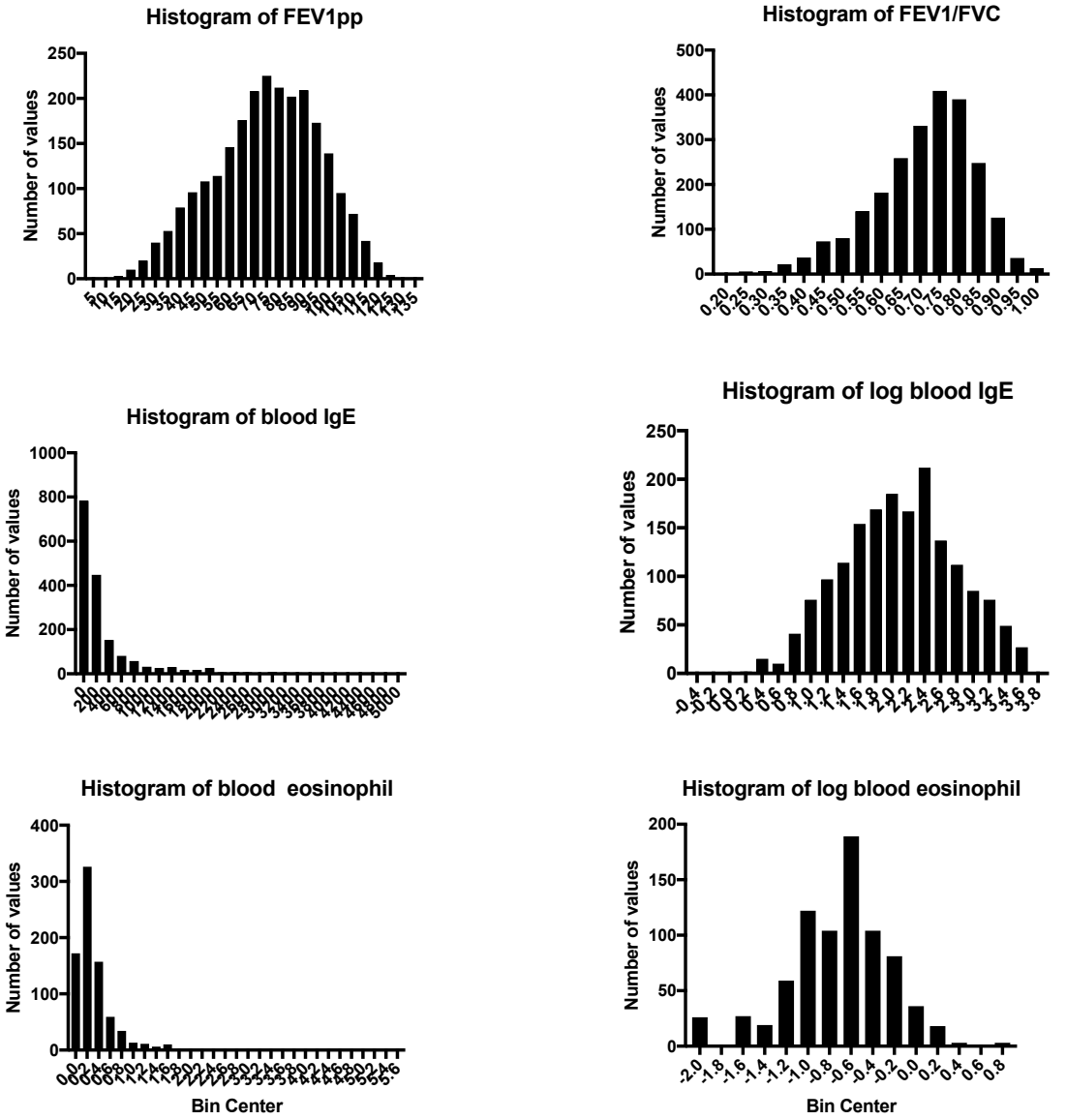

Figure 3.2: Distribution of the date. Lung functions looks normal. Blood IgE level and blood eosinophil counts presented before and after transformation for normality.

3.6 **Non-related asthma across GINA 2018**

To examine the relationship between clinical and immunological features of the patients and disease severity, the previous basic demographics were stratified based on GINA groups, see table 3.2. There was an increase in the number of subjects as this cohort was designed to be enriched for moderate-severe asthma. For example, here was a trend for increased prevalence of females in the more severe asthma groups; 59.8, 62, 72, 71% across GINA 1, 2, 3/4 and 5 respectively (P=0.008). Also, it seems in GINA 3 and 4 the number of subjects classified as moderate to severe were higher than others (985 subjects). Interestingly, looks like higher smoking in milder forms of asthma based on ever/never/current. Smoking pack years were increased in GINA 3 and 4, which is probably related to the high number of subjects after combined them together (table 3.2).

	GINA 1	GINA 2	GINA 3&4	GINA 5	P value
Gender	Female (59.8%) N= 52/87	Female (62.0%) N= 116/187	Female (72.0%) N= 716/995	Female (71.0%) N= 125 /176	0.008
Age	38.33 (19-59) N= 87	40.30 (16-60) N= 188	42.31 (16-60) N= 985	43.77 (16-60) N= 173	0.001
Smoking status	N= 39	N= 124	N= 905	N= 174	0.0191
Never	26 (66.7%)	85 (68.5%)	612 (67.6%)	141 (81.0%)	
Ex-smoker	9 (23.1%)	31 (25.0%)	200 (22.1%)	25 (14.4%)	
Current smoker	4 (10.3%)	8 (6.5%)	93 (10.3%)	8 (4.6%)	
Smoking Pack/Yrs	3.80 (0.08-9.0) N= 22	4.40 (0.06-39.0) N= 65	13.70 (0.05-120) N=266	5.24 (0.50-18) N= 26	<0.0001

Table 3.2: NRA basic demographics. Continuous variables are presented by mean and ranges. Categorical variables are presented by percentages (n/N). N = number of subjects with data field available.

3.7 **Blood counts and allergy tests across GINA groups**

The relationships between immunological markers in asthma, e.g., IgE with disease severity, was examined (Table 3.3). There were no statistical differences across the GINA groups. In addition, no significant overall association was found between blood IgE levels among all GINA groups. Similarly, no significant association appeared between blood eosinophil count and GINA group. The majority of the subjects were in group GINA 3/4. GINA 1 does not contain blood eosinophil, and, in group 5, only three subjects were recorded. No difference was found in the prevalence of hay fever across groups (60.9%–68.8%). ACQ scores were collected during patient visits, revealing a strong significant association with ACQ across GINA groups ($p < 0.0001$) (Fig. 3.3).

	GINA 1	GINA 2	GINA 3&4	GINA 5	P value
Blood IgE levels KU/L	231.93 (7-926) N= 6	404.87 (3.20-3726) N= 33	344.62 (2-4850) N= 363	366.10 (2-4779) N= 51	N/D
Blood Eosinophil Count (x10^9/L)	N/D	0.33 (0.02-0.79) N= 10	0.30 (0.02-1.60) N= 56	0.26 (0.03-0.59) N= 3	N/D
Hay Fever (yes)	(68.8%) N= 22/32	(61.6%) N= 61/99	(60.9%) N= 372/611	(61.3%) N= 84/137	0.850
Skin Prick Test (Yes)	(83.0%) N= 44/53	(80.6%) N= 75/93	(80.7%) N= 276/342	(65.2%) N=15/23	0.310
ACQ score	1.30 (0-4.43) N= 26	1.67 (0-4.86) N= 87	2.12 (0-5.71) N= 455	2.83 (0-5.83) N= 107	<0.0001

Table 3: Blood counts demonstration stratified by new GINA 2018 score.

- Continuous variables presented with mean and ranges. Categorical variables presented with percentages (n/N). During analysis, Blood IgE levels within GINA1 and GINA2 combined together due to lack of subjects in GINA1.
- During analysis, Blood Eosinophil within GINA1 and GINA2 combined together due to lack of subjects in GINA1. Also, GINA5 combined with GINA3&4. N/D= not determine because of the lack in numbers.

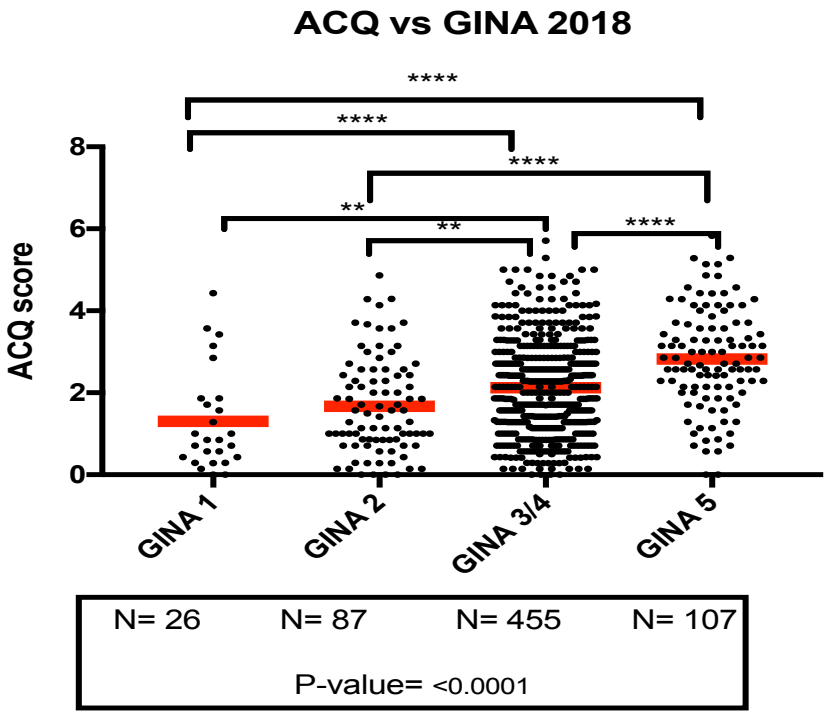

Figure 3.3: Scatter plot demonstrateing the association between median ACQ scores and new GINA groups classification. Test was performed by Kruskal-Wallis test followed by post-hoc test. **** represents P <0.0001 and ** represents P 0.0037.

3.8 **Lung function across GINA groups**

To examine the relationship between lung function and asthma severity, a stratification according to lung function was applied to the GINA groups (Table 3.4). The predicted FEV1 percentage showed a strong, significant association with the GINA scores ($p < 0.0001$) (Fig. 3.4). Interestingly, the significant association appeared within GINA groups even after the post-hoc test was applied. Similarly, FEV1/FVC pre-bronchodilator measures were significantly associated with GINA stratification ($p < 0.0001$). Figure 3.4 shows the stratification of lung functions performed with a post-hoc test, revealing significant association.

Lung Function	GINA 1	GINA 2	GINA 3&4	GINA 5	P value
FEV1 (% predicted)	88.72 (44-116) N= 82	84.81 (31-122) N= 167	75.62 (15-125) N= 846	68.18 (23-119) N= 126	<0.0001
FEV1/FVC prbd	0.79 (0.52-1) N= 85	0.76 (0.51-1) N= 177	0.73 (0.28-1) N= 730	0.69 (0.35-0.93) N= 143	<0.0001

Table 3.4: lung function stratified by new GINA 2018 scores. (%predicted)= percentage predicted. Lung functions are presented by the mean and range of each lung function test.

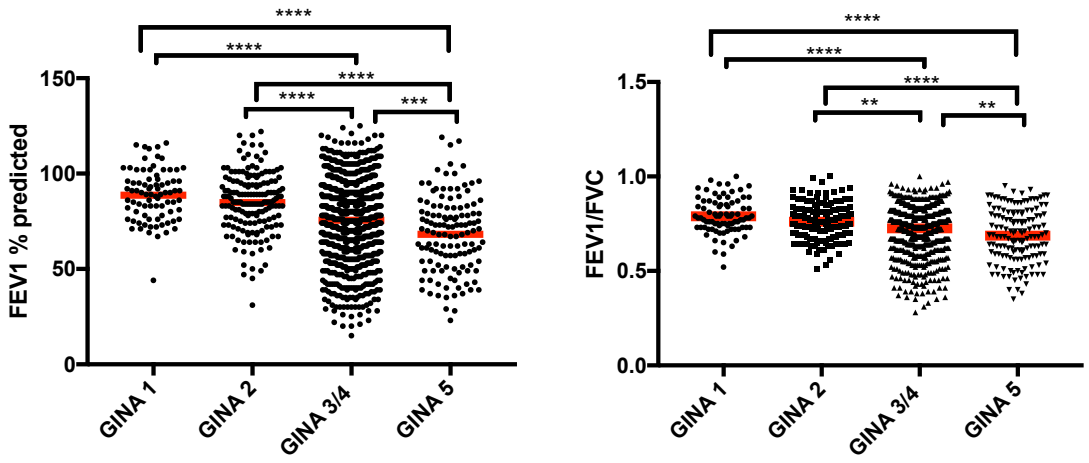

Figure 3.4: Scatter plots of the association between lung function and the new GINA 2018. . **** represents P <0.0001; *** represents P= 0.0005; and ** represents P= 0.0037. Test was performed by Kruskal-Wallis test followed by post-hoc test

3.9 Non-related asthma across the age of onset below or above 16 years old

To investigate how asthma presents in the adult-onset as compared to the childhood-onset disease, the subjects were stratified based on the age of onset (Table 3.5). The analysis found a significant association between the smoking status of the subjects and the age of onset below or above 16 years old. Additionally, there were more subjects recorded as current smokers in the adult-onset (14.5%) than in the child-onset (12.0%) group. Therefore, the association was significant (p=0.011).

	Age <16	Age >16	P value
Gender	Female (67.2%) N= 644/959	Female (70.6%) N= 633/897	0.113
Age	37.96 (16-60) N= 955/961	47.10 (18-60) N= 891/897	<0.0001
Smoking status	N= 953	N= 894	0.011
Never	646 (67.8%)	546 (61.1%)	
Ex-smoker	193 (20.3%)	218 (24.4%)	
Current smoker	114 (12.0%)	130 (14.5%)	
Smoking Pack/Yrs	10.71 (0.04-75) N= 263/961	17.71 (0.06-120) N= 289/897	0.944

Table 3.5: Basic demographics stratified by the age of onset below or above 16 years old. Continuous variables are presented by mean and ranges. Categorical variables are presented by percentages (n/N).

3.10 Blood counts and allergy tests across the age of onset below or above 16 years old

This section describes the stratification of blood counts based on the age of onset below or above 16 years old for all subjects examined with various blood tests (Table 3.6). The skin prick test was also performed to determine the allergy status of subjects included with an asthma diagnosis. A Mann-Whitney test was performed to identify the relationship between total IgE levels and the age of onset. Higher levels of serum total IgE were observed in childhood-onset than in adult-onset disease (424.69 vs. 239.01, respectively; p <0.0001) (Fig. 3.5). In another interesting finding, a chi-squared test found an association between subjects diagnosed as atopic after the skin prick test was performed (p <0.0001) (Fig. 3.5). Moreover, an association was found between the age of onset and the subjects diagnosed with hay fever.

	Age <16	Age >16	Total
Blood IgE levels KU/L	424.69 (0.60-4850) N= 462	239.01 (1-4190) N= 460	<0.0001
Blood Eosinophil Count (x10^9/L)	0.36 (0.01-5.15) N= 150	0.37 (0.01-5.42) N= 163	0.981
Hay Fever *(Yes)	(61.9%) N= 436/704	(53.4%) N= 339/635	0.002
Skin Prick Test *(Yes)	(84.7%) N= 326/385	(62.0%) N= 235/379	<0.0001
ACQ score	2.13 (0-5.83) N= 382	2.12 (0-5.71) N= 303	0.944

Table 3.6: Blood counts and allergy tests stratified by the age of onset below or above 16 years old. Continuous variables are presented by mean and ranges. Categorical variables are presented by percentage (n/N). *(Yes) indicated subjects had a positive result.

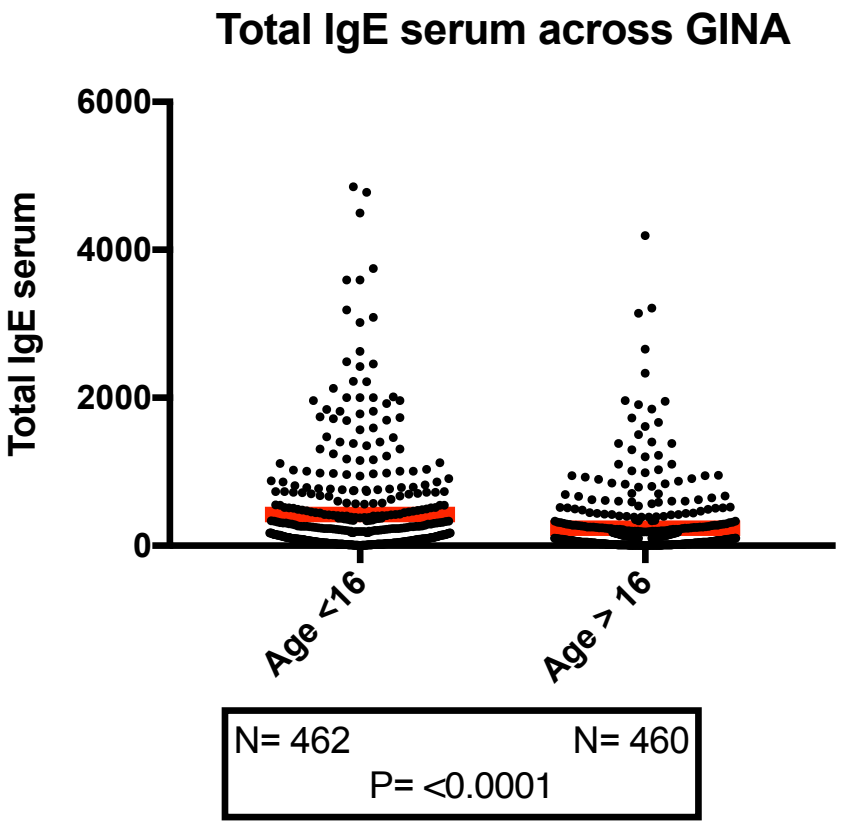

Figure 3.6: Scatter plots of total serum IgE stratified by the age of onset below or above 16 years old. The red line represents the mean of total IgE levels. The Mann-Whitney U test was used.

3.11 Lung functions stratifying by the age of onset below or above 16 years old

Simple statistical analysis was used to identify the relationship between the age of onset below or above 16 years old and various lung-function measurements (Table 3.7). A Mann-Whitney U test was used to analyse the relationship between predicted FEV1 percentage and FEV1/FVC pre-bronchodilator (PRBD) stratified by the age of onset. It revealed no significant association.

Lung Function	Age <16	Age >16	P value
FEV1 (% predicted)	74.15 (7-125) N= 818	73.98 (15-125) N= 790	0.874
FEV1/FVC prbd	0.71 (0.31-1.00) N= 769	0.72 (0.25-1.00) N= 737	0.188

Table 3.7: Lung function stratified by the age of onset below or above 16 years old. Continuous variables are presented by mean and ranges. Neither test showed an association.

3.12 **Discussion**

This chapter provided demographics of the NRA population in the GASP that will be used to address specific research questions, and it stratified the data to investigate the relationship between disease severity and clinical and immunological parameters. It also investigated the features of asthma patients with adult versus childhood onset to provide further understanding of those two populations.

In this cohort, the majority of the subjects were female, comprising 2280 of the 3841 recruited subjects (66.0%), which confirms what was mentioned in Chapter 2 regarding that gender's majority in the GASP. This was anticipated by most of the literature on asthma patients. Additionally, the recruited subjects were defined by basic demographics, including age (an age from 16 to 60 years was one of the study requirements), smoking history and, for smokers, how many packs per year they smoked. Asthmatic subjects were stratified by asthma severity using the GINA 2018 score. GINA classifies asthma severity based on symptom occurrence, medication use and lung function. This stratification of the database by the GINA provided the opportunity to identify potential subtypes of asthma and how they affect the various phenotypes in the GASP study. The analysis defined the relationship between the clinical and molecular features of one variable and those of another, which explicit each variable characteristic by another. Consequently, these interlinked relationships combined to describe the cluster of an asthma subset, similar to other updated research in asthma.

The results of the association test suggest that it is likely that more severe asthma is related to less smoking (p 0.0191). Smoking history was classified in three categories: never smoked, ex-smoker and currently smoking. The results indicate that asthma subjects with a currently smoking status are less frequent than ex-smokers and those who never smoked. Notably, the percentage of smokers in various GINA groups is similar except in GINA 3/4, where it was higher due to the combination of GINA 3 and 4, which was done at the start of this study because of a lack of some medication-dose records. These clusters are similar to what was found in one of the U-BIOPRED studies, which included smoking and severe non-smoking asthma and reported that patients with a smoking, non-smoking and ex-smoking history differed in their demographics, airway physiology and asthma symptoms (Shaw et al. 2015). Previous research has found that the therapeutic process of inhaled corticosteroids may be affected in patients who smoke and that those patients have poorer control of their asthma symptoms (Chaudhuri et al. 2003).

The current study found that lung function was significantly associated with the same stratification of GINA groups. The predicted FEV1 percentage declined with increasing severity (p <0.0001) (Table 3.4). Similarly, the results of the FEV1/FVC ratio demonstrated a significant reduction with increasing severity; the mean of the ratio was 0.79 in GINA 1 and 0.69 in GINA 5 (p <0.0001). The results of the GASP will now be compared to the findings of the previous study, which basically demonstrated the baseline features of severe and non-severe asthma in the SARP III cohort. The previous study reported that, among 313 severe patients and 213 non-severe patients, there was a significant

change in predicted FEV1 percentage in the severe asthma group. Moreover, SARP has reported that a severe airflow obstruction is most likely significantly associated with increased age (Teague et al. 2018). Another cluster analysis confirmed our results, indicating that, among five distinct characterised groups, the lowest predicted FEV1 percentages were in clusters 4 and 5, which involved the most severe asthma (Moore et al. 2010). Obviously, the results of Teague et al. (2008) and Moore et al. (2010) support the association of reduced lung function with increasing asthma severity.

The stratification of subjects in the GASP based on the age of onset below or above 16 was of particular interest because there is growing evidence that childhood and adult asthma may differ significantly in disease mechanisms and presentation. This classification aims to determine the clinical and molecular features of asthma as related to age of onset (e.g., child onset and adult onset). The analysis found that blood IgE level was significantly associated with age of onset. Interestingly, the blood IgE was statistically high in the child-onset group.

A previous cluster study defined diverse clinical features of asthma based on the age of onset. In the SARP study, there were three clusters (1, 2 and 4) pertaining to allergic asthma, and the subjects in those clusters were diagnosed with mild to severe airflow obstruction. The researchers reported that the skin prick test and elevation of blood IgE provided evidence that the majority of patients with allergic asthma had early-onset asthma (Moore et al. 2013). In parallel to the GASP results, Moore et al. (2013) stated that the ‘severity of allergic asthma appears to be associated with age of asthma onset; the earlier

the asthma, the worse the severity' (Moore et al. 2013). Another study reported that atopy and family history are the features most often associated with early-onset asthma (Tan et al. 2016).

The effects on lung function were greater in early-onset asthma, but they were not significantly different between the early- and late-onset groups. This finding was confirmed by Tan et al. (2016) when they assessed the features of asthma between two distinct ages of onset.

Conclusion:

As described in this chapter, this study developed a useful cohort within the GASP cohort, resulting in a high prevalence of asthma subjects. Based on diverse statistical analyses, the GASP identified relationships between severity groups, as proven by GINA. Symptoms worsen with the increasing severity of asthma. Moreover, this cohort is likely to have more female subjects than males. In addition, it was observed that atopy and allergic diseases are more common among early-onset than among adult-onset subjects. Moreover, the GASP identified similarities and differences in how adult and childhood asthma present. Finally, in agreement with previous studies, such as the SARP cohort, this chapter suggests that childhood onset may be more driven by allergic mechanisms than adult onset.

Chapter4: Relationship of clinical and immunological features of asthma

4 Introduction

In the last chapter I developed a subset of GASP representing non-related asthma subjects and presented summary demographics. In the current chapter I utilise this cohort to answer three clinically relevant questions as will be mention on the aim of the chapter.

Since years, research has been going through the asthma biomarkers and the pathway of allergens interacts focusing on the role of T cells in term of inflammation (Matucci et al. 2018). Specifically, the focus on cells that controlling immunoglobulin E (IgE) and its capability to produce the interleukin (IL)-4 and IL-13. While, promotion of the inflammation was reported by the presence of eosinophil and basophil, and that produced by the effect of IL-5 (Humbert et al. 2018, Matucci et al. 2018).

Asthma is one of the diseases that has been discovered to be related to an increased number of eosinophils as reported in research (Matucci et al. 2018). In addition, the relationship between the inflammations produced by increased numbers of eosinophil with asthma patients has been proved with frequent exacerbation. Moreover, eosinophilic asthma patients have been known and linked with the frequency of exacerbations (Seidel et al. 2014). Therefore, science led to improving the medication effects and tends to the molecular controllers of eosinophil acts (Brusselle et al. 2017). Furthermore, IL-5 has been

reported as a remarkable control of eosinophil development and responsible for the transmission from the bone marrow to the inflammation colony. However, these tend scientists to discover novel drugs which are anti-IL-5. Anti- IL-5 for example, Reslizumab which given intravenously as an antibody, and mepolizumab which given as injection, has been developed to stop the pro-inflammatory activation of IL-5 on eosinophil (Brusselle et al. 2017). Interestingly, anti-IL-5 such as Reslizumab and mepolizumab effects is correlated with its capability to link with high correspondence worked on the area where the bind has occurred, which noticed on the surface of eosinophil, and to prevent the interaction between IL-5 and its receptor (Flood-Page et al. 2003).

On the other hand, asthma severity has been assessed and treated as a reason for the influences of frequent exacerbation of asthma. Although, besides focusing on asthma management, symptoms treatments and reducing the exacerbations danger, a few countries, such as USA and Korea, recorded that number of hospital admission rates because of asthma more than twice. These records lead to establishing a sign of failure in the prevention of asthma care (Kang et al. 2018). Therefore, it is highly recommended to record the history of hospital admission related to asthma by clinicians.

Moreover, essential to review the comorbidities reasons and treating these conditions, and by treating those conditions, possible improvements in asthma management occurs. Recently, routine assessment of asthma severity and control are essential since they linked to the current failure in management and prevent any further consequences (National Asthma Education and Prevention

Program 2007). Besides, research stated that medication monitoring is essential to improve asthma control (Zeiger et al. 2016). In the link to previous information, a cross-sectional study reported that asthma-related healthcare and a high cost of treatment are most likely presented because of different severity (Lee et al. 2017).

The aim of the chapter:

The purpose of this chapter is to examine the relationship between:

• Blood eosinophils and clinical features of asthma

• The impact of smoking on asthma presentation

• Clinical and immunological features of asthma subjects that are prone to exacerbation.

Method

In this chapter, basic demographics tables demonstrated into three different classifications. First of all, subjects demonstrated based on their stratification of blood eosinophil counts and then followed by statistical analysis on specific variables such as lung functions FEV_1% predicted and FEV_1/FVC pre-bronchodilator) and symptoms using mean ACQs score. Another stratification performed based on smoking behaviour followed by different statistical analysis to find out relationships. Finally, subjects subdivided into different groups that rely on the hospital admission to identify the exacerbation of asthma cohort. As mentioned in previous chapters, subjects were recruited with criteria, included subjects between age 16-60 years old and non-related family members.

4.1 Statistical analysis

Statistical analysis in this chapter performed by using SPSS software. As mentioned in the beginning of this thesis, all variables assessed in the chapter were tested for normality using the K-S test, those that did not show a normal distribution. Therefore, non-parametric tests performed in this chapter to find out the relationship between asthma phenotypes and to achieve the aim of the chapter. The analysis was performed by Spearman rank correlation test for non-parametric continuous variables. Furthermore, the Kruskal-Wallis test used for comparing more than two sample groups for non-parametric data. However, the multiple comparisons test used to find the association between each group in one-single analysis test. Additionally, the Mann-Whitney U test performed to test

whether two sample mean ranks are equal or not. Chi-squared usage was to compare percentages of two categorical variables. Graphs were presented by using the software of GraphPad Prism.

4.2 Results

4.3 What clinical and immunological features are related to blood eosinophil counts?

To answer the three clinical questions we generated three cohorts from the non-related asthma subjects in GASP. Each group were demonstrated with basic demographics tables and graphs for the significant associations after statistical analysis performed. First of all, the stratification of subjects based on the blood eosinophil presence (Table 4.1). There was 796 out of 3804 non-related asthma subjects presented with blood eosinophil. Majority of subjects were females with a number of 528 out of 780 subjects, which represented 67.7% of involved subjects. The mean of age recruited was 44.96 years old. Interestingly, smoking history was recorded and presented with 56.5% non-smoker which represented the highest group in eosinophil stratification. In opposite, smoker subjects were the lowest group and formed 18.7%.

Subjects with blood IgE level has been presented with a total of 635 subjects. Furthermore, skin prick test performed to identify the atopic and non-atopic subjects and presented with a high number of subjects diagnosed as atopic (68.8%) a total of 440 out of 640. Lung function assessed using FEV_1 % predicted and FEV_1/FVC. Finally, GINA score was taken but not counting in the analysis because of the lack of subjects in some classification, such as in GINA 1 no subjects found with blood eosinophil.

	Subjects with blood Eosinophil Count (x10^9/L) N= 796
Gender	
Female	(67.7%) N= 528/780
Male	(32.3%) N= 252/780
Age	44.96 (17-60) N= 778
Smoking behaviour	
Never	441 (56.5%)
Ex-smoker	193 (24.7%)
Current smoker	146 (18.7%)
Blood IgE levels KU/L	449.27 (1.0-4900) N= 635
Hay Fever (yes)	(52.4%) N= 186/355
Skin Prick Test	
Atopic	(68.8%) N= 440/640
Non-Atopic	(31.3%) N= 200/640
Lung Function	
FEV1 (% predicted)	69.80 (15-125) N= 705
FEV1/FVC prbd	0.67 (0.23-0.99) N= 736
GINA score	
GINA 1	N/D
GINA 2	(14.5%) N= 10/69
GINA 3/4	(81.2%) N= 56/69
GINA 5	(4.3%) N= 3/69

Table 4.1: Basic demographics of the cohort used to investigate the relationship between blood eosinophils and clinical and immunological features of asthma. Continuous variables were presented with mean, ranges and the number of subjects involved (N). Categorical variables were presented with percentage of presence among each group (n/N) with subjects involved. Note: N/D means not determined because of no subjects involved to analyses in that group.

4.4 The relationship between blood eosinophil levels and lung function and serum IgE

To further define the relationship between blood eosinophils and lung function, we showed no significant correlation between blood eosinophil and FEV1% predicted; P-value 0.120 and R^2= 0.004. Similarly, no correlation found between blood eosinophil and FEV1/FVC; results showed p-value 0.234 and R2= 0.002. However, Spearman correlation showed a positive significant correlation of blood eosinophil with Blood IgE levels (Figure 4.1) with p-value <0.0001 and R^2= 0.050 (Table 4.2). In this figure, there was an outlier, and unclear distribution of the data and needs more resolution between data. Therefore, both axis scales changed as a log/log scale only without transforming the data, as showed in Figure 4.2. However, still data non-normal and outliers, but changing the axis scale has masked them. Besides, to have a more accurate graph, blood eosinophil and serum IgE transformed using log 10 (figure 4.3). That transformation does meet the Pearson assumption with R^2= 0.044 and p-value <0.0001. The scatter plot where data transformed has no outliers, and appeared to be more accurate but potentially less interpretable.

Eosinophil correlation	
FEV1 (% predicted)	N= 706 R= -0.059 R^2= 0.004 P-value= 0.120
FEV1/FVC prbd	N= 736 R= -0.044 R^2= 0.002 P-value= 0.234
Blood IgE levels KU/L	N= 635 R= 0.232 R^2= 0.050 **P-value= <0.0001**

Table 4.2: Eosinophil correlations with continuous variables. Correlation was performed by Spearman correlation rank test. N= number of subjects involved in the correlation test. R, R^2 and p-values presented.

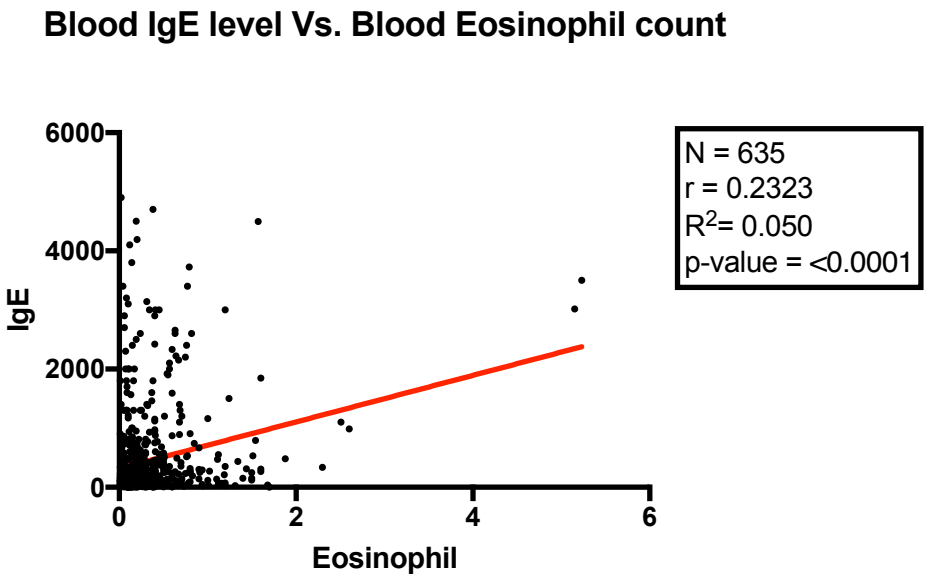

Figure 4.1: scatter plots demonstrated the correlation between blood IgE level and blood Eosinophil. The correlation was positive significant with p-value <0.0001 and R 0.2323.

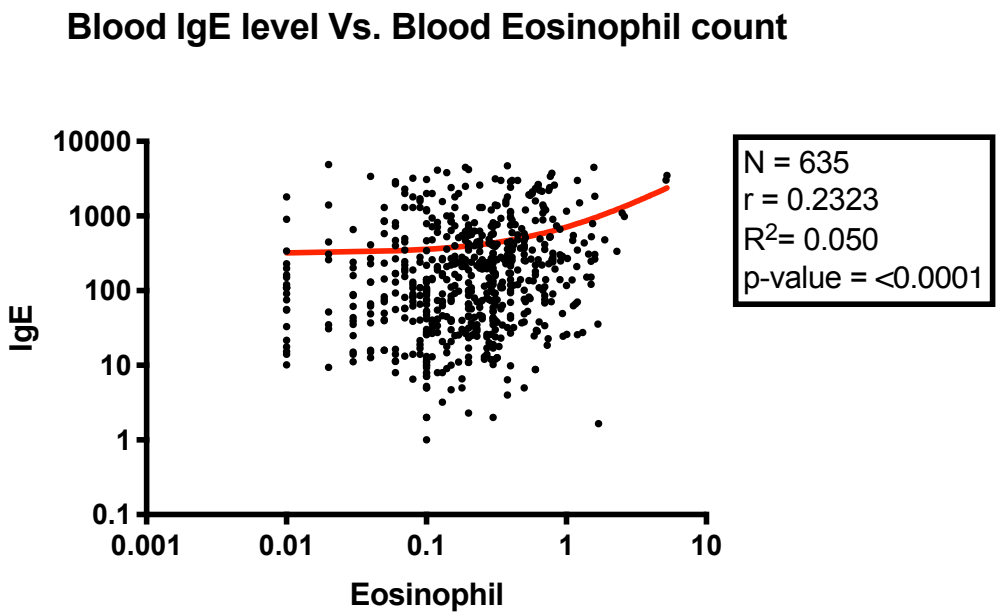

Figure 4.2: scatter plots demonstrated the correlation between blood IgE level and blood Eosinophil with changing axis to log/log scale. The correlation was positive significant with p-value <0.0001 and R 0.2323.

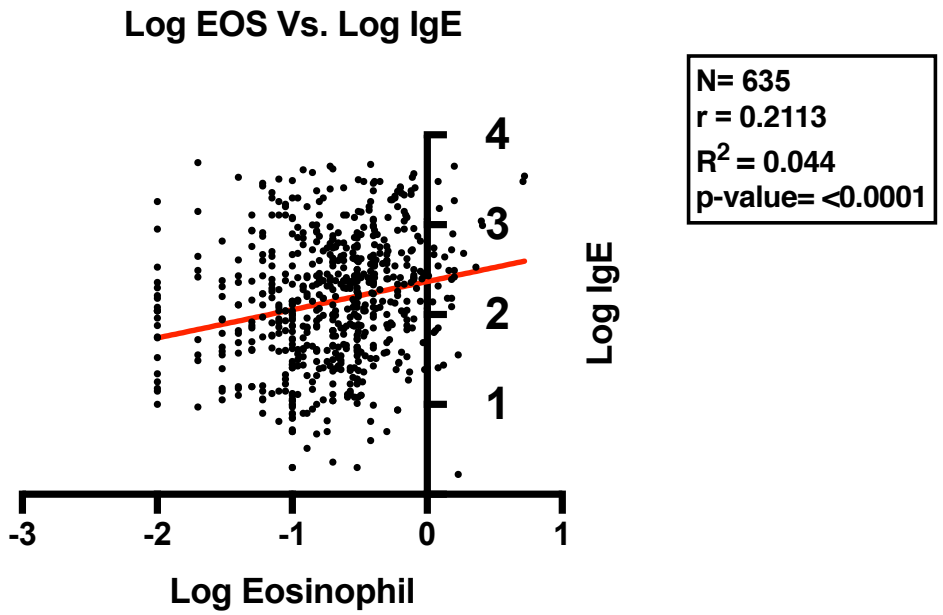

Figure 4.3: scatter plots demonstrated the correlation between blood IgE level and blood Eosinophil after transformation. The correlation was positive significant with p-value <0.0001 and R 0.2113. Both eosinophil counts and serum IgE were logarithmic transformed.

Moreover, the correlation was significant in skin prick test (Figure 4.4), and was more evident among subjects had atopy, this significant presented with p-value 0.004. Atopic subjects were high in prevalence with 440 out of 640 (Table 4.3).

Eosinophil correlation			P-value
Skin prick test	**Atopic**	N= 440 median= 0.23 (0.01-5.23)	0.004
	Non-atopic	N= 200 Median= 0.195 (0.01-1.88)	

Table 4.3: Eosinophil correlation with skin prick test. Skin prick test demonstrated into atopic and non-atopic presented with median, ranges and the number of subjects involved in the test.

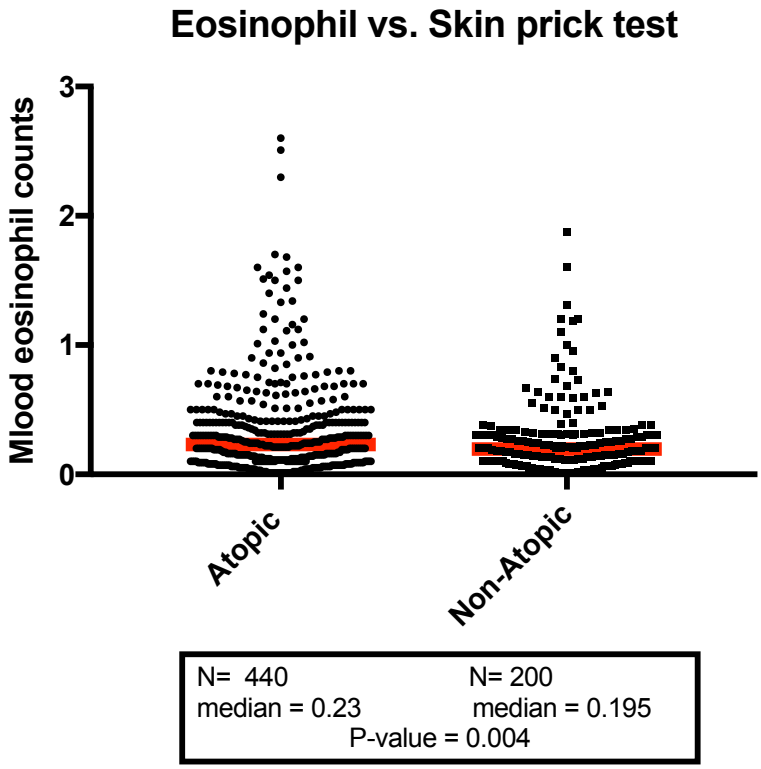

Figure 4.4: scatter plots demonstrated the association between skin prick test and blood Eosinophil counts. The association was significant with p-value 0.004. Atopic and non-atopic demonstrated with median and subjects involved.

4.5 **The effect on smoking on asthma presentation**

Stratification of asthma subjects in this section based on smoking behaviour (never, ex-smoker and current smoker) and specific tests were chosen to identify the nature of these phenotypes and how it affected by smoking (Table 4.4). There was 2878 out of 3804 subjects presented with smoking behaviour history. Majority of subjects were females, which represented 68.5% never smoked, 66.8% ex-smoker and 58.8% currently smoker. The mean of age recruited was 41.68 and 43.11 years old. Smoking behaviour is associated with greater asthma symptoms. Interestingly, mean ACQ was recorded and showed decline with smoker (mean= 1.94) more than never (mean= 2.153) or ex-smoker (mean= 2.155). In addition, lung function deteriorated in both FEV1 % predicted and FEV1/FVC with current smokers.

Subjects with blood IgE level has been presented but there was no significant findings. Furthermore, skin prick test performed to identify the atopic and non-atopic subjects and presented with a high number of subjects diagnosed as atopic never smoke (75.9%), ex-smoker (65.5%), and current smoker (64.8%). Finally, GINA score was demonstrated but similar to the previous stratification with eosinophil, it couldn't analyses because the lack of subjects in some classification.

	Smoking behaviour (N= 2878)		
	Never	**Ex-smoker**	**Current smoking**
Gender (Female)	(68.5%) N= 1237/1806	(66.8%) N= 428/641	(58.8%) N= 227/386
Age	41.68 (16-60) N= 1808	44.63 (17-60) N= 647	43.11 (17-60) N= 383
Blood IgE levels KU/L	397.03 (0.60-4900) N=1046	362.85 (1.66-4850) N= 409	383.24 (2-3800) N= 251
Pack/yrs	N/A	8.41 (0.03-108) N= 461	21.56 (0.06-120) N= 277
ACQ	2.153 (0.00-5.71) N= 531	2.155 (0.00-5.83) N= 163	1.94 (0.28-4.29) N= 17
FEV1 % predicted	75.22 (15-133) N= 1426	73.27 (15-123) N= 492	69.92 (16-116) N= 307
FEV1/FVC prbd	0.71 (0.23-1.00) N= 1354	0.70 (0.24-1.00) N= 459	0.67 (0.25-0.95) N= 311
	GINA score		
GINA 1	(3.0%) N= 26	(3.4%) N= 9	(3.5%) N= 4
GINA 2	(9.8%) N= 85	(11.7%) N= 31	(7.1%) N= 8
GINA 3/4	(70.8%) N= 612	(75.5%) N= 200	(82.3%) N= 93
GINA 5	(16.3%) N= 141	(9.4%) N= 25	(7.1%) N= 8
	Skin Prick test		
Atopy	(75.9%) N= 623	(65.5%) N= 205	(64.8%) N= 129
Non-atopic	(24.1%) N= 198	(34.5%) N= 108	(35.2%) N= 70
Hay fever (Yes)	(63.3%) N= 705/1114	(58.1%) N= 222/382	(45.5%) N= 115/253

Table 4.4: Basic demographics, atopy test, and GINA scores demonstration after stratification based on smoking behaviour. Continuous variables were presented with mean, ranges and the number of subjects involved (N). Categorical variables were presented with percentage of presence among each group (n/N) with subjects involved.

4.6 Current smoking is associated with a lower lung function in asthma subjects

Stratification of asthma subjects in this section based on smoking behaviour (never, ex-smoker and current smoker) and specific tests were chosen to identify the nature of these phenotypes and how it affected by smoking. The analysis was performed using Kruskal-Wallis test for comparing more than two sample groups for non-parametric data. There was a significant association between FEV_1 % predicted with smoking status (Table 4.5). P values were 0.0002, and after multiple comparisons test, the association was presented between a never smoke group and current smoker group with p-value 0.0002 (figure 4.3). Additionally, the same test performed on FEV_1/FVc and presented a significant association with p value <0.0001 (table 4.4), also multiple comparisons test a proven the association between a never smoke grouped and current smoker grouped (Figure 4.5).

Subgroup	Median	Range	P-value
Never	76.56	(15-133) N= 1425	0.0002
Ex-smoker*	74.41	(15-123) N= 492	
Current smoker*	70.49	(16-116) N= 307	

Table 4.5: FEV1% predicted vs. smoking behaviour association. P value presented by using Kruskal-Wallis test. Median and ranges showed for each group**.** *post hoc test test showed significant association between never and current smoker groups with P-value 0.0002.

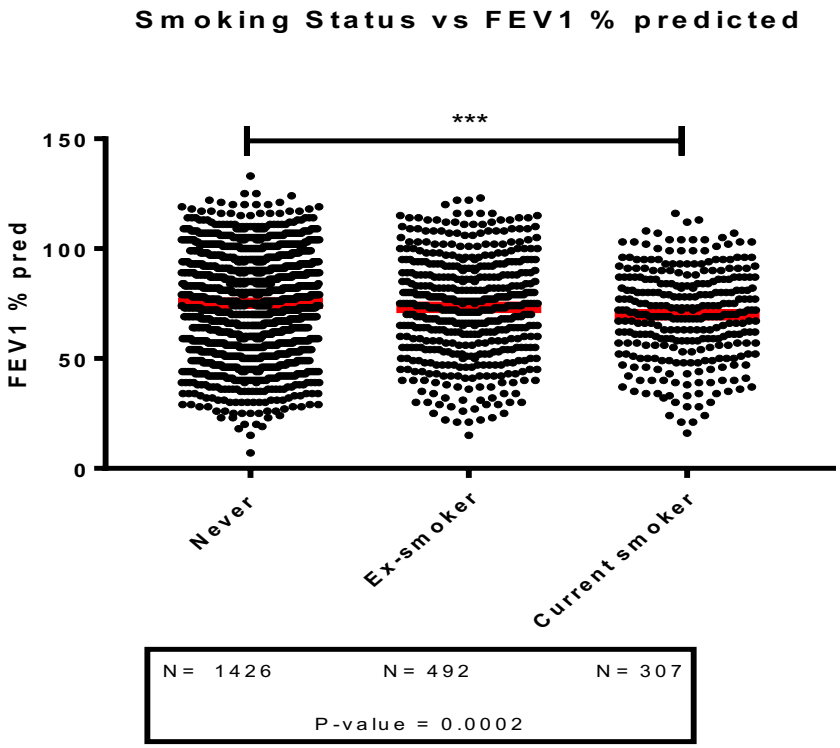

Figure 4.5: Scatter plots demonstrated the association between FEV1% pred and smoking behaviour. The association was significant with p-value 0.0002. Results demonstrated with median and subjects involved. Analysis performed using Kruskal-Wallis test *** represents p-value 0.0002.

Subgroup	Median	Range	P-value
Never*	0.73	(0.23-1.00) N= 1354	<0.0001
Ex-smoker	0.72	(0.24-1.00) N= 459	
Current smoker*	0.69	(0.25-0.95) N= 307	

Table 5: FEV1/FVC vs. smoking behaviour association. P value presented by using Kruskal-Wallis test. Median and ranges showed for each group**. ***Multiple comparison test showed significant association between never and current smoker groups with P-value <0.0001.

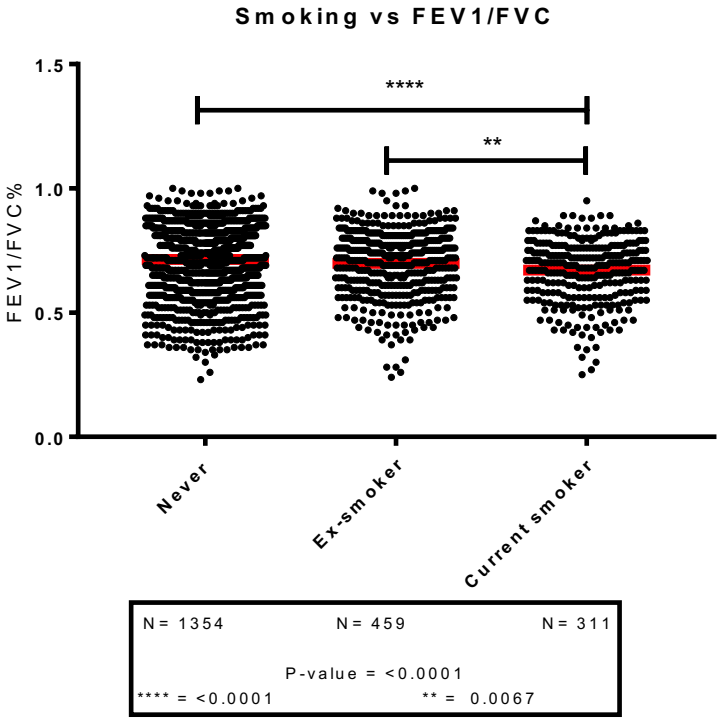

Figure 4.4: Scatter plots demonstrated the association between FEV1/FVC and smoking behaviour. The association was significant with p-value <0.0001. Results demonstrated with median and subjects involved. Analysis performed using Kruskal-Wallis test. *** represents p-value <0.0001.

4.7 The effect of ever smoking on clinical and immunological features of asthma

The following section identify the impact of smoking on asthma after stratified subjects into only two groups, including never and ever groups. Table 4.6 presented the basic demographics of asthma subjects after stratified them into only two groups. Generally, age of subjects on everyone group was 44.07 years old which is slightly higher than never smoke group. Additionally, lung function values reduced with smokers with obvious reduction values.

		Smoking behaviour	
		Never	**Ever**
		N= 1835	**1043**
Gender	**Female**	(68.5%) N= 1237/1806	(63.8%) N= 655/1027
	Male	(31.5%) N= 569/1806	(36.2%) N= 372/1027
Age		41.68 (16-60) N= 1808	44.07 (17-60) N= 1030
Blood IgE levels KU/L		397.03 (0.60-4900) N=1046	370.61 (1.66-4850) N= 660
Pack/yrs		N/D	13.34 (0.03-120) N= 738
ACQ		2.153 (0.00-5.71) N= 531	2.14 (0.00-5.83) N= 180
FEV1 % predicted		75.22 (15-133) N= 1426	71.98 (15-123) N= 799
FEV1/FVC prbd		0.71 (0.23-1.00) N= 1354	0.69 (0.24-1.00) N= 770
		GINA score	
GINA 1		(3.0%) N= 26/864	(3.4%) N= 13/378
GINA 2		(9.8%) N= 85/864	(10.3%) N= 39/378
GINA 3/4		(70.8%) N= 612/864	(77.5%) N= 293/378
GINA 5		(16.3%)	(8.7%)

	N= 141/864	N= 33/378
	Skin Prick test	
Atopy	(75.9%) N= 623/821	(65.2%) N= 334/512
Non-atopic	(24.1%) N= 198/821	(34.8%) N= 178/512
Hay fever (Yes)	(63.3%) N= 705/1114	(53.1%) N= 337/635

Table 4.6: Basic demographics, atopy test, and GINA scores demonstration after stratification based on never and everyone smoke. Continuous variables were presented with mean, ranges and the number of subjects involved (N). Categorical variables were presented with percentage of presence among each group (n/N) with subjects involved.

4.8 The effect of ever smoking on Lung function

Stratification of asthma subjects based on smoking behaviour (never and ever) has been analysed to find the association with lung function values. The analysis was performed using Mann-Whitney U test for comparing more two sample groups for non-parametric data (table 4.7). Median of the never group presented with 76.56, however, everyone group (ever) reported almost high reduction in the median with 72.72.

As the table 4.7 shows, there is a significant difference between FEV1 % predicted with smoking behaviour of two groups with P values of 0.0003 (Figure 4.5). Additionally, the same test performed on FEV1/FVC and reported a significant difference with p value <0.0001 (Table 4.8 & Figure 4.6).

Subgroup	Median FEV_1% pred	Range	P-value
Never	76.56	(15-133) N= 1425	0.0003
Ever	72.72	(15-123) N= 799	

Table 4.7: FEV1% predicted vs. smoking behaviour association (Never Vs. Ever). P value 0.0003 presented using Mann-Whitney U test. Median and ranges showed for each group.

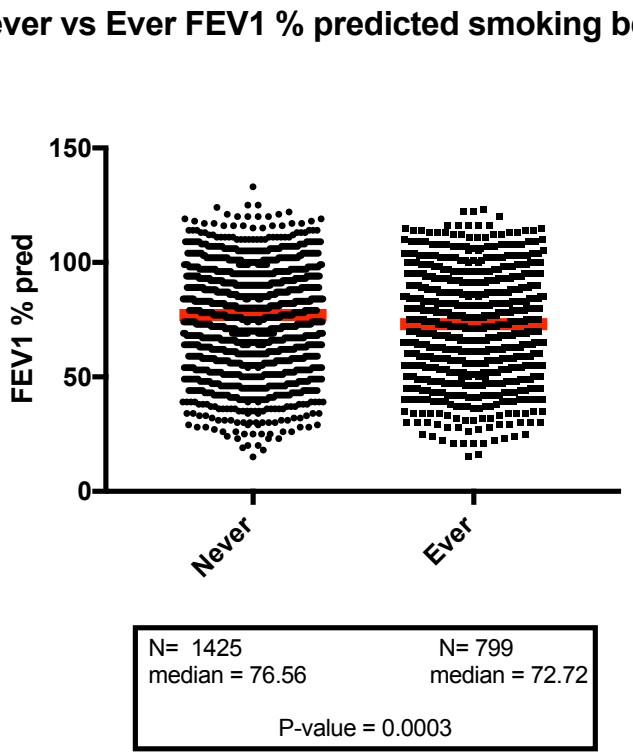

Figure 4.5: Scatter plots demonstrated the association between FEV1% pred and smoking behavior (Never Vs. Ever). The association was significant with p-value 0.0003. Results demonstrated with median and subjects involved. Analysis performed using Mann-Whitney U test.

Subgroup	Median	Range	P-value
Never	0.73	(0.23-1.00) N= 1354	<0.0001
Ever	0.70	(0.24-1.00) N= 770	

Table 4.8: FEV1/FVC vs. smoking behavior association (Never Vs. Ever). P value presented by using Mann-Whitney U test. Median and ranges showed for each group.

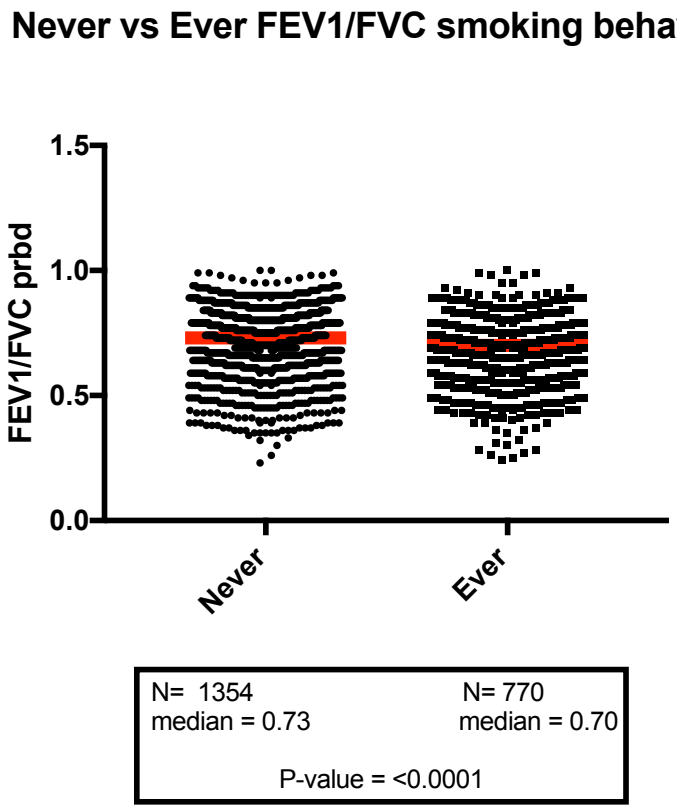

Figure 4.6: Scatter plots demonstrated the association between FEV1/FVC and smoking behaviour (Never Vs. Ever). The association was significant with p-value <0.0001. Results demonstrated with median and subjects involved. Analysis performed using Mann-Whitney U test.

4.9 What are the clinical and immunological features of asthma patients that exacerbate?

Patients diagnosed as severe or difficult to control asthma are most likely needing close care or emergency, and often will need to be admitted to hospital. However, even moderate to severe asthma sometimes will need to admit to the hospital and seeking close monitoring and to control their asthma medication. Table 4.8 shows an overview of basic demographics, clinical and immunological phenotypes stratified by the hospital admission. First of all, hospital admission or hospitalization have been divided into four groups, and these categories or classifications were based on the number of hospital stay records. Those groups are never, once, more than once, and high dependency/intensive care unit (ICU). The average scores of the age were compared in order to each group, and the mean averages were 42.75 y/o never admitted to the hospital, and they formed about 504 subjects. However, subjects represented high dependency/ICU has been recorded as it an important indicator of exacerbation, and a total of 51 subjects admitted to the hospital with a mean of age 39.63.

Interestingly, child-onset asthma has been found as correlated to the high percentage of GASP in term of asthma hospitalization. In the link to the previous point, percentages of hospital admission in each group seem high and more than 50% in subjects with child-onset asthma. To assess the symptoms of asthma, ACQ questionnaire used for most of subjects and results suggested that symptoms are getting worse with increasing the number of hospital stays. For example, subjects with a history of never admitted have a mean of ACQ

2.06, while it was getting high up to a mean of 2.90 with ICU admission. Moreover, lung functions have been tested using FEV_1% predicted, and FEV_1/FVC and the table below demonstrates the different scores among each group. Therefore, table 8 illustrates the findings and proof that the more hospital stay is worsened lung function. Furthermore, medication has been recorded and tested for each group because of the importance of the medication that controlling asthma.

		Hospitalization			
		Never	Once	More than once	High dependency/ ICU
Gender (Female)		(68.4%) N= 349/510	(73.2%) N= 112/153	(89.2%) N= 74/83	(72.5%) N= 37/51
Age		42.75 (16-60) N= 504	39.86 (16-60) N= 151	39.58 (17-60) N= 83	39.63 (18-60) N= 51
Age of Onset	<16	(53.6%) N= 263/491	(51.7%) N= 75/145	(64.2%) N= 52/81	(58%) N= 29/50
	> 16	(46.4%) N= 228/491	(48.3%) N= 70/145	(35.8%) N= 29/81	(42.0%) N= 21/50
Blood IgE levels KU/L		279.60 (2-3187) N=183	514.32 (5.20-4850) N= 52	298.88 (2-1844) N= 24	331.36 (5.64-1960) N=10
ACQ		2.06 (0.00-5.29) N= 411	2.30 (0.00-5.71) N= 121	2.38 (0.228-5.14) N= 54	2.90 (0.29-5.29) N= 29
FEV1 % predicted		79.75 (20-121) N= 410	77.78 (30-119) N= 118	74.10 (21-121) N= 65	73.62 (30-104) N= 38
FEV1/FVC prbd		0.74 (0.33-1.00) N= 419	0.73 (0.35-0.94) N= 118	0.76 (0.43-0.99) N= 70	0.72 (0.51-0.95) N= 38
Skin prick test	Atopic	(80.7%) N= 121/150	(70.6%) N= 24/34	(92.9%) N= 13/14	(62.5%) N= 5/8
	Non-Atopic	(19.3%) N= 29	(29.4%) N= 10/34	(7.1%) N= 1/14	(37.5%) N= 3/8
Hay fever (Yes)		(67.7%) N= 291/430	(61.7%) N= 66/107	(71.4%) N= 50/70	(59.5%) N= 25/42
GINA score					
GINA 1		(5.6%) N= 27/484	(2.1%) N= 3/141	(0.0%) N= 0	(0.0%) N= 0
GINA 2		(12.6%) N= 61/484	(12.1%) N= 17/141	(11.0%) N= 9	(8.2%) N= 4/49
GINA 3/4		(65.7%) N= 318/484	(68.1%) N= 96/141	(65.9%) N= 54/82	(49.0%) N= 24/49
GINA 5		(16.1%) N= 78/484	(17.7%) N= 25/141	(23.2%) N= 19/82	(42.9%) N= 21/49

Table 4.8: Basic demographics, clinical, and immunological features demonstration after stratification based on hospital admission. Continuous variables were presented with mean, ranges and the number of subjects involved (N). Categorical variables were presented with percentage of presence among each group (n/N) with subjects involved.

4.10 **Gender stratification on the hospital admission**

Turning now to the statistical analysis on asthma subjects stratified based on the hospital admission. Interestingly, the gender was examined using chi-square test to find if hospital admission or exacerbation of asthma are driven by particular subject's sex. The results, as shown in Table 4.9, indicate that more females exacerbate in patients with asthma. This result is significant at p value 0.002 level.

Gender	Never	Once	More than Once	High dependency/ICU	P-value
Male	(31.6%) N= 161	(26.8%) N= 41	(10.8%) N= 9	(27.5%) N= 14	0.002
Female	(68.4%) N= 349	(73.2%) N= 112	(89.2%) N= 74	(72.5%) N= 37	

Table 4.9: Gender demonstration after stratified by the hospital admission. P value presented by using chi-square test. Number of subjects and ranges showed for each stratification.

Interestingly, for those subjects with ACQ scores performed using Kruskal-Wallis test, there was a significant difference among groups stratified by the hospital admission. The result is significant at the p-value 0.007 level (Table 4.10). Post hoc analysis revealed that between never admitted subjects group and high dependency/ICU group was a significant difference at p-value level 0.0179 level (Figure 4.8).

ACQ	Never*	Once	More than Once	High dependency/ICU*	Total	P-value
Mean ACQ	2.06 (0.00-5.29) N= 411	2.30 (0.00-5.71) N= 121	2.38 (0.28-5.14) N= 54	2.90 (0.29-5.29) N= 29	N= 615	0.007

Table 4.10: Mean ACQ illustration across the hospital admission stratification groups. Average mean, number of subjects and ranges showed for each stratification. P-value presented by using Kruskal-Wallis test. * represents p-value at 0.0179 level after multi-comparison test performed.

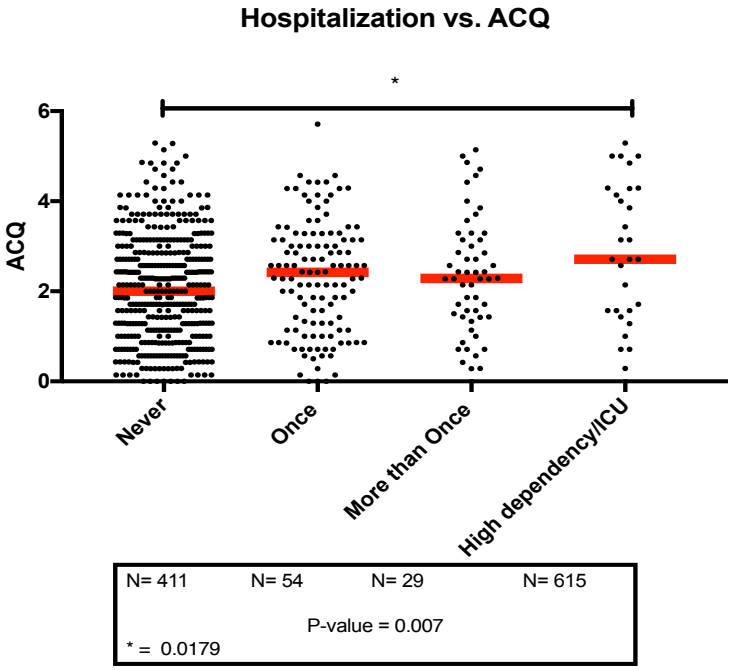

Figure 4.8: Scatter plots explained the significant differences among ACQ scores and hospital admission. The difference was significant at p-value 0.007 level. Results demonstrated with mean, ranges and subjects involved. Analysis performed using Kruskal-Wallis test. * represents p-value at 0.0179 level after multi-comparison test performed.

4.11 **Discussion**

This chapter set out with the aim of assessing three topics: first, the importance of blood eosinophils and clinical features in asthma; second, the impact of smoking on asthma phenotypes; and, third, the relationship of clinical and immunological features of asthma subjects that are prone to exacerbation. As is known, the purpose of asthma treatment is to control symptoms, to decrease exacerbations, and to improve the subject's quality of life (Matucci et al. 2018). Therefore, this chapter was intended particularly to study the different relationships between the clinical and immunological features of asthma subjects. Ultimately, the GASP results in this chapter demonstrated different correlations and findings to provide a better understanding of asthma treatments.

The GASP study met its first aim and provided an interesting finding, which is that asthma subjects possess elevated blood eosinophil counts. This is especially the case with atopic individuals, as demonstrated in tables 4.2 and 4.3. In particular, this elevation of eosinophil was linked directly to an increased level of IgE. Both elevations contribute physiologically to processing asthma. Therefore, the GASP results proved a parallel between increased eosinophil counts and IgE levels. This correlation has been proved by previous groups such as Khadadah et al. (2000), who presented the same principle and findings, convening the relationship of allergic sensation. Similarly, the SARP cohort confirmed in their study of three different clusters that the majority of their subjects were atopic. Moreover, they appeared with early (childhood) onset, and 76% of their patients were atopic (Moore et al. 2010). Likewise, the U-BIOPRED reported that in their cohort, the incidence of atopy was high at 70% in the

asthma groups compared to only 46% in the healthy control group. In addition, they noted that blood eosinophil counts were significantly higher in asthma groups compared to the control group. The significances were: severe asthma non-smoker vs. healthy control, p value 0.002; severe asthma smoker/ex-smoker vs. healthy control, p value 0.005. However, they stated that the severe asthma group was less frequently diagnosed with atopy than the mild to moderate group (Shaw et al. 2015).

The second aim of this chapter was to determine the impact of smoking on asthma presentation. There is a lack of research on the effects of smoking on asthma patients and, hence, little knowledge on the relationship between smoking and asthma. In the GASP study, a comparison of non-smokers and ex-smokers with current smokers showed that current smoking is associated with a reduced lung function in asthma subjects. In our result, the FEV1% predicted and FEV1/FVC were statistically analysed, and both showed a significant reduction when the subject was a current smoker. These results match those observed in earlier studies. For example, a clinical study by Tommola et al. (2016) demonstrated a relationship between smoking and reduced lung function during a follow-up of 12 years; they found that cigarette smoking is significantly associated with the rapid reduction of the lung function, especially with adult-onset asthma subjects. Interestingly, it has been reported that the reduction of FEV_1% predicted (p=0.006) and FEV1/FVC (p=0.045) occurred at a significantly faster rate in subjects with $\geqslant$10 pack-years of smoking history than subjects with <10 pack-years of smoking history (Tommola et al. 2016).

Moreover, the Copenhagen General Population Study cohort reported that within 4.5 years of follow-up, the acceleration in the reduction of FEV1 was

higher in smoking asthmatic groups than amongst those with asthma who had never smoked (Çolak et al. 2015). Recently, an epidemiologic study reported that among a birth cohort followed until 38 years old, there was an association between smoking and reduced FEV1/FVC, which appeared within young adult asthmatics (Hancox et al. 2016). The GASP results confirm that smoking asthmatic patients are associated with accelerated lung function reduction, especially in late-onset asthma, as suggested by previous studies (Tommola et al. 2016, Çolak et al. 2015, Hancox et al. 2016).

The third aim of this chapter was to determine the relationship between clinical and immunological features of asthma subjects that are prone to exacerbation. The GASP results suggested that asthma patients that exacerbate are predominantly female and have higher baseline symptom scores. These findings are in agreement with Patel et al.'s (2014) study, which showed that females, especially of older age, are associated with a higher risk of exacerbation. Patel et al. further reported that symptoms worsen with high ACQ scores, increasing the risk of severe exacerbation as reported and predicted (Patel et al. 2014B). As many studies have reported, the prevalence of asthma, exacerbation rates, and hospital admission overall are higher in females than males. However, the exacerbation rate has been reported as highest among males from birth to 14 years old (Davidson et al. 2010, Patel et al. 2014B). The U-BIOPRED cohort confirmed our findings related to gender; they observed that females are more prone to asthma, particularly when composed with obesity in a cluster, and have high exacerbation rates (Lefaudeux et al. 2017). Furthermore, the clustering analysis in adult asthma patients identified that females are most likely to have more severe asthma and

be weaker in corticosteroids responsiveness than males (Moore et al. 2010, Wu et al. 2014). While it is unknown why asthma is more prevalent in females, the cause has been linked to hormonal or immunological factors (Zein and Erzurum 2015A).

The GASP study's findings have important implications for developing new insights into the treatment of asthma. In the GASP and previous studies, subjects were categorized and divided into groups that displayed the clinical and immunological relevant differences in the result. Eventually, these subjects will be treated for the underlying cause of their disease. Currently, IgE, IL-5, and, possibly shortly, IL-13 are the most available therapeutic agents that work as allergy mediators. Furthermore, these are existing evidence of molecular medicine. For example, omalizumab, an IgE-targeting therapeutic, is used for allergic exacerbation patients with asthma. In addition, omalizumab is a highly recommended add-on therapy to the current treatment due to its ability to eliminate both early and late allergic responsiveness (Buhl 2005; Humbert et al. 2018). Omalizumab has been reported as an exacerbation inhibitor, to improve asthma symptoms and lifestyle, and to eliminate the use of systemic corticosteroids (Humbert et al. 2005; Kulus et al. 2010).

Another treatment has recently been launched that primarily works on eosinophilic asthma and is known as anti-IL-5, such as mepolizumab and reslizumab (Matucci et al. 2018). Anti-IL-5 drugs inhibit interaction with the surface of eosinophil and occur by binding with high affinity to IL-5. Recently, GINA (2018) recommended using anti-IgE (omalizumab) and IL-5-targeting biologics (mepolizumab, reslizumab, and benralizumab) as add-on therapy to LABA/ICS for the long-term control of asthma. The addition of both drugs

probably played a role in reducing the use of oral corticosteroid in patients with severe allergic asthma and eosinophilic asthma, respectively (Bel et al. 2014; Katsaounou et al. 2019).

Conclusion

In conclusion, this chapter has demonstrated various results concerning non-related asthma subjects. The relationship between clinical and immunological features with various parameters of asthma presented several significant outcomes. Firstly, among the non-related asthma subjects involved in this cohort, there was a significant association between the elevation of blood eosinophil counts in atopic subjects and the total serum IgE. Moreover, a decline in lung functionality and high baseline symptoms are significantly associated with current smoking.

Chapter 5: General discussion

This is a valuable cross-sectional study that combines clinical and immunological data from multiple cohorts spanning the UK in addition to our own cohort, which included asthma patients from 20 clinical centres across the UK. This study involved several patient populations: control, asthma, COPD, and asthma-COPD. Also, it was necessary to include a moderate-severe asthma population to answer the key questions of the thesis. The aim of this study was to identify the needs of these demographics in order to achieve better understanding of asthma features and treatments. This study using data from moderate-severe asthma subjects, analysed: 1) the relationship between blood eosinophil counts and different clinical features; 2) the impact of smoking on asthma presentation; and 3) the clinical and immunological features of asthma that are prone to exacerbation.

The subjects in the GASP cohort presented with clinical, immunological and genetic features of chronic respiratory diseases, with a focus on asthma, COPD, and asthma-COPD overlap. The GASP cohort was enriched by having moderate-severe asthma subjects recruited from all over the UK. In reviewing the literature, no amount of data as large as that provided by the GASP was found regarding the association between the clinical and immunological features of asthma in the UK. There were a total of 8533 patients in the GASP, including 3841 subjects who presented with moderate to severe symptoms of unrelated asthma subjects. The initial objectives of the project were to summarise the clinical and immunological findings of the GASP cohort regarding control, asthma, COPD and asthma-COPD subjects. Moreover, with the inclusion of

non-related asthma subjects, it shows the relationship between blood eosinophil counts and clinical variables of asthma, the impact of smoking on asthma presentation, and the essential features of asthma that are prone to exacerbation.

The results of this study indicate that the majority of asthmatic patients in the UK are females (66%) and that females are more likely to experience asthma exacerbation than males. These findings have been confirmed in previous studies, such as the U-BIOPRED cohort study, which demonstrated that females demonstrate higher asthma prevalence and are more likely to experience exacerbation (Lefaudeux et al. 2017). However, it is difficult to explain why this is, except that it might be related to hormonal factors (Zein and Erzurum 2015B). However, blood counts were found to be elevated amongst atopic subjects, and this is positively associated with elevated total IgE serum. The findings of this current study are consistent with those of the Leicester cluster cohort, which indicated that subjects with late-onset severe asthma were more likely to have an eosinophilic phenotype (Haldar et al. 2008). Clusters of atopic subjects that presented with late-onset severe asthma showed allergic markers. However, in comparison with the GASP, the SARP cohort featured more factors, such as medication usage and obesity, that were not accounted for in this study (Moore et al. 2010).

In this study, I identified the impact of smoking on asthma presentation. Many studies in the literature have featured this, but it is rare to find results except amongst moderate to severe asthma cohorts. The GASP results showed that

moderate to severe asthma subjects are most likely to experience worsening lung function, and continuing to smoke may lead them to develop COPD. Our results suggested that smoking accelerates the reduction of both FEV_1 percentage predicted and FEV_1/FVC. Moreover, the GASP cohort's large lung function records indicated that the most likely patients in this demographic are in mid-age (41–43 years old). This reduction in lung function, in particular FEV_1/FVC, is indicative of the airway obstruction and could be linked to an increase in total IgE serum (IgE is one of the biomarkers contributing to asthma). These results have been confirmed by many studies, such as one clinical study done by Tommola et al. (2016) and one by the Copenhagen General Population Study cohort in 2015. Unfortunately, it has been found that many studies included the number of pack-years of their subjects, for example in Tommola et al. (2016). However, the GASP excluded the pack-years variable because that information was not included in the records.

Asthma is as same as other diseases have confounders, that associated with many conditions, which may affect the clinical manifestations of patients. One of the confounders is upper respiratory tract infections (URI) and rhinosinusitis. Rhinoviruses are the leading cause of URI and rhinosinusitis. Although it can be healed within three weeks, it leads to asthma exacerbation by increasing the viral load and causing airway inflammation (FitzGerald and Gibson 2006). Also, obesity considered as one of the diseases commonly associated with asthma. Decline lung volumes are one of the characteristics of obesity-associated asthma (Gherasim, Dao and Bernstein 2018). Moreover, GERD is one of the common causes of respiratory symptoms. GERD occurred with chest tightness

and chronic cough. GERD can cover-up as asthma, and when it happened, further symptoms will appear, such as heartburn and regurgitation (Mastronarde 2012). However, patients with GERD frequently deny symptoms. Besides, differentiating between variant cough asthma and GERD is complicated because both happen during day and night (Broers, Tack and Pauwels 2018).

Inhaled corticosteroids are using to treat patients with COPD. Additionally, ICS has an influence in decreasing the risk of exacerbations. Several studies showed that a high dose of inhaled corticosteroids is associated with a significant decrease in the percentage of sputum eosinophil and an increase in neutrophils (Brooks et al. 2017). Recently, three reason has increases the interest in targeting ICS (Pavord and Agusti 2016). First, there was an increase in potential adverse effects of ICS, such as increases the risk of pneumonia and tuberculosis (Suissa et al. 2013). Second, in the last ten years the high dose usage of ICS has increased, and that out of the recent guidelines. Finally, the combination of long-acting β2-agonists (LABA) and long acting anti-muscarinic agents (LAMA) is considered as an alternative options for patients with COPD (Singh 2015). Interestingly, Bafadhel et al. stated that out of many biomarkers examined, the blood eosinophil count was the hallmark biomarkers of exacerbation associated with an elevation in sputum eosinophil counts (Bafadhel et al. 2011).

Furthermore, several studies investigated the relationship between ICS and blood eosinophil in linked with lung function. ICS reduce the rate of decline in FEV_1 and the number of hospital admission (Vestbo et al. 2016). For example,

findings from Korean COPD Subtype Study (KOCOSS) cohort showed that FEV_1 increased in patients with high eosinophil counts. However, the reduction of FEV_1 is faster with subjects on ICS medication (Song et al. 2017). Further study on blood eosinophil and FEV_1 reported that patients using ICS with high blood eosinophil recorded with lower decline of FEV_1 (Barnes et al. 2016)

The strength of this study was demonstrated by the large number of subjects included in the GASP cohort, in comparison with other studies. Also worth noting was the large amount of subject information available, which provided the chance to express many phenotypes by continually adding to the database. Furthermore, the presence of a significant association between the results provides more support for the study. Also, the number of genotyping were high which might help in the further study including genetics features in asthma but because of the time limit this, as the thesis needed to be done in a year, genetics study was cancelled or disponed to future work. Besides, the GASP enriched with a large number of blood and saliva samples. Therefore, GASP can be one of the most extensive published paper in term of genetics, and that because of the number of genotyping that can be done with large sample sizes cohort such as NRA cohort.

However, this cohort was missing some data, which eliminates the chance to examine more variables. This limitation is one of the reasons for the lack of information provided by the clinical centres or the cohort itself at the time the study began. One of the issues is the long time spent correcting the data of any errors or abnormal values. A further limitation that faced GASP was the lack of

dosage information for most of the medications, which affects the exact number of subjects' GINA scores. A big limitation is that the study is cross-sectional, not longitudinal. More importantly, there was a huge lack of pack-year records, which did not allow us to examine subjects' smoking either below or above 10 pack-years, as many other cohorts have done. Another limitation is the small sample sizes particularly for subgroups such as ACOS.

Future Work

This study has offered many questions that require further answers and investigations. More research is necessary to identify the efficacy of current asthma medications. Moreover, it would be beneficial to look for changes in the associations within the asthma-COPD overlap, as this aspect is controversial. It would be interesting to do further research to identify the genetic features of asthma, and to learn more about their associations with the clinical and molecular findings in this study.

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