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THE DANGERS OF ALLERGIC ASTHMA

JESÚS MIGUEL GARCÍA-MENAYA, MD, PHD
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Editor

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To my family.

Contents

Preface ix

Chapter 1 **Epidemiology of Asthma** 1
Tomás Chivato Pérez, Francisco J. Caballero Segura,
Natalia Gutierrez Albadalejo and Rodrigo Jiménez García

Chapter 2 **Immunology of Allergic Asthma and T2 Inflammation in Allergic Asthma** 17
Paula Galvan-Blasco, Victoria Cardona Dahl
and Moisés Labrador-Horrillo

Chapter 3 **Pollens Causing Asthma** 33
Jesús Miguel García-Menaya
and Concepción Cordobés-Durán

Chapter 4 **Mites and Fungal Spores Causing Asthma** 55
Teresa Carrillo Diaz and María del Pilar Cuesta Apausa

Chapter 5 **Diagnosis of Asthma: Clinical Symptoms and Tests** 77
Alejandra Carrón Herrero,
María Daniela de Jesús Escalante Carrero,
Patricia Gutiérrez Canales and Darío Antolín-Amérigo

Chapter 6 **Occupational Allergic Asthma** 93
Santiago Quirce

Chapter 7 **Severe Asthma** 107
Carmen Panaitescu, Roxana Silvia Bumbacea,
Rama Boustani, Maria-Roxana Buzan, Laura Haidar,
Catalina Cojanu, Alexandru Laculiceanu,
Daniela Spinu and Ioana Agache

Chapter 8 **An Update on Thunderstorm Allergy and Asthma** 125
Gennaro D'Amato, Isabella Annesi-Maesano,
Lorenzo Cecchi, Benedetta Biagioni
and María D'Amato

Chapter 9 **Asthma and Climatic Change** 137
Javier Subiza and Martha Cabrera

Chapter 10 **Air Pollution and Asthma** 149
Francisco Feo Brito, Pilar Mur Gimeno,
Javier Fernández Sánchez, José Antonio Lucas García,
Teresa Alfaya Arias and José Miguel Urra Ardanaz

Chapter 11 **Allergic Asthma and Viruses** 167
Alicia Armentia-Medina, Cristina Díaz-Rodríguez
and David Pérez-Torres

Chapter 12 **United Airway Disease**..... 181
Ricardo Madrigal-Burgaleta, F. Runa Ali,
Bryan N. Fernandes, Matt Lechner,
Nara Orban and Paul Pfeffer

Chapter 13 **Allergic Bronchopulmonary Aspergillosis**..... 193
Álvaro Moreno-Ancillo, Marta Jiménez Arroyo,
Miriam Artero Clemente, Jesús Jurado Palomo,
José Manuel Bravo Nieto and Ana Carmen Gil-Adrados

Chapter 14 **Aspirin-Exacerbated Respiratory Disease**..... 217
Inmaculada Doña, Ibon Eguiluz
and María José Torres

Chapter 15 **Pharmacological Treatment of Asthma** 237
Manuel J. Rial Prado, Carolina Gómez Fariñas
and Raquel Eiras Leal

Chapter 16 **Immunotherapy for Asthma** 249
Carmen Vidal and Paula Méndez-Brea

Chapter 17 **Biological Treatments for Asthma**..... 265
Cristina Martin-García, Alicia Gallardo-Higueras
and Ignacio Dávila

Chapter 18 **Clinical Pharmacogenomics in Asthma Therapy**..... 285
Pedro Ayuso, Jesús Miguel García-Menaya,
Concepción Cordobés-Durán, José A. G. Agúndez
and Elena García-Martín

Index 307

About the Editor..... 319

Preface

Asthma is an important, complex and frequent disease, mainly characterized by airway inflammation with symptoms such as wheezing, shortness of breath, cough and chest tightness together with expiratory airflow limitation. In developed countries, between 5% and 10% of the population suffer from asthma, and allergic asthma corresponds to an important percentage of total asthma that varies depending on the age and population characteristics.

This book describes in depth the potential dangers of allergic asthma, like thunderstorm-asthma outbreaks, occupational asthma, allergic bronchopulmonary aspergillosis and new potential threats like climate change and air pollution-influencing asthma. There are also other chapters describing epidemiology, immunology and allergens related with allergic asthma. The concept of united airway disease is reviewed and special situations like severe asthma and aspirin-exacerbated respiratory disease are also covered. Chapters about diagnostic approach and the main treatments, including biologics and clinical pharmacogenomics as asthma therapy, contributes to a more complete knowledge about this complex, fascinating and sometimes dangerous disease.

Chapter 1

Epidemiology of Asthma

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Abstract

The World Health Organization considers asthma a major noncommunicable disease, affecting both children and adults, and the most common chronic disease among children.

The current evidence suggests that asthma is a complex multifactorial disorder, and its etiology is increasingly attributed to interactions between genetic susceptibility, host factors, and environmental exposures.

Asthma is a disease with a high social impact, due to its effect on the quality of life, work absenteeism, healthcare resources use, and mortality. In addition, it is estimated that 70% of the healthcare costs of asthma are due to poor control of the disease.

Recent studies suggest that the prevalence and severity of asthma may be decreasing due to better diagnosis and treatment, especially in high income countries. However, climate change, pollution from cities and global warming are conditioning a worsening of the quality of the air we breathe and may increase the burden of asthma in the near future.

Keywords: asthma, epidemiology, prevalence, risk factors, mortality

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Introduction

Asthma has been described for more than 3,000 years and in recent decades it has become a major public health problem. In childhood, asthma is the most common chronic disease [1-3]. Bronchial asthma is a chronic disease that affects millions of people of all ages. It has been described that in 2019 it affected more than 260 million people worldwide and caused almost half a million deaths [1]. Therefore, around 1,150 people die in the world every day because of bronchial asthma. Most of these deaths could be avoided with a correct diagnosis and the use of the appropriate treatments currently available [1-3]. The current evidence suggests that asthma is a complex multifactorial disorder, and its etiology is increasingly attributed to interactions between genetic susceptibility, host factors, and environmental exposures [3].

Asthma is a disease with a high social impact, due to its effect on the quality of life, work absenteeism, healthcare resources use, and mortality [3]. In addition, in Spain it is estimated that 70% of the healthcare costs of asthma are due to poor control of the disease [4].

Definition

Bronchial asthma is a chronic inflammatory disease of the airways in whose pathogenesis various cells and inflammatory mediators are involved, conditioned in part by genetic factors, and which presents with bronchial hyperresponsiveness and variable airflow obstruction, totally or partially reversible, either by drug action or spontaneously [2].

Most commons manifestations of asthma are wheezing, intermittent dyspnea, cough, nocturnal cough and exercise-induced wheezing and dyspnea [2].

Two types of asthma are commonly described: allergic (extrinsic) and nonallergic (intrinsic) [2]. The main characteristics of each type of asthma are described below.

Allergic asthma is clinically characterized by appearing in childhood or youth, presenting seasonal or perennial symptoms, and having a personal and/or family history of other atopic diseases. From the immunological point of view, presents high levels of total IgE and positive specific IgE to different allergens, does not usually require the use of oral corticosteroids, does not have sinusitis, naso-sinusal polyposis or intolerance to non-steroidal anti-inflammatory drugs [2].

Non-allergic asthma is clinically characterized by appearing from the 3rd decade of life, with no personal or family history of other atopic diseases. Immunologically, presents normal total IgE levels and negative IgE to aeroallergens. Usually require cycles of oral corticosteroids, present sinusitis or naso-sinusal polyposis and are frequently associated with intolerance to non-steroidal anti-inflammatory drugs [2].

Risks Factors

The risk factors for the development of asthma are those that are related to the onset of the disease. The triggering factors of asthma symptoms are those whose exposure causes the appearance of symptoms in asthmatic patients.

Different risk factors have been described: patient-specific, perinatal and other environmental [3-18].

Patient-specific factors:

- Atopy [5].
- Premature menarche [6].
- Obesity [7].
- Bronchial hyperresponsiveness [8].
- Rhinitis [9].
- Chronic rhino-sinusitis [10].

Perinatal factors:

- Prematurity [11].
- Neonatal jaundice [12].
- Lactation [13].
- Caesarean section [14].
- Tobacco in pregnancy [15].

Environmental factors:

- Aeroallergens [16].
- Occupational allergens [17].
- Respiratory infections [18].
- Smoking [19].

Different asthma triggers have also been described [20]. It is important to know these factors to avoid them and establish possible preventive measures. These triggers can be environmental, occupational or systemic factors.

Among the environmental triggers have been described:

- Atmospheric factors (pollutant particles and pollens).
- Domestic factors (house dust mites and animal epithelia).
- Infectious agents (fungi, bacteria and viruses).

Occupational triggers include:

- Low molecular weight substances (anhydrides, diisocyanates, wood and metals).
- High molecular weight substances (flours, animal enzymes, vegetable gums, and foods).

Among the systemic triggering factors have been described:

- Drugs (antibiotics, non-steroidal antiinflammatory drugs, non-selective beta blockers).

- Food (cow's milk, eggs, nuts, cereals, fish, shellfish, plant panallergens such as profilins or LTP).
- Hymenoptera venom (*Apis mellifea, Vespula* spp., *Polistes).*

Epidemiological Studies

Epidemiology can be defined as the study of disease in human populations. Its application includes description of the occurrence and natural history of disease, investigation of the causes of disease, and evaluation of therapies and prevention program for disease [21].

The indicators of disease occurrence are:

- *Prevalence*: proportion of the population having the disease at a point in time.
- *Incidence*: rate of appearance of new cases in a time period.
- *Hospitalizations*: Number of hospital admission caused by a disease
- *Mortality*: rate at which persons die from the disease.

Different epidemiological studies have been carried out on bronchial asthma. Information is collected using different methos such as: self-reported asthma, symptom questionnaires, bronchial hyperreactivity measurement tests, or mixed methods.

It is relevant to use standardized and validated questionnaires such as those included in the European Community Health Study (ECRHS) for adults and, the International Study of Asthma and Allergy in Childhood (ISAAC) in children [2, 22, 23].

Prevalence and Incidence

As for any chronic condition the utility of prevalence is much higher than the incidence. In fact, all changes in the incidence will be reflected in the prevalence after several years. Therefore, most reports provide data on prevalence rather than incidence [1, 3].

The ideal methodology to assess prevalence would be to use specific symptom questionnaires. To confirm the diagnosis the provocation test has greater specificity and therefore would be the test of choice [2, 3, 22, 23].

However, these methods are expensive and self-reported diagnosis of asthma is very frequently used to estimate the prevalence of asthma in population-based interview surveys [3].

The prevalence of asthma ranges from 1% to 18% of the population, with great variability between countries and regions [24]**.** The disease remains under-diagnosed globally, particularly in low- and middle-income countries. According to the Global Burden of Disease Study 2019 around 262 million people worldwide had asthma [1, 3, 24].

In Spain, the prevalence varies between different regions and ages, for example from 2.5% of adults of working age in Barcelona to 13.4% of adolescents in rural areas of Navarra**,** or 6.3% in Madrid for all age groups [25-27]**.** Differences in the asthma definitions, sampling methods used, and study populations explain great variations between or even within countries [3].

Prevalence in Children

The prevalence of bronchial asthma in childhood has been studied in depth thanks to several important international groups [23, 28-31]. The International Study of Asthma and Allergies in Childhood (ISAAC). It is an excellent program that was started in 1991 to investigate asthma, rhinitis and eczema in children due to considerable concern that these conditions were increasing in western and developing countries. ISAAC includes more than 100 countries and nearly 2 million children and one of its aims is to develop environmental measures and disease monitoring [23, 29, 30].

The ISAAC findings have shown that these diseases are increasing in developing countries and that they have little to do with allergy, especially in the developing world [23, 29, 30]. Further population studies are urgently needed to discover more about the underlying mechanisms of non-allergic causes of asthma, rhinitis and eczema and the burden of these conditions [23, 29, 31].

The 20-year ISAAC program found that childhood asthma is a common disease in both high income and lower income countries. It is relatively more severe and increasing in prevalence in many lower income countries [23, 29, 30]. Some environmental factors have been identified as risk factors; therefore, it is vital to continue surveillance of asthma, research its causes and reach all asthma sufferers with good management as summarized in the Global Asthma Report 2018 [28].

Table 1. Prevalence of self-reported physician diagnoses asthma among Spanish children interviewed in the Spanish National Health Interview Surveys (SNHIS) conducted in 2006, 2011/12 and 2017, according to sex and age groups

	SNHIS 2006	SNHIS 2011/12	SNHIS 2017
Both sexes	2006 (%)	2011/12 (%)	2017 (%)
Total	6.58	5.20	4.53
0-4 years	4.08	4.44	2.61
5-9 years	7.39	5.69	4.47
10-14/15 years*	7.99	5.47	6.29
Boys			
Total	7.52	6.04	5.35
0-4 years	5.09	5.02	3.45
5-9 years	8.63	6.77	5.27
10-14/15 years*	8.63	6.30	7.10
Girls			
Total	5.58	4.32	3.66
0-4 years	3.03	3.82	1.71
5-9 years	6.13	4.50	3.62
10-14/15 years*	7.28	4.62	5.43

SNHIS. Spanish National Health Interview Survey

* Data for the SNHIS 2006 include the age group 10 to 15 years.

Data obtained from the Spanish Statistics Institute [37].

In more developed countries, a significant increase in asthma prevalence was observed in the 1980s and 1990s, with slower rates of increase in the 2000s and a plateau thereafter [32]. In this regard, asthma prevalence rates in children under 18 years increased in the United States from 2001 to 2009 (from 8.7 to 9.7 percent), and then declined, with a prevalence of 7.5 percent in 2018 [33, 34]. It is generally in English-speaking countries (Australasia, Europe and North America), and parts of Latin America where the highest prevalence (≥ 20%) are collected. In contrast, lower prevalence (< 5%) are observed in the Indian subcontinent, Asia-Pacific, Eastern Mediterranean, and Northern and Eastern Europe [35]. In Spain, there is a high prevalence of asthmatic symptoms, with an increase in adolescents and a stabilization in Spanish schoolchildren (15.3% at 13-14 years of age and 10.4% at 6-7 years of age) [36].

As can be seen in Table 1, according to the Spanish National Interview Health Surveys (SNHIS) conducted in Spain from year 2006 to year 2017 the prevalence of self-reported asthma has decreased overall and for boys and girls [37]. The prevalence is higher among boys than girls in all years analyzed and increases with age in both sexes. In the last SNHIS, conducted in year 2017 the proportion of parents that reported that their sons had been diagnosed with asthma by a physician was 5.35% and for their daughters it was 3.66% [37].

Prevalence in Adults

Three important multinational studies have been carried out, used common protocols to report comparisons of adult asthma prevalence between countries [2].

- The European Community Respiratory Health Survey (ECRHS) assessed the prevalence of asthma symptoms, asthma attacks, and the use of asthma medication in the general population aged 20-44 years in 48 centers in 22 European countries from 1991-4 [31].
- The Global Allergy and Asthma Network of Excellence (GA2LEN) survey of 15-74 year old subjects in 19 centers in 12 European countries in 2008/09 using similar methods to those in the ECRHS showed, again, marked variation in prevalence of asthma across Europe [10,38].
- In 2002/2003, the World Health Survey (WHS) assessed the prevalence of wheeze and of asthma diagnosis in adults in over 60 countries, including low- and middle-income countries. It showed wide variations in the prevalence of wheeze- and asthma regardless of overall national income [39].

Like commented for children, in Spain the prevalence of self-reported physician diagnosed asthma has been collected by the SNHIS, and by the European Health Interview Surveys for Spain (EHISS) [37, 40]. The results of these surveys can be found in Table 2. Unlike found for children, women have higher prevalence than men (EHISS2020 4.59% vs. 3.44%). The prevalence of asthma among adults seems to have remained stable over time.

Table 2. Prevalence of self-reported physician diagnoses asthma among Spanish adults interviewed in the Spanish National Health Interview Surveys (SNHIS) conducted in 2006, 2011/12 and 2017 and the European Health Interview Surveys for Spain (EHISS) conducted in 2014 and 2020, according to sex and age groups

Both sexes	2011/12 SNHIS (%)	2014 EHISS (%)	2017 SNHIS (%)	2020 EHISS (%)
Total	4.08	4.37	4.68	4.03
15-24 years	4.72	3.95	5.22	4.47
25-34 years	4.49	4.80	5.31	5.10
35-44 years	4.03	4.71	4.19	3.66
45-54 years	2.97	3.02	3.98	3.52
55-64 years	3.38	4.19	3.78	3.25
65-74 years	4.33	5.06	4.79	3.89
75-84 years	4.72	5.81	6.84	4.62
≥85 years	6.97	4.43	5.68	5.86
Men				
Total	3.38	3.90	3.74	3.44
15-24 years	4.67	3.81	3.45	4.46
25-34 years	3.61	5.11	4.31	4.16
35-44 years	4.12	3.91	4.36	3.53
45-54 years	1.80	2.46	2.70	2.95
55-64 years	2.28	2.93	2.65	2.12
65-74 years	3.18	4.15	4.15	3.62
75-84 years	3.99	6.73	5.36	4.05
≥85 years	6.05	4.68	5.42	3.56
Women				
Total	4.75	4.82	5.57	4.59
15-24 years	4.76	4.10	7.08	4.48
25-34 years	5.40	4.49	6.30	6.06
35-44 years	3.95	5.54	4.02	3.79
45-54 years	4.12	3.58	5.25	4.10
55-64 years	4.42	5.40	4.86	4.32
65-74 years	5.32	5.86	5.36	4.11
75-84 years	5.22	5.15	7.92	5.09
≥85 years	7.46	4.29	5.81	7.09

Data obtained from the Spanish Statistics Institute [37, 40].

Hospitalizations

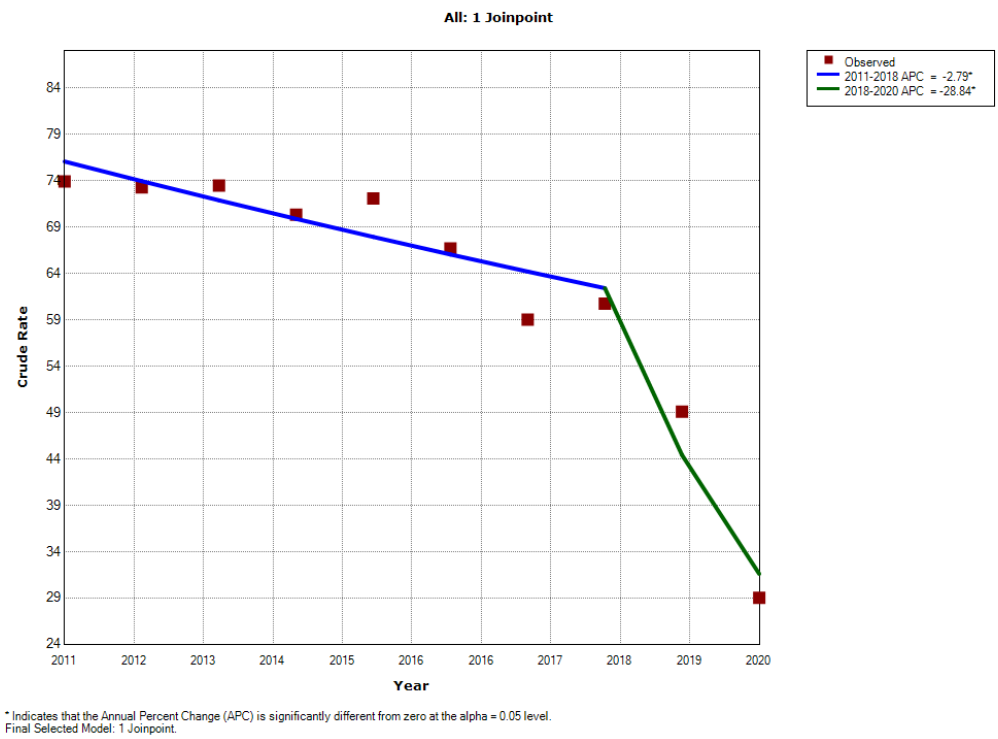
Data from hospital admissions for asthma are one of the best sources of information on the evolution of asthma control in a territory [41, 42]. One of the key sources of information in this regard in Spain is the Spanish National Hospital Discharge Database (SNHDD), which provides information on multiple variables and indicators for the analysis of hospitalizations with a diagnosis of asthma at the national level [42].

The time trend in hospital admission for asthma exacerbation among Spanish children in Spain from 2011 to 2020 are shown in Figure 1.

As can be seen in Figure 1A, the incidence of hospitalizations declined significantly in children, with an annual percentage of change of 2.79% from 2011 to 2018 and, of 28.84% from that year until 2020. The trend found for boys and girls is almost identical (Figures 1B and 1C), showing a constant reduction overtime that became more intense from 2018 onwards.

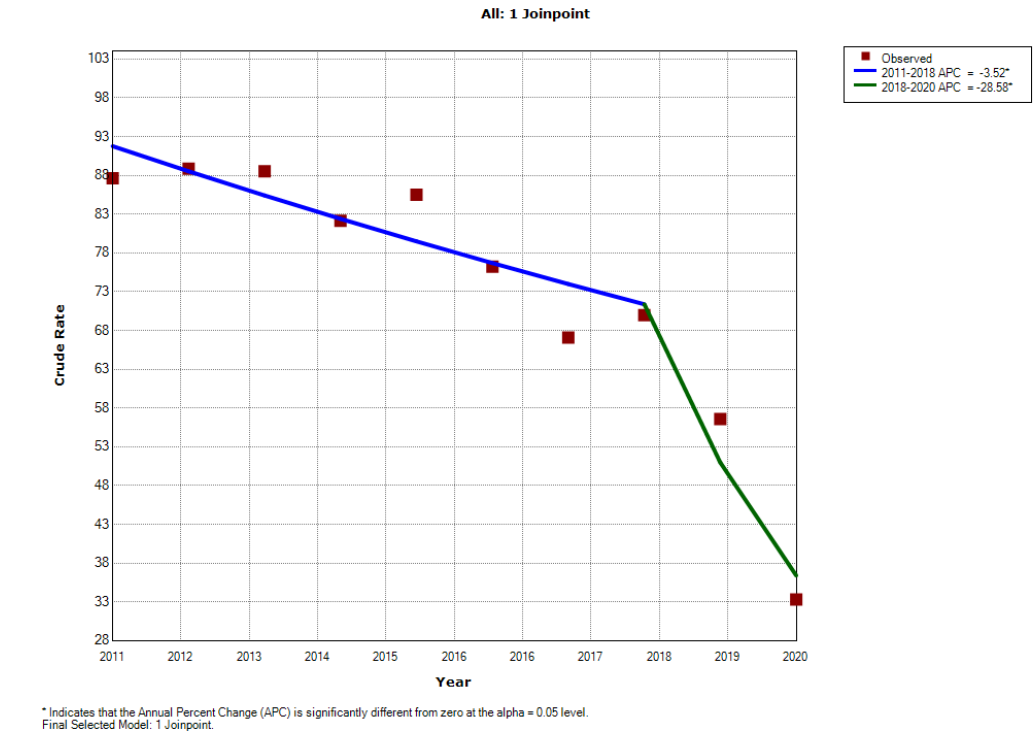
In Figure 2 are shown the trends of asthma exacerbations hospitalizations among adults from 2011 to 2020 according to sex.

Even if a decrement is also observed for men and women the reduction seems to be smaller than among children.

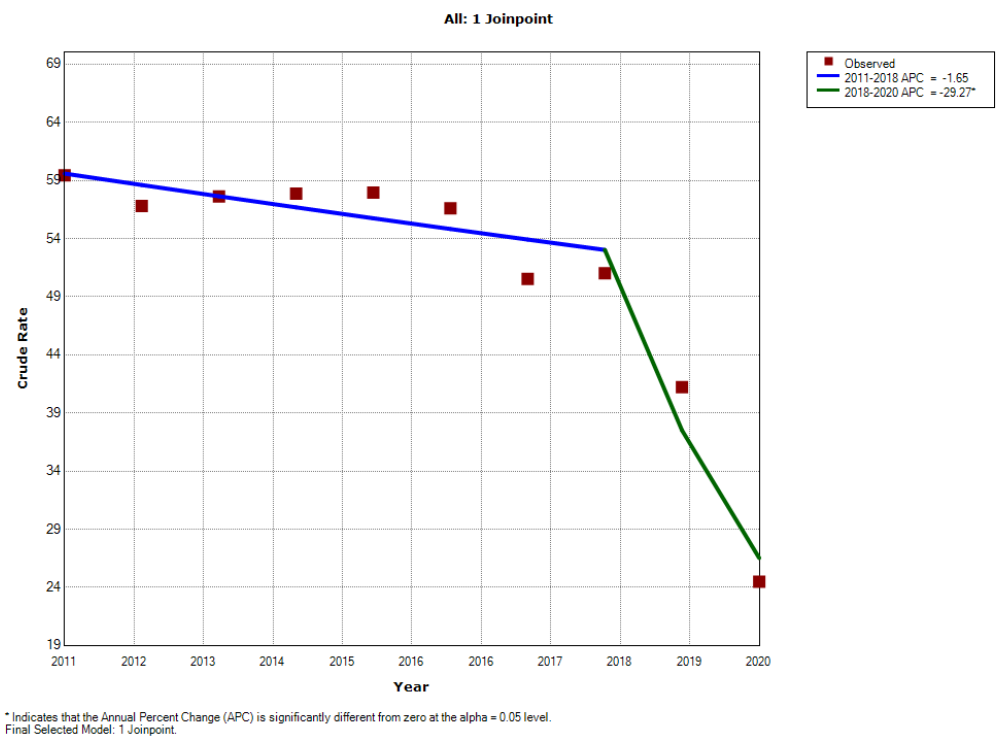


A

Figure 1. (Continued).

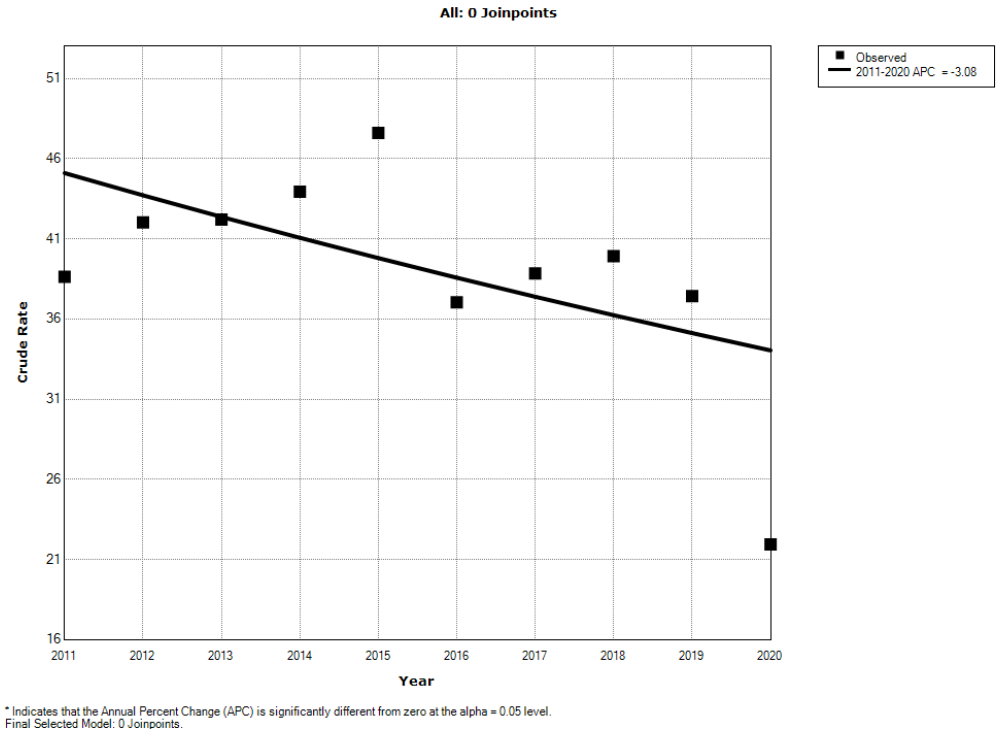


B

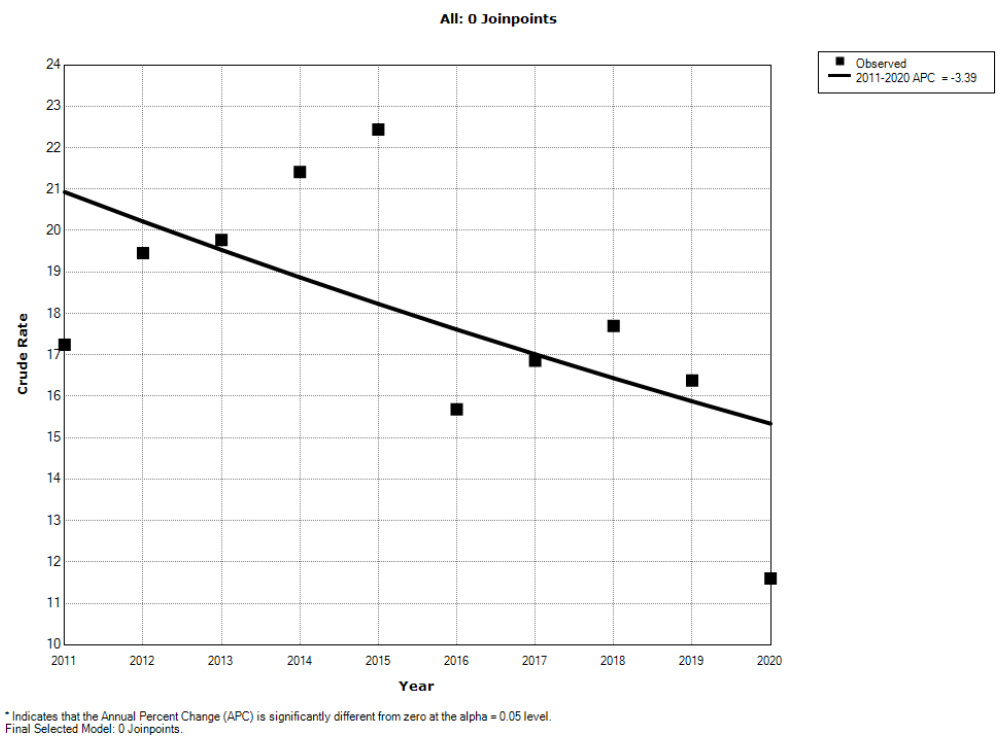


C

Figure 1 (A, B, C). Time trends in the incidence of hospitalizations for asthma exacerbations among Spanish children from 2011 to 2020 according to sex (1A both sexes, 1B boys, 1C girls). Data of the Spanish National Hospital Discharge Database.

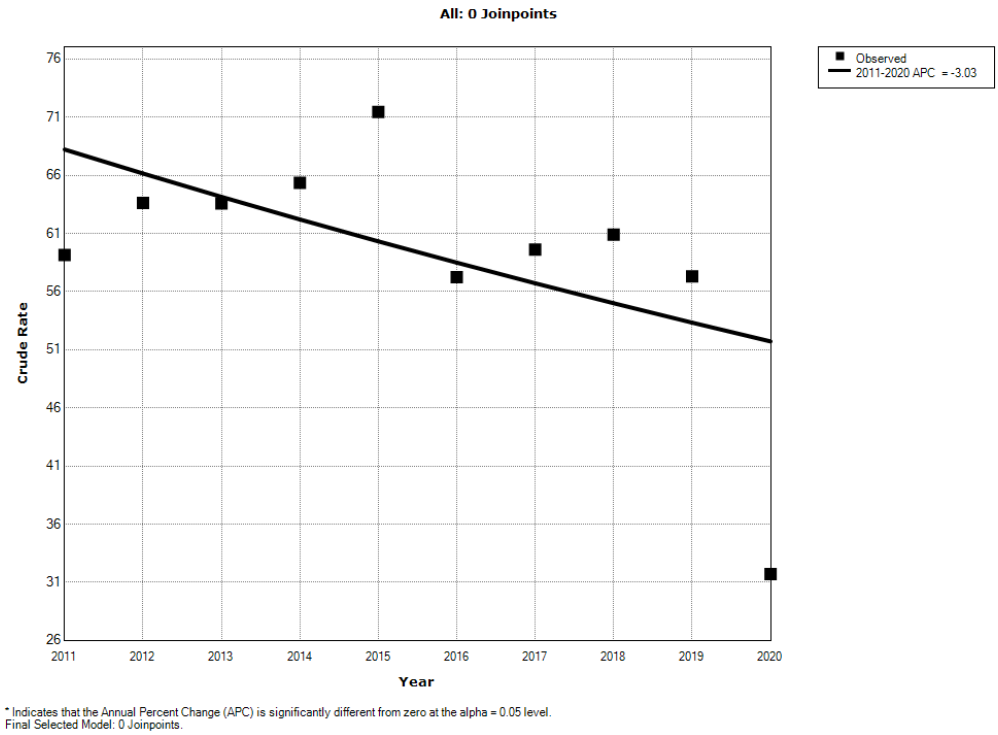


A



B

Figure 2. (Continued).



C

Figure 2 (A, B, C). Time trends in the incidence of hospitalizations for asthma exacerbations among Spanish adults from 2011 to 2020 according to sex (2A both sexes, 2B men, 2C women). Data of the Spanish National Hospital Discharge Database.

Similar trends in hospitalizations are found in studies conducted in children and adults in high and low income [43-48]. Reasons suggested for this reduction in hospitalizations include:

i) The higher availability of asthma medication
ii) The emergence of biological therapies in the treatment of this disease that has meant an important change in the management of patients with severe asthma, who are the ones with the highest number of exacerbations and hospitalizations, and
iii) The value of international and national clinical practice guidelines, which optimize asthma management and are frequently updated with available evidence, is not negligible either, probably contributing to avoid hospitalizations [43-48].

Mortality

Asthma is characterized by a high morbidity and relatively low mortality compared with other chronic diseases. Mortality secondary to asthmatic pathology in children is rare, ranging between 0.1 and 0.7/100,000 in different parts of the world [49, 50]. It's also found differences in asthma mortality rates based on sex and age, with results almost several times higher in adults

compared to children, and in the latter, it is observed that the rate in boys is higher than that of girls [49-51].

In the United States the rate of asthma deaths decreased from 15 per million in 2001 (n = 4,269) to 10 per million (n = 3,518) in 2016. Adults were nearly five times more likely than children to die from asthma. The asthma death rate was highest among the 65 years and older age group compared with all other age groups [52].

In Spain, according to the National Statistics Institute, the age-standardized asthma mortality rates decreased from 7.38 for the period 1980-1984 to 2.03 deaths per 100,000 for the period 2015-2019 [53].

In a study carried out in Australia, between 2004-2013, in which numerous factors associated with mortality in asthmatic children were collected, it was found that 90% of them were atopic, 70% had a family history of allergy or atopy in a first-degree relative, 70% were male and 55% were 10 to 14 years old. A close family member or caregiver smoked in 35% of cases, and 55% had psychosocial problems [54].

The variations observed in the different countries depending on the economic conditions of the patients and their families should call the attention of the political and health authorities. Bronchial asthma is a treatable disease and therefore most deaths could be prevented [49-52].

Asthma and COVID-19

People with asthma do not appear to be at increased risk of acquiring COVID-19, and systematic reviews have not shown an increased risk of severe COVID-19, hospitalizations or mortality in people with well-controlled, mild-to-moderate asthma [55-59].

Overall, studies to date suggest that people with well-controlled asthma are not at increased risk of COVID-19- related death [55-59].

Conclusion

Asthma is an important public health problem that affects people of all ages and from all countries of the world. It leads to morbidity, loss of quality of life, work absenteeism, healthcare resources use, and mortality, all of these resulting in an important economic cost. This is relevant considering that it is a disease that can be adequately diagnosed and treated.

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Chapter 2

Immunology of Allergic Asthma and T2 Inflammation in Allergic Asthma

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Abstract

Allergic asthma is a condition characterized by a type 2 chronic inflammatory response, conditioned by the interaction of multiple elements. The basis of the inflammatory response begins in the bronchial epithelium, where the interaction of infections, microbiota, allergen exposure and epithelial damage with the rupture of the intercellular junctions will lead to the release of pro-inflammatory cytokines like alarmins [thymic stromal lymphopoietin (TSLP), interleukin (IL) IL-25 and IL-33].

The type of bacterial species that make up the microbiota will have a protective or harmful effect on the bronchial epithelium, something that has been demonstrated using murine and human models and comparing asthmatic patients with healthy individuals and comparing asthmatic patients with different degree of asthma severity. These alarmins and cytokines cause the activation of innate lymphoid cells type 2 (ILC2) and promote the antigenic presentation of allergens by dendritic cells, stimulating the proliferation of T lymphocytes (Th2) that will coordinate the immune response and B lymphocytes that will secrete specific immunoglobulin E (IgE) antibodies that will bind to the effector cells, mast cells and basophils.

Allergens can induce inflammation through an IgE-mediated mechanism, but also through non IgE-dependent mechanisms, since many of them are active enzymes such as proteases, capable of destroying the integrity of the intercellular junctions of the epithelium.

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Although type 2 (T2) adaptive responses are orchestrated by Th2 cells, new types of lymphocytes have been described both in innate [natural killer (NK) cells, invariant natural killer T (iNKT) cells and mucosal associated invariant T (MAIT) cells] and adaptive responses (Th9 lymphocytes). These cells modulate the inflammatory response and could represent new therapeutic targets.

Many T2 cytokines (IL-4, IL-5, IL-13) and T2-related inflammatory mediators (periostin, prostaglandin-D2, and exhaled nitric oxide) have been studied regarding their usefulness as asthma biomarkers and as therapeutic targets. The use of anti-IL-5 and anti-IL-4 receptor monoclonals has helped to understand their interaction with different cells of the immune system and their effect on airway remodeling.

FOXP3+ Tregs play a vital role in modulating and regulating immune responses by inducing immune tolerance and inhibiting toxic inflammatory reactions; regulatory responses are essential to maintain routine tissue repair. Asthmatic patients have been reported to show decreased FOXP3 protein expression within their CD4+ CD25 high regulatory T cell repertoire, highlighting the potential therapeutic value of Tregs to reverse established allergen-induced pulmonary inflammation.

Keywords: immunology, allergic asthma, T2 inflammation, epithelium, microbiota, allergen, mast cell, basophils, eosinophil, Tregs, T2 cytokines

Introduction

The knowledge of immunology of asthma has been redefined in recent years. Both a humoral and a cellular response are known to be involved in the immunology of asthma, leading to a state of airway hyperreactivity (AHR). Nowadays, many publications have shown an increase in the number of cells of the immune system that are involved in the inflammatory cascade of asthma, including, in addition to the T and B lymphocytes of the adaptive response, cell types belonging to the innate response. These innate cells include airway epithelial cells, macrophages, dendritic cells, mast cells, neutrophils, eosinophils and basophils. Also, different lymphoid cells such as innate lymphoid cells (ILC), natural killer (NK) cells, invariant natural killer T (iNKT) cells and mucosal-associated invariant T (MAIT) cells along with the different subpopulations of T and B cells and a network of cytokines, chemokines and their corresponding co-stimulatory and regulatory signals are known to orchestrate this process [1-2].

Asthma can be divided into two main endotypes based on cell subpopulations and network of cytokines involved: T2 (or high T2) asthma and non-T2 (or low T2) asthma. The first type of asthma, T2 asthma, includes allergic and non-allergic eosinophilic asthma, and the second type, or non-T2 asthma, includes neutrophilic and paucigranulocytic asthma. Almost all new biological treatments available are aimed at high T2 asthma [3-5].

T2 Asthma (Or High T2)

Classically, a large percentage of patients with asthma show a pathophysiological pattern mediated by Th2 lymphocytes. The main clinical characteristics of this asthma are the association with atopy, allergens, eosinophilic inflammation, and good response to corticosteroids. This type of patients shows a predominance of Th2 responses, present in both

early and late-onset responses. Currently, the term T2 asthma instead of Th2 asthma is favored, since the quintessential Th2 cytokines, IL-4, IL-5 and IL-13, among others, are produced not only by CD4+ T lymphocytes (Th2), but also by other cells of the innate immune system such as mast cells, eosinophils, basophils, and type 2 innate lymphoid cells (ILC2) [4-6].

Early-onset T2 asthma includes what was previously called allergic asthma and is considered the most common type of asthma. It is generally induced during childhood by a type I hypersensitivity response after a sensitization to environmental allergens. The main environmental aeroallergens are house dust mites (HDM), grass pollen, weed and tree pollen, fungal spores and animal dander [7].

In a type I hypersensitivity response, sensitization is the first process that occurs, which leads to the production of specific immunoglobulin E (IgE) against allergens in patients with a genetic predisposition (atopic). This phase is asymptomatic. The allergic disease would become clinically apparent on subsequent exposures to the allergens that would activate the effector response [8].

The initiation of type I hypersensitivity reactions is triggered by the different allergens and begins with the activation and differentiation of allergen specific Th2 lymphocytes and *T follicular helper cells* (Tfh) in the regional lymph nodes that lead to IgE production against these allergens. In a later phase, this IgE migrates to the respiratory tissue and sensitizes effector cells as mast cells and basophils through the high-affinity IgE receptor. In a new exposure to these allergens in the peripheral tissues, these cells are activated, and their mediators (cytokines and chemokines) are released, which will recruit other cells, initiating an inflammatory response that ends up causing asthma symptoms [8].

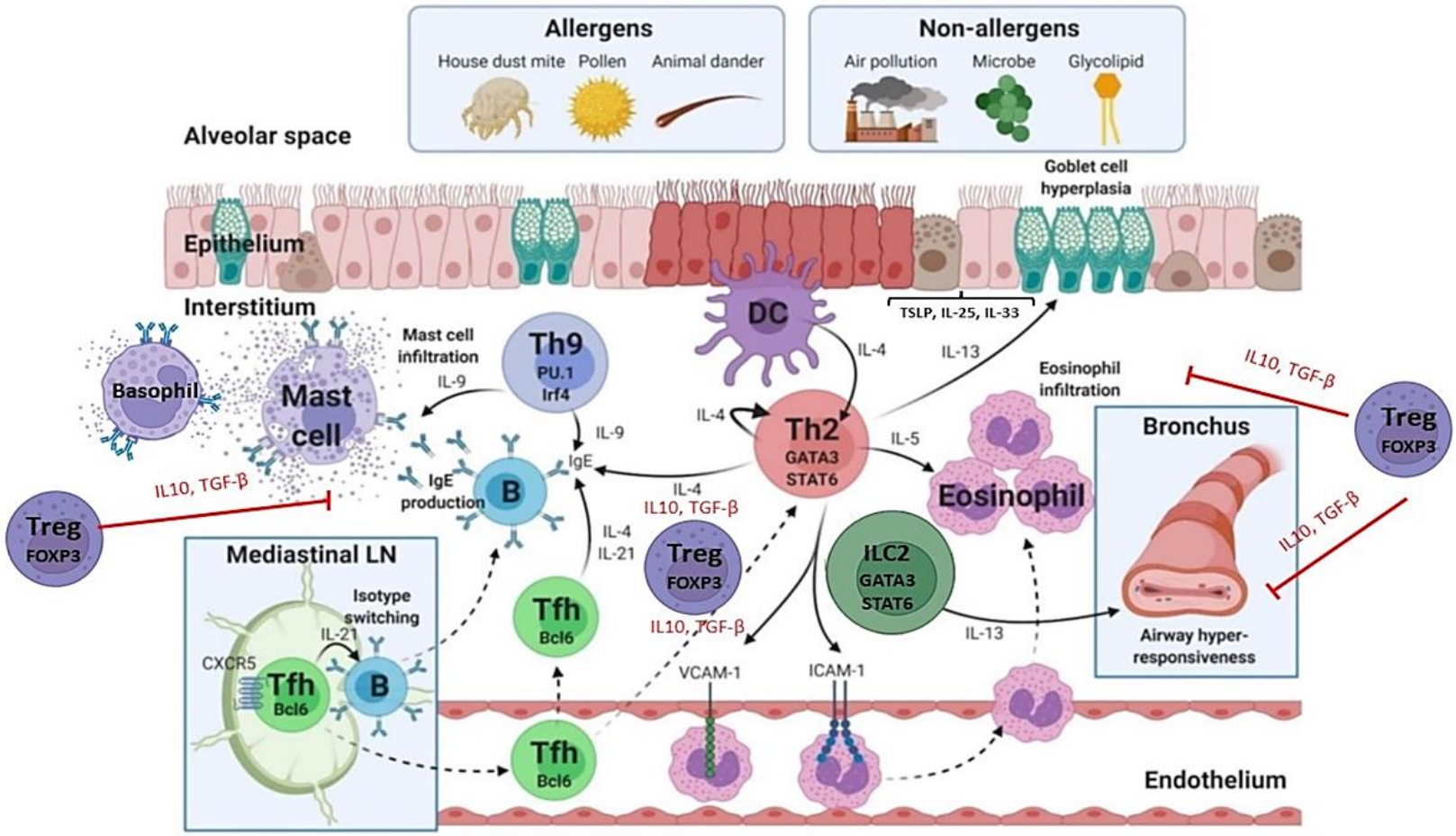
The main components and mediators involved in this early-onset T2 asthma are in Figure 1.

Lung Microbiota

It is estimated that more than 10,000 different microbial species live on humans, representing 100 billion organisms. This so-called human microbiota (microbiome if referred to the codifying genes) in holds a commensal symbiotic relationship with the human host and is thought to be essential for many host functions, including immune regulation [9].

Microbiota colonization during the first days of life is as important as dysregulation in later stages. The impact of airway bacteria on the development of asthma may be related to the early establishment of bacterial colonies. Furthermore, colonization by certain bacteria strains has been associated with increased blood eosinophil counts and elevated total IgE at 4 years of age [10].

The lung microbiota may contribute not only to the presence of asthma, but also to the phenotypes and severity of asthma [11, 12]. Some bacterial exposures showed to be protective against a type 2 airway response in mouse models of ovalbumin-induced allergic asthma by toll-like-receptor-4 (TLR4)-dependent induction of γδT cells, decreased activation of dendritic cells in the lung, and decreased production of T2 cytokines, which collectively protected mice from allergic airway inflammation [13]. Recent studies have expanded our understanding that dysbiosis of the lung microbiota can play an important role in the pathogenesis of asthma and bacterial composition in the airway is associated with disease severity in asthmatics [11, 12].



Modified from Jeong, J.; Lee, H. K. The Role of CD4+ T Cells and Microbiota in the Pathogenesis of Asthma. *Int. J. Mol. Sci*. 2021, 22, 11822. https://doi.org/10.3390/ijms222111822.

Figure 1. The main cells and mediators involved in T2 allergic asthma.

The Lung-Gut Axis

It has been shown that the lung-gut axis correlates with the pathogenesis of asthma. Epidemiologic studies have revealed several potentially protective environmental factors, such as growing up on a farm, vaginal birth, breast-feeding, the presence of household pets, birth order, and the number of siblings, as well as an increased risk of asthma being associated with antibiotic use during late pregnancy and the first year of life. These factors are strongly associated with gut dysbiosis [14].

The mechanism by which gut microbiota influence the initiation and development of asthma, however, remain largely unknown. Microbes, bile salts and other immune stimuli from the digestive tract might play a vital role in mucosal immunity of the respiratory system [15]. Increasing evidence suggests that gut microbiota has an important role in coordinating both the innate and adaptive immunity that is involved in the development of asthma, whereas the underlying molecular mechanisms need to be further identified [16]. The epithelial mucosa and dendritic cells, as well as antimicrobial peptides secreted by immune cells, are major effectors in the response to environmental agents in the airway lumen [17]. The epithelium controls the local respiratory immune activities producing several cytokines called alarmins: thymic stromal lymphopoietin (TSLP), IL-25 and IL-33; which may lead to a T2 type inflammation, thus facilitating the development of asthma [18].

Given the role of gut microbiota, whether manipulation of gut microbiota represents a promising therapeutic strategy for lung diseases has been validated by increasing clinical and experimental studies. Interventions including antibiotics, probiotics, prebiotics, and natural products or diets that target gut microbiota have been attempted in subjects with lung diseases, including asthma. Some have shown a suppression of various immune effector cells [19]. More studies are needed to define the efficacy of such measures.

Epithelial Cells

The function of the airway epithelium goes beyond its classic barrier function. The barrier function of the lung epithelium is mainly provided by the so-called “tight junctions” (TJ) or hermetic junctions. These TJ are formed by complexes of different proteins that form the sealing interface between adjacent epithelial cells. In human adults they transport approximately 8000 liters of air per day between the environment and alveoli while forming an effective barrier against microorganisms and/or different particles either inspired from outside or aspirated from the digestive system.

The epithelial surface lines the entire airways, from the nasal cavity to the lower respiratory tract. In larger proximal airways, with a pseudostratified columnar epithelium, cells come into contact with the basement membrane, but not all are contiguous with the airway lumen. In smaller airways, the epithelium becomes columnar and cuboidal [20].

In addition to its barrier role, the epithelium is a central player in the initiation of innate and adaptive immune responses in the airways. Some of the theories that support the preponderant role of the epithelium in the development of asthma suggest that the characteristics of this epithelium, its immaturity and fragility, would be responsible of the initiation of the disease. In a second stage, due to their destructuration and dysfunctionality, allergens, pollutants, and other harmful elements would be capable of inducing a chronic

inflammatory response, frequently of the T2 type, and in many individuals, inducing allergic sensitization.

Airway epithelial cells respond to various environmental stimuli. Epithelial pattern recognition receptors (PRRs) recognize highly conserved pathogen-associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPS), that include alarmins. PRRs include toll-like receptors (TLRs) that recognize cell surface pathogens, intracellular TLRs, and nucleotide-binding and oligomerization domain (NOD) receptors that recognize intracellular pathogens. Activation of these receptors causes the release of a series of host defense effector molecules including antimicrobial factors (lysozyme, defensins, collectins, and pentraxins), antiviral cytokines (interferons), eicosanoids, peptidases, nitric oxide, and proinflammatory cytokines (tumor necrosis factor α:TNF-α, IL-1, IL-6) as well as granulocyte-macrophage colony-stimulating factor (GM-CSF) [20,21].

The epithelium releases cytokines called alarmins (TSLP, IL-25 and IL-33) that attract and activate dendritic cells and T cells, placing epithelial cells at an important interface between innate and adaptive immune responses [21].

IL-25 and IL-33 directly activate innate lymphoid cells to produce T2 cytokines. Certain viral infections, such as rhinoviruses, can also induce IL-33 and promote T2-type inflammation [22]. IL-4 and IL-13 can cause epithelial barrier dysfunction of the human respiratory tract. IL-13-producing ILC2s can significantly impair the epithelial barrier of human bronchial epithelial cells. TSLP-stimulated CD11c+ dendritic cells (DC) can activate memory CRTH2+ (Chemoattractant receptor-homologous molecule expressed on TH2 cells) Th2 lymphocytes causing increased Th2 polarization amplifying allergic inflammation. In addition, TSLP is a hematopoietic differentiation and proliferation factor for B lymphocytes. TSLP can also act directly on CD4+ T lymphocytes and CD8+ "naive" cells to become Th2 and Tc2 lymphocytes, respectively. TSLP increases the expression of GATA3 in human ILC2 and induces the production of IL-4 and other T2 cytokines. TSLP can significantly exacerbate eosinophilic inflammation, promoting eosinophil activity and chemotaxis by delaying eosinophil apoptosis, upregulating CD18 and ICAM-1 adhesion molecule expression, and downregulating L-selectin [23].

Periostin is an extracellular matrix protein that is secreted by activated airway epithelial cells. Its gene expression is upregulated in bronchial epithelial cells by IL-13 and IL-4. It acts on fibroblasts promoting airway remodeling, increasing mucus secretion and collaborates in recruiting eosinophils. Periostin has been strongly positioned as an emerging biomarker of asthma, being associated with eosinophilic inflammation and the T2-high molecular phenotype, because of a clinical trial with lebrikizumab (anti-IL-13) in 2011, in which the best responders were patients with high pretreatment periostin levels. However, periostin has yet not met such high initial expectations, especially as a substitute for bronchial eosinophilia. Periostin also lacks specificity for asthma, and it seems that it does not discriminate healthy subjects from asthmatic patients with a clear cut-off point. However, periostin is positioning itself in the recent years as a possible biomarker of airway remodeling, measurable by non-invasive methods [24].

Allergens

Allergens are capable of inducing inflammation through an IgE-mediated mechanism (dependent on adaptive immunity: dendritic cells, Tfh, Th2, and B lymphocytes), but also

through mechanisms independent of this immunoglobulin. Many allergenic sources, such as house dust mites, animal dander, spores of fungi or pollens, contain active proteases that can break the integrity of the intercellular junctions of the epithelium (i.e., the hermetic junctions). This facilitates the penetration of substances, but also modulates the activation of the innate and acquired immune response. The activity of these proteases is critical for their allergenicity. Allergenic proteases can activate the release of pro-inflammatory cytokines through the activation of protease-activated receptors (PAR), such as PAR-2, which are located on epithelial cells [7].

Some allergens, such as house dust mites, major sensitizer in many geographical areas, are inhaled together with particles of bacterial and fungal origin, such as lipopolysaccharides (LPS) or endotoxins. These LPS have the capacity to activate the TLR-4 of the epithelial surface, triggering the production and release of multiple cytokines and chemokines (IL-6, CCCL2 and 20, TSLP, GM-CSF, IL-25 or IL-33) capable of inducing the recruitment and activation of basophils, eosinophils, mast cells and dendritic cells, and shifting the immune response to a T2 profile. Thus, either through PAR-2 and TLRs, or through activation of the immune system through IgE, the result is a characteristic T2 inflammatory profile [25].

Dendritic Cells (DCs)

Airway dendritic cells (DCs) play a crucial role in allergic responses because they are able to capture (FcεRI is expressed in DC membrane) and process allergens, transport them to regional lymph nodes, and help generate an allergen-specific Th2 cell response [26]. Therefore, the regulation of dendritic cells is a key factor in the allergic response. Although DCs can be activated by direct interaction with the allergen, it is recognized that their functional activation critically depends on interactions with other cells of the innate immune system [27]. The DC lineage consists of two main types: conventional DC (cDC) and plasmacytoid DC (pDC). Lung conventional dendritic cells are a heterogeneous cell population and comprise two different cDCs; conventional dendritic cells type 1 (cDC1s) and type 2 cDCs (cDC2s). Compared to other DC subsets, cDC1 have less capacity to process allergens and are associated with tolerance generation. In contrast, cDC2s are important for the induction of cell differentiation of both Th2 and Th17 lymphocytes in a murine model of mite-induced asthma. Different studies show differences in the frequency of these subtypes of dendritic cells in the lung and in the development of allergic asthma due to their different capacity to induce Th2 responses [28].

Th2/Th9/Tfh Lymphocytes

Type 2 helper (Th2) lymphocytes are CD4+ lymphocytes belonging to the adaptive immune response that have traditionally been implicated in allergic asthma. The generation of allergen specific Th2 cells is a complicated process that requires numerous interactions between various cell types in the lungs and regional lymph nodes. It occurs in two phases: a sensitization phase and an effector phase, that in turn has an early and a late phase [28].

Th2 lymphocytes are cells that produce T2 cytokines, mainly IL-4, IL-5 and IL-13, considered crucial in the pathophysiology of allergic asthma.

IL-4 is a cytokine that induces differentiation of "naive" or virgin T lymphocytes into Th2 lymphocytes, an important process in the development of a type I hypersensitivity response. After activation by IL-4, Th2 lymphocytes subsequently produce additional IL-4 in a positive feedback loop. The cell that initially produces IL-4, which induces Th2 differentiation, has not been identified, but recent studies suggest that basophils may be responsible of such initiation. IL-4 is closely related to and has similar functions to interleukin 13. The IL-4 receptor shares a chain with the IL-13 receptor. IL-4 has many biological functions, including the stimulation of activated B lymphocytes and the proliferation of T lymphocytes. It is also involved in the differentiation of B lymphocytes into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces the class switch of B lymphocytes to IgE producer plasma cells [23].

IL-5 is the main cytokine responsible for the maturation and release of eosinophils from the bone marrow as well as their chemotaxis to lung tissue. It is a cytokine produced mainly by Th2 lymphocytes and mast cells [23].

IL-13 exhibits secondary structure characteristics similar to that of IL-4; however, it has only 25% sequence identity with IL-4 and is capable of IL-4-independent signaling. IL-13 is a cytokine secreted by Th2 cells, other CD4+ cells, NKT cells, mast cells, basophils, eosinophils, and ILC2. IL-13 is a central regulator of IgE synthesis (since it induces the proliferation of IgE-producing B lymphocytes) and is also involved in goblet cell hyperplasia, mucus hypersecretion, bronchial hyperreactivity, and fibrosis [23].

The concentration of other cytokines such as IL-9, IL-31 and IL-31R, along with SCF (stem cell growth factor) produced by Th2 lymphocytes are also increased in the serum of patients with allergic asthma. In Th2 lymphocytes of asthmatic patients, an increase in chemokine receptors such as CCR4, CCR8, CXCR4 and CCR3 can also be observed [23].

CCR4 regulates the chemotaxis of Th2 lymphocytes and its ligands CCL17 and CCL22, which are increased in patients with allergic asthma.

CCR8 can induce eosinophilia and AHR and can be elevated in Th2 cells in the lungs and airways of allergic asthmatics.

CXCR4 participates in the attraction of Th2 lymphocytes to the lungs. In murine models, treatment with selective inhibitors of CXCR4 has been shown to significantly reduce AHR and the inflammatory response [29].

Type 9 helper T (Th9) cells are closely related to Th2 cells and may differentiate from naïve T cells through a switch from Th2 cells. They mainly produce IL-9, another T2 cytokine, which shares the potential to induce eosinophilic inflammation, mucus hypersecretion, and bronchial hyperreactivity. Patients with allergic asthma have higher numbers of Th9 lymphocytes and a higher concentration in peripheral blood. IL-9 decreases IFN-γ production, a classical T1 cytokine, and synergistically promotes IL-4-induced IgE secretion. Although it has been shown that IL-9 plays a role in steroid-resistant asthma, a study with an anti-IL-9 monoclonal antibody therapy (MEDI-528) did not demonstrate efficacy in controlling symptoms and reducing exacerbations in a group of asthmatic patients [29].

T follicular helper cells (Tfh) are localized in B-cell follicles in the secondary lymphoid organs and are responsible for regulating antibody isotype switching, affinity maturation and B-cell memory generation. They are characterized by the high expression of CXC chemokine receptor 5 (CXCR5), programmed cell death protein 1 (PD-1), B-cell lymphoma 6 (Bcl-6), and IL-21 in both mice and humans [30]. Recent studies on mice and humans have revealed that IL-4+ Tfh cells are required for IgE production [31]. More recently, it has been shown that IL-13-producing Tfh cells, having both IL-4 and IL-13 production, are responsible for the

production of high -but not low-affinity IgE in house dust mite (HDM)-sensitized mice [32]. Studies performed in asthmatic patients during exacerbations have showed that differentiation of Tfh cells was enhanced in acute exacerbation of asthma patients and ameliorated after treatment, implying their involvement in the allergic inflammatory response [33].

Regulatory T Cells (Tregs)

FOXP3+ Tregs have emerged as an important cell type with the potential to mediate targeted immunosuppression and are key cells in maintaining the homeostatic balance during dysregulated immune responses, which is a critical feature of asthma inflammation [27].

Tregs are generated in the thymus as a functionally mature T cell subset, while in the periphery they derive from naive T cells. They are crucial in maintaining immunological unresponsiveness to self-antigens, and suppressing heightened immune responses destructive to the tissue during asthma inflammation. Tregs play a vital role in modulating and regulating immune responses by generating immunotolerance and inhibiting toxic inflammatory reactions, essential to maintain routine tissue repair [34].

Tregs (CD4+/CD8+) are characterized by intracellular expression of FOXP3, and secrete various key regulatory cytokine, which include IL-10 and Transforming Growth Factor β (TGF-β) to suppress heightened immune responses and trigger inducible Treg expansion. Treg-mediated immunosuppression mainly operates through the secretion of suppressive soluble factors (IL-10, TGF-β, IL-35, fibrinogen-like protein 2, CD39, and CD73), cell contact-mediated suppression (through galactin-1, CTLA-4, LAG-3), and competition for growth factors (i.e., IL-2). IL-10 mainly suppresses the effects of pro-inflammatory cytokines, restores epithelial layer integrity, tissue healing, and inhibits the survival and migration of eosinophils during allergic inflammation [34].

IL-10 also down-regulates IL-4 induced isotype switching of activated B-cells. Besides, Tregs have been associated with the maintenance of immune responses, and secreted immunosuppressive cytokines such as TGF-β, IL-10, and IL-35 are involved in immune responses following antigens/allergen exposure. IL-10 can subdue the release of major pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, and Tumor Necrosis Factor α (TNF-α) produced by Th1 cells, activated T helper cells, mast cells, NK cells, endothelium, eosinophils, and macrophages. In addition, IL-10 augments IgG4 release, which plays a key protective role in allergic responses and inhibits IgE production. Decreased IL-10 has been observed in allergic and asthmatic patients compared with healthy controls [35].

In addition to the cytokine-mediated suppressive activity, Tregs also mediate suppressive functions through the release of perforin and granzyme B and the release of cyclic adenosine monophosphate (cAMP). Such studies proved the therapeutic value of Treg to resolve established allergen-induced pulmonary inflammation (eosinophilia, Th2 infiltration, IL-5, IL-13, and TGF-β), and to prevent the progression of airway remodeling, reduce mucus hypersecretion and peribronchial collagen deposition [36].

Studies in mice, subsequently reproduced in humans, have shown how androgen Treg modulation is important in asthma inflammation and could explain sex differences in asthma. These studies showed that 5α-dihydrotestosterone (DHT), an androgen, decreased ST2/IL-33 receptor expression in lung, Tregs and decreased IL-33 secretion in human bronchial epithelial

cells. These findings showed that androgen receptor signaling stabilized Treg suppressive function [37].

Unconventional T Cells: NKT Cells (Natural Killer T Cells), MAIT Cells (Mucosal Associated Invariant T Cells) and Tγδ Lymphocytes

NKT cells represent a subset of T lymphocytes but are involved in the innate response. NKT cells are activated by binding to the nonclassical major histocompatibility complex (MHC) class I molecule CD1d and include several subpopulations, notably CD4+ and CD4−CD8− (DN) cells. One subtype of these cells are iNKT (invariant natural killer cells). iNKT cells and MAIT cells respond rapidly to antigens bound to CD1d and MHC class 1 molecules, respectively, and immediately exert effector functions by releasing various cytokines and granules. Up to 60% of CD4+ T cells present in bronchoalveolar lavage (or induced sputum) from severe asthmatic patients are iNKT cells. These cells produce type 2 cytokines, IL-4 and IL-13 [38].

MAIT cells are abundant in peripheral blood and comprise 10% of T cells in the pulmonary mucosa. Although their exact role in asthma remains to be defined, their numerical deficiency correlates with the severity of the disease [38].

Tγδ lymphocytes are resident cells in mucous membranes; in the lung they have been characterized as double negative CD4 and CD8 cells: Lung resident Tγδ lymphocytes are potent producers of IL-17 and have been associated with neutrophil recruitment in exacerbations. However, in asthmatic patients they are able to increase the production of IL-4 and reduce the production of IFN-γ. Nonetheless, their exact role in asthma remains to be defined [39].

Immunoglobulin E

IgE antibodies that are generated after a type I hypersensitivity response are primarily responsible for the "early phase" of an allergic reaction but considered to play a minor role in the "late phase". The biological role of IgE is complex and is related to its ability to influence the functions of various immune and structural cells that have been involved in the pathogenesis of chronic allergic inflammation through specific receptors, such as the high affinity IgE receptor (FcεRI) and low affinity receptor (CD23 or FcεRII) [40].

FcεRI is constitutively expressed by mast cells and basophils, although in inflammatory conditions such as asthma it has been shown that other cells such as dendritic cells (DC), airway smooth muscle cells, epithelial cells, endothelial cells, neutrophils, and eosinophils may also express it. This high affinity IgE receptor is expressed in an inducible way under certain circumstances. Instead of the tetramer described in mast cells and basophils (αβγγ), it may be a trimer without the β chain (αγγ) and its function is probably different. For example, on dendritic cells (DCs) IgE facilitates antigen presentation by DCs. The allergen captured by DCs binds to FcεRI receptors and is then presented to memory Th2 lymphocytes. IgE bound to DC leads to up to a 1000-fold increase in T cell activation. This process is called IgE-facilitated antigen presentation [41].

In addition, the activation of this FcεRI receptor in DCs is capable of blocking, or at least reducing, intracellular signals involved in the defective production of type I interferons in antiviral, response managing to restore this antiviral response in case of viral infections [42].

The expression of the low-affinity receptor for IgE or CD23 (FcεRII) on B lymphocytes has been shown to be an important part of the adaptive immune response against inhaled house dust mite (HDM) allergens in the induction of allergen-specific Th2 responses. IgE, especially acting through CD23, also has a direct effect on eosinophil functions such as activation, eosinophil peroxidase release, increased integrin expression, and TNF-α release. IgE directly activates airway smooth muscle to produce IL-4, IL-5, IL-13, TNF-α, TSLP, and chemokines (CCL5, CCL11, CXCL8, CXCL10). This causes contraction and proliferation of airway smooth muscles which can lead to airway remodeling. CD23 is also constitutively expressed on airway epithelial cells and has been involved in transporting IgE-allergen complexes across this IL-4-polarized mucosal barrier. An increased number of IgE+ memory B lymphocytes and plasmablasts are found in allergic patients correlating with the number of Th2 lymphocytes [43].

Innate Lymphoid Cells Type 2 (ILC2)

Innate lymphoid cells type 2 (ILC2) are derived from a lymphoid progenitor and thus belong to the lymphoid lineage. These cells, like Th2 lymphocytes, produce type 2 cytokines such as IL-4, IL-5 and IL-13. ILC2s activated by alarmins produced by epithelial cells (IL-25, IL-33, and TSLP) are currently considered to play an important role in the development of asthma as well as many allergic diseases [21]. Like Th2 lymphocytes, ILC2s are induced under the control of the transcription factor GATA3. In a mouse model with T and B lymphocyte deficiency, activated ILC2s were able to induce eosinophilia and AHR [44]. Numerous studies carried out, especially in the last decade, have shown their involvement in early-onset or allergic T2 asthma both in pediatric and adult patients. ILC2s play a critical role in the initiation, maintenance, and possibly in corticosteroid resistance of allergic airway inflammation [45].

Eosinophils

The presence of eosinophilic bronchial inflammation in asthmatic patients was described decades ago. In the last 10 to 15 years, the importance given to this inflammation has changed radically, and is now considered to be the main feature of the disease. Eosinophilic inflammation is usually found in early-onset T2 asthma. Currently, monitoring eosinophilic inflammation seems the best procedure to control the disease and to prescribe the most appropriate treatment. Traditionally, the study of the inflammatory processes of lung diseases involved invasive or semi-invasive methods such as bronchoscopy with biopsy and/or bronchoalveolar lavage. Currently, bronchoscopy is reserved for static histological studies of bronchial inflammation in critically ill patients or in the context of clinical trials. Non-invasive methods for studying inflammation in asthma include induced sputum. Consensus has been reached to classify eosinophilic inflammation when ≥ 3% of eosinophils are found in induced sputum samples from asthmatic patients [46].

Eosinophils are effector cells of the innate immune system that contain different granules with cytotoxic function. Upon eosinophil degranulation, numerous proteins are released, such as eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), major basic protein (MBP). Eosinophil activation is mediated by cytokines (IL-5) and lipid mediators, such as *cysteinyl leukotrienes (CysLT)* [47].

In addition to blood eosinophilia, tissue eosinophilia is also considered to be an important feature of allergic inflammation and asthma. Eosinophils tend to accumulate where allergic inflammation occurs and contribute to the development of bronchial asthma. They may also play a role in airway remodeling through the production of the TGF-β and cysteinyl leukotrienes (CysLTs) that induce AHR and MBP production. CysLTs are involved in the accumulation of eosinophils in the airways of asthmatic patients, i.e., inhalation of LTE4 has been shown to stimulate accumulation of eosinophils in the airways of asthmatic patients and LTD4 induces transendothelial migration of eosinophils and the release of specific granular proteins mainly through the β2 integrin and the cysLT1 receptor. The development and maintenance of eosinophilic inflammation in the airways is the contribution of cysLTs along with the T2 cytokine network [48].

Several independent studies have shown that both serum IL-5 as well as different eosinophil proteins, such as EDN and ECP was down-modulated after treatment with anti-IL-5 therapy. The observed decrease in eosinophil count results in a significant reduction in concentrations of EDN and ECP [49]. This indicates that cytotoxic granule proteins are not released after achieving eosinophil depletion. The recruitment of eosinophils is probably related to the adhesion of eosinophils to endothelial cells, through VCAM-1 integrin. VCAM-1 is upregulated by IL-4 and IL-13 on endothelial cells. The interaction of eosinophils with VCAM-1 induces eosinophil activation. Eotaxin and its receptor, CCR3, are more strongly expressed in the airways of asthmatic patients than in controls [50].

Mast Cells

Mast cells are essential in the development of asthma and have substantial effects on smooth muscle, mucosal hypersecretion, and airway remodeling through the release of proteases such as tryptase and growth factors. This is supported by multiple lines of evidence, including clinical studies and studies in murine models of mast cell deficiency. However, there are still knowledge gaps on the exact effector mechanisms by which mast cells influence asthma [47].

Mast cells contain large numbers of secretory granules, which are filled with a variety of bioactive compounds including histamine, cytokines, lysosomal hydrolases, proteoglycans as well as several mast cell-restricted proteases. When mast cells are activated i.e., through the IgE receptor, the content of their granules is released and may cause an inflammatory reaction. Mast cell-restricted proteases include tryptases, chymases, and carboxypeptidase A3, and these are expressed and stored at remarkably high levels. There is currently emerging evidence supporting a prominent role for these enzymes in the pathogenesis of asthma [47].

Mast cells also express high levels of the IL-33 receptor ST2 which is activated by IL-33 (alarmin released from epithelial cells). IL-33 stimulates mast cells to produce T2 cytokines, particularly IL-13. The number of mast cells increases in both allergic and non-allergic asthma, but the accumulation of mast cells has been shown mainly in allergic asthma. In addition, mast

cells are more active in the bronchial mucosa of allergic patients than in non-allergic patients [51].

It is important to highlight that mast cells are one of the main cellular sources of PGD2. Both mast cell numbers and PGD2 concentrations are increased in the airways of patients with severe asthma. Another prostaglandin such as prostanoid D (DP) and the CRTH2 receptor, which are receptors for PGD2, are expressed among other cells in Th2 lymphocytes. Recently, the role of this receptor, CRTH2, has been highlighted in the pathogenesis of asthma. It has been shown that the expression of CRTH2 in lymphocytes [Th2, T cytotoxic (Tc) and Treg cells], ILC2s and eosinophils is higher in patients with allergic eosinophilic asthma than in patients with non-allergic asthma and healthy controls. In addition, it has been shown in patients with allergic asthma, intensive treatment reduces the type 2 immune response and corrects the increased expression of CRTH2 and its deregulated functions in mast cells, lymphocytes (Th2, Tc and Treg), ILC2s and eosinophils [52].

Basophils

For more than 40 years, basophils have been reported to be enriched in sputum samples from patients with asthma. Numerous scientific approaches have attempted to elucidate the role of basophils in the pathophysiology of asthma. The small number of basophils in peripheral blood and the technical limitations of previous studies have made it difficult to increase our understanding of how basophils affect the course of asthma [48].

Furthermore, asthma is a highly heterogeneous inflammatory disorder, and the role of basophils may vary among phenotypes. Recent studies using flow cytometry to analyze the cellular content of induced sputum samples have concluded that basophils may be particularly important in eosinophilic asthma. An increase in the number of sputum basophils has been found in patients with eosinophilic asthma, correlating with the severity of the disease [53].

Conclusion

Nowadays there is plenty knowledge about the effector immune response in allergic asthma and type 2 inflammatory response. Gut microbiota impacts both the innate and adaptive immunity in airway epithelia. The danger or injure stimuli in the epithelium induces the release of inflammatory cytokines, recently grouped under the name of alarmins (TSLP, IL-25, and IL-33), which will trigger an inflammatory cascade that will lead to a cellular and humoral response characterized by production of IgE. Type 2 immunity cells include type 2 innate lymphoid cells (ILC2s), CD4(+) T helper 2 (Th2) cells, eosinophils, mast cells, and the least understood basophils. Beside them, new subsets of dendritic cells and lymphocytes (Th9, Tγδ lymphocytes, etc.) have been discovered in the last decade and their particular phenotype is important to understand their role in type 2 inflammation.

Eosinophil inflammatory cytokines play the main role in airway remodeling, and peripheral and tissue eosinophilia continue to be our guide to control the disease and to prescribe the most appropriate treatment. The vast majority of new therapies for allergic asthma focuses on decreasing tissue eosinophilia to revert T2 inflammation. Strategies to increase the presence of

an inhibitory or tolerogenic T lymphocyte subset, known as Treg i.e., allergen immunotherapy, have also achieved good results in allergic asthma and rhinitis.

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Chapter 3

Pollens Causing Asthma

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Abstract

Grasses, trees, and weeds produce pollen grains. They are the male partners of plants that aim to transport the male gametes to the female reproductive structures. Pollination occurs through the wind (anemophily) or a vector, such as insects (entomophily), but only anemophilous plants have allergic importance. Charles Blackley, 1873, recognized that wind-pollinated pollens were the etiology agents of the allergy symptoms caused by the so-called hay fever.

Recent reports describe the unquestionable relationship of pollens with asthma and rhinitis. Pollens first contact the immune system from ocular, nasal, and oral mucosal surfaces, starting sensitization. The humid milieu from these mucosae facilitates the release of soluble aeroallergens and other bioactive compounds in the pollen matrix. Grasses are considered a leading aeroallergen in Europe and other extensive geographic areas, and *Phleum pratense* is among the best-studied grasses.

P. pratense pollen contains a hundred proteins, and 11 groups of allergens have been described among these proteins. Birch trees belong to the family Betulaceae. The pollen from those trees is an important cause of allergic rhinitis and asthma, especially in central and northern Europe and North America. The main birch allergen, Bet v 1, and other major allergens from the homologous birch group are proteins belonging to the pathogenesis-related protein class 10 (PR-10) family.

Olea europaea pollen is an important cause of respiratory symptoms in Mediterranean countries and other areas where *Olea europaea* is intensively cultivated. Up to date, 15 olea allergens have been described.

Cupressus arizonica pollen, from the Cupressaceae family, is the most relevant and better studied of this family. These trees are responsible for winter pollinosis in Europe and other zones. In Japan, from an allergy point of view, Japanese cedar (*Cryptomeria*

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japonica) is the most important member of this family. Platanus pollen is also considered an important allergenic pollen, and 3 main allergens have been described in *Platanus acerifolia* pollen.

Ragweed pollen represents an important health problem in North America and now, also in Europe and other continents. Eleven ragweed pollen allergens have been described, with 2 of these considered major allergens. The *Parietaria* pollen season is long, extending about 6-7 months, and *Parietaria* is one of the most relevant aeroallergens that causes rhinoconjunctivitis and asthma in patients in the Mediterranean basin.

Keywords: pollens, asthma, allergens, sensitization

Introduction

Male partners of gymnosperms and angiosperms plants are represented by pollen grains. The role of the pollen grain is to transport the male gametes to the female reproductive structures [1]. Pollen grains develop in the anthers of plants after several sequential steps. They are transported to the stigma through a process called pollination. After germination, a pollen tube enters the embryo sac inside the ovules [2]. Pollination occurs through the wind (anemophily) or a vector, such as insects (entomophily). Only anemophilous plants have allergic importance [3], although some plants use both methods to pollinate. So, pollen grains are produced by grasses, trees, and weeds. Charles Blackley, 1873, recognized that wind-pollinated pollens were the etiology agents of the allergy symptoms caused by the so-called hay fever. Blackley carried out skin and provocation tests [4]. Moreover, anemophilous allergenic pollens should be abundantly distributed, produced in large quantities, and able to travel considerable distances.

The outermost layer of the pollen wall is called the pollen coat. It is sticky and mainly composed of extremely hydrophobic lipids. Inside the pollen coat is the outer wall of the pollen grain, with a sporophytic origin, called exine, formed of 2 layers. Beneath the exine is the intine. It surrounds the vegetative cell and comprises fibrillar cellulose, hemicellulose, and pectin. The intine has enzymes acting during pollen tube growth and germination [1].

More than 90% of flowering plants use animals to achieve pollination [2], so anemophilous pollens are characterized by inconspicuous flowers. These anemophilous plants have small flowers with drab colors without an obvious scent. Instead, brightly colored petals from flowering plants with sugary scents attract insects, being entomophilous but almost without allergic importance [5].

Pollens contain aeroallergens, and the impact on the upper and lower airways depends on their size, varying from less than 10 μm to more than 100 μm. Aeroallergens larger than 5 μm impact the ocular and nasal mucosa producing symptoms of conjunctivitis or rhinitis, respectively [6]. More controversy exists about how they can reach the lungs, although it is known that pollens can break, producing paucimicronic and submicronic particulates that can reach the lower airway, contributing to asthma symptoms and asthma exacerbations [7].

After Charles Blackley's work, many other researchers related pollens as one of the main etiologic agents of asthma, and recent reports describe the unquestionable relationship between pollens with asthma and rhinitis. In this respect, recent publications have concluded that exposure to grass pollen is an important trigger for childhood asthma exacerbations that can require emergency department attendance [8], highlighting the importance of grass and birch exposures as triggers of childhood asthma hospitalization [9] or the association between

different tree pollens exposures and reduced lung function in children living in Sidney [10]. Another recent research by Idrose et al. in grass pollen-sensitized patients from Melbourne concluded that grass pollen exposure was associated with airway inflammation 1-2 days after exposure mediated by eosinophils and, even more important, the observation of airway obstruction 2-3 days later exposure. These findings highlight the relationship between pollens, lung function changes, and airway inflammation [11]. In the same way, 2 very recent systematic reviews and meta-analyses have provided extra evidence that pollen grains exposure increases various respiratory symptoms in asthmatic patients [12] and that asthma patients' exposure to ambient pollen triggers type-2 upper and lower inflammation [13].

So, there is an important body of evidence about the relationship between pollens and asthma symptoms and asthma exacerbations. Moreover, one study has reported an association between early grass pollen exposure in the first months of life and a decreased lung function at 12 and 18 years, respectively, although more evidence is needed to confirm these results [14].

Pollen concentrations have been recorded all over the world for decades. There are several methods to sample pollen, but today the most common method is the so-called volumetric method using the Hirst device, used since the 1950s (Figure 1). Using this method, concentration data can be obtained daily or hourly [15]. Although not yet routinely implemented, the number of available automated pollen counting methods is expanding. These new methods range from color and shape information, flow cytometry, fluorescence, and Raman microscopy to mass spectrometry [16].

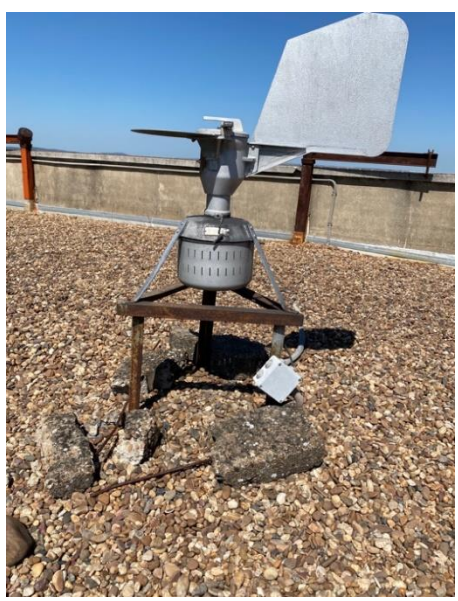

Figure 1. Burkard volumetric device.

Pollen Sensitization

Wind-pollinated pollens first contact the immune system from ocular, nasal, and oral mucosal surfaces, starting sensitization. The humid milieu from these mucosae facilitates the release of soluble aeroallergens and other bioactive compounds into the pollen matrix. This pollen matrix

is composed not only of intrinsic molecules (proteins, carbohydrates, lipids and metabolites) but also of extrinsic compounds (pollen-linked microbiome, viruses and air pollutants particles). Nowadays, this specific context is considered important to produce a Th2 polarization. After the contact with mucosa surfaces, pollens hydrate and release a hydrophilic mix of allergenic and non-allergenic proteins and other bioactive molecules favoring this Th2 polarization in a specific inflammatory milieu [17]. So, there is sufficient evidence that the allergenicity of pollens is not only produced by the different allergens but is also important the presence of other protein and non-protein substances in addition to allergens. Some of these substances interact with epithelial cells, acting as dangerous signals facilitating the synthesis and liberation of alarmins like thymic stromal lymphopoietin (TSLP) and interleukin 25 (IL-25) and IL-33. Some of these pollen substances are enzymes, such as oxidases implicated in the synthesis of reactive oxygen species (ROS) or are proteases that disrupt epithelial tight junctions, which facilitates the transport of different allergens to sub-epithelial layers where contact with different immune cells [like antigen-presenting cells (APC_s), such as dendritic cells (DC_s)] is easier [18] (Figure 2). APC_s and epithelial cells possess a repertory of specific receptors called pattern recognition receptors (PRRs), such as protease-activated receptors (PARs) and Toll-like receptors (TLRs) necessary to provide the first innate response against different pathogens.

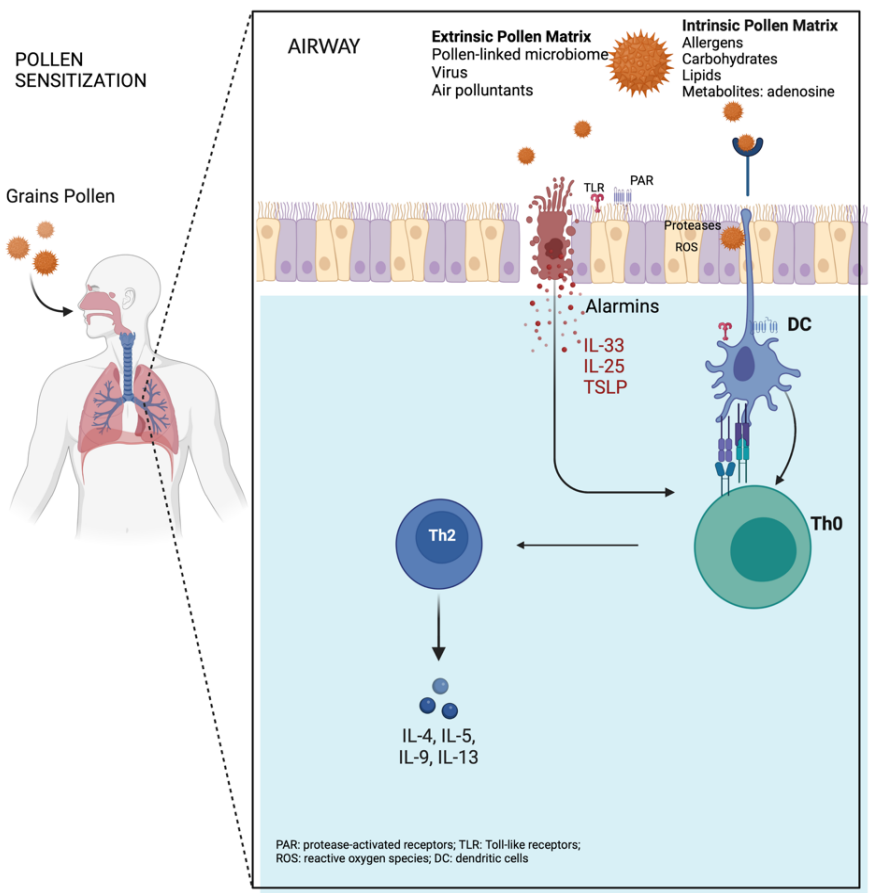

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Figure 2. Pollen sensitization.

Studies highlight the importance of the pollen matrix, suggesting that the allergic inflammatory response depends on the specific context and the sources in which the allergen is presented to the immunologic system. In this respect, an *in vitro* study demonstrated that a purified recombinant Bet v 1 could not produce DC_s maturation in contrast to a complete aqueous birch pollen extract, and recombinant Bet v 1could not get a Th2 polarization [19].

One of the metabolites included in the intrinsic matrix of pollens is adenosine. It is considered an immunomodulator with dual properties and a potent immunoregulatory substance in pollen [20]. In this way, a study in mice using also *in vitro* normal human bronchial epithelial cells reported that a complete ragweed pollen extract could produce a Th2 immune polarization, but this was not observed with the purified natural allergen Amb a 1. Authors showed that allergic lung inflammation was aggravated by adenosine and the presence of pollen-derived adenosine was essential for the *in vitro* migration of human neutrophils and eosinophils toward the supernatants of bronchial epithelial cells stimulated with ragweed extract, concluding that adenosine from pollens constitutes a critical factor in allergic airway inflammation produced by ragweed grains pollen [21].

Main Pollens Causing Asthma and Their Allergens

Poaceae Pollen

Grasses belong to the Poaceae family. This great family comprises more than 700 genera and around 12,000 different species, included in 12 subfamilies. Most of these species belong to the subfamilies Pooideae, Panicoideae, Chloridoideae, Bambusoideae, and Arundinoideae, and the subfamily Pooideae is the most important in temperate climate areas. Other important allergenic subfamilies are Chloridoideae and Panicoideae. Grasses cover around one-fifth of the world's land surface. Some of the most important genera are *Phleum spp.*, *Lolium spp.*, *Dactylis spp.*, *Trisetum spp.*, *Poa spp.*, *Festuca spp.*, *Cynodon spp.*, *Anthoxanthum spp.*, *Holcus Lanata*, and *Trisetaria spp.* [22]. The Poaceae family includes the most important crop species, like wheat, barley, rye, oat, rice, maize, bamboo, and sugar cane, although grains pollen from cultivated species are larger and so of minor allergic importance. Grasses are considered one of the leading aeroallergens in Europe and other extensive geographic areas [7].

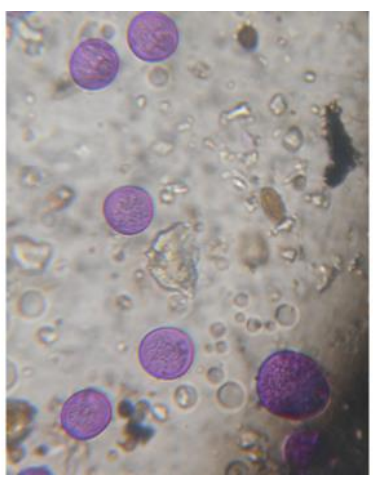

Figure 3. Grass pollen grains.

Grass pollen shape is spheroidal to suboblate, presenting a single pore surrounded by an annulus (a thickening of the outer and inner walls) (Figure 3). The mean size of grains is around 35 to 40 μm [22]. Two types of cytoplasmic granules compost grass pollen cytoplasm: the starch granules, with a mean diameter of 1.1 μm and the polysaccharide particles [23]. An optical microscope makes it impossible to distinguish the different species or genera [24].

In the Mediterranean countries, grass flowering begins in April and finishes in June, although in other regions of Europe, this period starts and finishes 1 month later [25]. In this respect, different publications are reporting an advance in the flowering period of grasses, an extension of the flowering period and an increase in the Annual Pollen Index (API) related to an increase in temperature and climate change [26, 27, 28]. Some years ago, researchers published a large multicentric study analyzing geographical and temporal variations in pollen exposure throughout Europe. Pollen data from 13 different European cities from 1990 to 2009 were analyzed in that study. The most abundant pollen was Betulaceae, being the most frequent pollen in 9 of the 13 cities included in the study, but the second more abundant pollen belonged to the Poaceae family, being the dominant pollen in 3 of the 13 cities of the study [29]. However, API > 5,000 pollen grains were only registered in the cities of Derby and Leiden. In this respect, at the Aerobiology Station of Badajoz, belonging to the Aerobiology Committee of the Spanish Society of Allergology and Clinical Immunology, we realize pollen grain counts continuously since 2003, and in most years, the grass API recorded has been >5,000 pollen grains (data not published) (Figure 4).

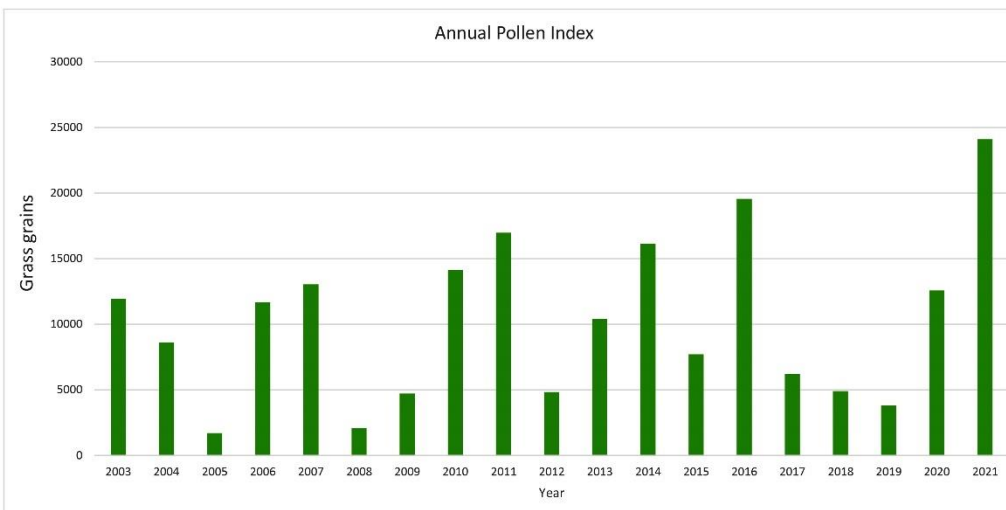

Figure 4. Annual grass/pollen index in Badajoz.

As mentioned above, grasses are considered one of the world leaders in airborne allergens [7], although the sensitization rate for Poaceae varies considerably between countries and even regions. A multicentric study realized in 13 different cities in Europe showed different rates of grasses sensitization, being highest in the two German cities included in the research and in the south of Sweden (above 20%) and being the lowest (around 10%) in Poland [30]. Previously published in another multicentric European study involving 17 centers, the allergen with the highest rate of clinical relevance was grass. It was demonstrated that the sensitization to grass

was clinically relevant in 88% of patients suffering symptoms if exposed to grasses [31]. Regarding North America, it has been reported that about 50% to 70% of patients with allergic rhinitis are sensitized to grass pollen [32]. Recently, one research performed in China with pollinosis patients has been published. Authors studied 546 pollinosis patients describing that 71.1% of them (389) showed positive specific IgE to grass pollen, especially to bermudagrass (97%) and timothy grass (78%) [33]. High rates of sensitized patients have also been found in a recent German study showing that the highest sensitization rate corresponded to grass pollen and rye (*Secale cereale*) pollen, representing 55.3% and 59.6% of their symptomatic patients, respectively [34]. Also, another recently published research in Madrid has shown that the most frequent sensitization in the foreign-born population was grass pollen (75.2% of the patients), especially concerning South American patients [35]. Even in desert areas, such as Qatar, many patients (55.8%) are sensitized to Poaceae, as shown in a recently published article [36]. So, an important body of evidence indicates that grasses continue to be a very relevant aeroallergen in different and extensive zones of the world, sensitizing an important percentage of patients suffering from upper and lower respiratory symptoms.

Phleum pratense (timothy grass) is one of the better-studied grasses. Due to similarity and cross-reactivity with other Poaceae species, *P. pratense* allergens have been considered a good model for studying the allergens and grass pollen allergies. Timothy grass contains a hundred proteins with molecular weights ranging from 10 to 94 kDa. Among these proteins, 11 groups of allergens have been described, named from group 1 to group 13 (there is no group 8 nor 9), based on their biological function and structural properties, with groups 1 and 5 being the most important from an allergy point of view (Table 1).

Table 1. Some grass major and minor allergens

Allergens	Major/Minor	ST (%)	Family name	Function	MW (kDa)
Phl p 1	Major	83-95	CCD-bearing protein	Beta-expansin	27
Phl p 4	Major	70-75	CCD-bearing protein	Berberine bridge enzyme	55
Phl p 5	Major	50-95	Grass group 5	Ribonuclease	30
Phl p 7	Minor	7-10	Polcalcin	Calcium-binding protein	6
Phl p 12	Minor	15	Profilin	Actin-binding protein	15

ST: Sensitized patients; MW: Molecular weight; CCD: Cross-reactive carbohydrate determinants.

Only the concentration of Phl p 5 has been determined in different studies. It has been calculated a medium concentration of 3.2±0.5 μg/mg for this allergen, and it has been estimated that the sum allergens of group 5 and group 2 constitute about the 7-8% of the total protein content of a *P. pratense* pollen grain [23]. In this respect, only a few months ago, one European study was published showing that a Phl p 5-specific ELISA system is appropriated, in terms of accuracy and precision, to quantify native and recombinant Phl p 5 preparations. So, the Phl p 5 ELISA system described by the authors is appropriate to be used as a standard method and can be included in the European Pharmacopoeia, the second allergen-specific standard method after the described for the major birch pollen allergen Bet v 1 [37]. Grass allergens from group 5 are cross-reactive allergens present in most Poaceae species. These allergens are monomeric proteins of 30 kDa molecular weight localized in the amyloplast. These allergens can produce mast cell and basophil degranulation at low concentrations. It has been proposed that they have a role in pollen germination. They can be released from the pollen grains after rainfall in inhaled submicronic particles, causing severe asthma attacks in asthma patients [38]. The N- and C-

terminal domains of the isoforms Phl p 5a and Phl p 5b from *Phleum* are constituted by anti-parallel helix bundles, and these domains contain IgE-binding sites [39]. About 50-95% of patients sensitized to grass present specific IgE to Phl p 5 [22, 40, 41].

Phl p 1 is considered the most prevalent sensitizing allergen from grasses and a marker of genuine primary grass sensitization. It has been demonstrated that 98% of children with specific IgE against timothy grass extract also had specific IgE to Phl p 1, and sensitization to Phl p 1 in children has been shown as an early indicator able to predict grass pollen allergy years later [42]. Usually, sensitization to Phl p 1 is produced before sensitization to the rest of the allergens from grass pollen, being considered an initiator molecule before the next sensitization to Phl p 4 or Phl p 5 and later sensitization to some of the other grass pollen allergens. It shares more than 80% of homology to another group 1 allergens of grasses, sharing different epitopes with group 1 allergens from the Pooideae subfamily and showing important IgE cross-reactivity. Its molecular weight is about 27 kDa and consists of 2 isoforms. Concerning its function, Phl p 1 belongs to the beta-expansin family and is bound to the grain wall, helping pollen tube penetration [41]. About 83-95% of patients sensitized to grass show specific IgE to Phl p 1 [22, 40, 41].

Phl p 7 and Phl p 12 are panallergens from the plants but are minor allergens from *P. pratense.* Phl p7 is a calcium-binding protein from the polcalcin family in many pollens, responsible for a broad cross-reactivity pattern with different allergens from trees and weeds. It has a low molecular weight of about 6 kDa, and its allergenicity is also low (7-10%). Phl p 12 belongs to the profilin family, an actin-binding protein present in the vegetal king. Profilin sensitization is responsible for broad cross-reactivity between pollen and plant food, frequently causing the so-called oral allergy syndrome characterized by oropharynx pruritus. Its molecular weight is around 15 kDa, and it has 3 different isoforms [41]. Although its general described allergenicity is low, about 15% (41), in Spanish patients, the prevalence, intensity and cross-reactive response of T-cell against Phl p 12 are like the major allergen Phl p 1, which suggests its importance in the induction of allergy syndromes, such as the pollen-food syndrome [43], as previously published [44].

Tree Pollen

Betulaceae

Birch trees belong to the family Betulaceae, which is included in the order of Fagales as the family Fagaceae. In addition to birch, alder, hazelnut, and hornbeam are also trees from the Betulaceae family and oak, chestnut, and beech are the main trees from the Fagaceae family. The pollen from all those trees is an important cause of allergic rhinitis and asthma, especially in central and northern Europe [45] and North America [41]. Birch pollen has a maximum axis of not more than 24 μm and three pores, although sometimes only two are visible with the optic microscope, depending on the position. The oncus width is not greater than 10.1 μm. These morphological characteristics allow for to differentiation of birch pollen from alder and hazel pollens [46].

Most sensitized patients suffer symptoms when exposed to a birch pollen concentration of 80 grains/m^3; allergy symptoms can appear when only exposed to 20 grains/m^3 [47]. The flowering period begins about the end of March in western Europe, but in central and eastern Europe, the onset is delayed by about 2 weeks. In northern Europe, the pollination period starts from late April to late May, delaying the onset with increasing latitude [7]. Some years ago,

researchers published a large multicentric study analyzing geographical and temporal variations in pollen exposure across Europe. Pollen data obtained from 13 different European cities from 1990 to 2009 were analyzed in that research. The most abundant pollen was Betulaceae, the most frequent pollen in 9 of the 13 cities included in the report [29].

Similarly, as previously mentioned concerning grasses [26, 27, 28], different studies report a trend toward the earlier onset of birch pollen season in different cities of Europe related to climate change and increasing temperatures. In this respect, a recently published study performed in eastern Poland recording birch pollen counts from 2001 to 2019 obtained similar results, showing that the beginning of birch pollen season is presenting earlier and concluding that a relationship was found between the monthly temperatures preceding a season and the start date of that season. As previously reported about birch pollen seasons, these researchers also found a biennial rhythm consisting of the alternative between seasons of high and low counts of pollen grains [48].

About 8% to 16% of the general European population is estimated to be sensitized to birch pollen, as described by Biedermann et al. in an interesting review of birch pollen allergy in Europe [45], although wide ranges (from 6.8% to 57.4%) of people sensitized to birch pollen in different European countries have been previously reported [31]. The percentage of sensitized people to the Betulaceae family seems to be increasing. For example, 2 cross-sectional studies realized in Sweden reported an increase in the sensitized population from 13% in 1994 up to 18% 15 years later [49], and similar studies conducted in Denmark showed that the rate of birch pollen sensitization increased from 12.1% in 1990 up to 13.7% in 1998 [50]. In the same way, a recently published meta-analysis including 6,163 children from 4 European birth cohorts showed increased risks of sensitization to birch pollen related to several air pollutants [51].

Different analyzes have shown that the main birch allergen, Bet v 1, and other major allergens from the homologous birch group are proteins with a molecular weight of 17 kDa belonging to the pathogenesis-related protein class 10 (PR-10) family. The allergens from this family have a strong identity concerning the amino acid composition and the extracts of other trees from the order of Fagales, such as alder, hazel, chestnut, beech hornbeam, and beech. All of these have homolog allergens cross-reacting with Bet v 1. This fact is important from a therapeutic point of view when deciding on treatment because immunotherapy with Bet v 1 could effectively cover sensitivities to different trees of the order of Fagales [45]. The expression of the PR-10 family is induced by pathogen attacks or different abiotic stress. These proteins are expressed in high concentrations in different pollens, seeds, and fruits, causing in patients with respiratory symptoms cross-reactivity with fruits and vegetables, such as the Rosaceae family (pear and apple), Apiaceae family (carrot and celery), and Fabaceae family (peanut and soybean). These patients often suffer from oral allergy syndrome after eating plant food containing PR-10 proteins. Some studies have suggested that allergy immunotherapy could improve food-related symptoms in those patients [41].

It is estimated that 93% of patients with allergies to birch pollen have specific IgE to the major allergen Bet v 1 [41], and the use of both this allergen and the natural birch extract allows the detection of the 99.2% of patients allergic to birch [52].

The main minor allergens from Betulaceae are Bet v 2, Bet v 4, Bet v 6, and Bet v 7 (Table 2). Bet v 2 is profilin with a molecular weight of 15 kDa. This allergen is recognized by the 22% of patients sensitized to birch, although this percentage is higher in zones where grass pollen is the primary sensitizer. Patients sensitized to profilin can suffer oral allergy syndrome

with various fruit and vegetables [41]. Bet v 4 is a panallergen belonging to polcalcins, with a molecular weight of 7 kDa that sensitized about 5% of patients to birch [41]. Polcalcins are cross-reacting panallergens considered markers of sensitization to multiple pollens with a variable clinical relevance. Fewer than 10% of patients with pollen allergies are sensitized to polcalcins, although this proportion may differ among patients with sensitization to specific pollen sources [53]. Bet v 6 is a minor allergen with a molecular weight of 35 kDa, sensitizing about 32% of birch-allergic patients and Bet v 7 is another minor cross-reactive allergen belonging to the cyclophilin family, with a molecular weight of 21 kDa that sensitizes about 21% population allergic to birch pollen [41].

Table 2. Main allergens from Betulaceae

Allergens	Major/Minor	ST (%)	Family name	MW (kDa)
Bet v 1	Major	93	Pathogenesis-related protein class 10	17
Bet v 2	Minor	22	Profilin	15
Bet v 3	Minor	10	Polcalcin-like protein	24
Bet v 4	Minor	5	Polcalcin	7
Bet v 6	Minor	32	Isoflavone reductase-like and phenylcoumaran benzylic ether reductase	35
Bet v 7	Minor	21	Cyclophilin	21

ST: Sensitized patients; MW: Molecular weight.

Oleaceae

The Oleaceae family includes 4 main genera: olive (*Olea europaea*), common privet (*Ligustrum vulgare*), European ash (*Fraxinus excelsior*) and lilac (*Syringa vulgaris*). Trees from this family are present in the 5 continents, being an important cause of respiratory symptoms in the Mediterranean countries and other areas where *Olea europaea* is intensively cultivated [41]. It is native to southwestern Asia, although it was cultivated in the Mediterranean countries centuries ago in classical cultures (Figure 5). Centuries later, it was introduced as a commercial cultivar in California and other areas of the southwest the United States [54]. *Olea* pollen is trizonocolpate-colporate, has a medium polar axis of about 20 μm and its exine is reticulate. Furrows are broad, flecked, and short, and the intine under them shows marked thickening [54, 55].

In a study in Spain, exposure to a threshold of 162 *Olea* pollen grains/m^3 could cause significant respiratory allergic symptoms in all the studied patients (56), although a superior threshold has been previously reported [57]. In Europe, the main *Olea* pollen season is from middle April to June [7, 41, 56]. Although the percentage of the allergic population sensitized to *Olea* is variable in different countries, and within regions, it is assumed that 30-40% of Italian patients are sensitized to *Olea*, and this percentage is over 80% of patients in areas with very high exposure as in southern Spain [41, 56].

So far, 15 *Olea* allergens (ole e 1 to 15) have been described (Table 3). Several of these (for example, Ole e 7) are minor allergens, although they become major allergens in areas with high exposure and can be associated with severe symptoms and adverse reactions to specific immunotherapy [57]. The last *Olea* allergens that have been characterized are Ole e 14, in 2018, which is a polygalacturonase with high cross-reactivity with Salsola polygalacturonase [58], and Ole e 15, which is an allergen belonging to the family of cyclophilins and sensitized about the 13% allergic population to *Olea* pollen [59].

Figure 5. Olive (*Oleae Europeae*).

Table 3. Main *Olea* allergens

Allergens	Major/Minor	ST (%)	Family name	MW (kDa)
Ole e 1	Major	80	Ole e 1-like protein family member	16
Ole e 2	Major/minor	50	Profilin	15
Ole e 3			Calcium-binding protein	9
Ole e 5	Minor	35	Superoxide dismutase	16
Ole e 7	Minor/major	47	NsLTP	9
Ole e 8	Minor	5	Calcium-binding protein	18.8
Ole e 9	Major	65	1,3-β-glucanases	46
Ole e 10	Major	90	X8 domain containing protein	11
Ole e 14	Minor	19	Polygalacturonase	47
Ole e 15	Minor	13	Cyclophilin	19

ST: Sensitized patients; NsLTP: Non-specific lipid transfer protein. MW: Molecular weight.

Ole e 1 is the major allergen from *Olea*, sensitizing about 80% of the *Olea*-pollen allergic patients. It is a glycoprotein of 145 amino acids with a molecular weight of 16 kDa and a microheterogeneity in function on the variety of the cultivated *Olea*. Ole e 1 has high homology with other allergens from de Oleaceae family (Syr v 1 from lilac, Fra e 1 from ash and Lig v 1 from common privet). This homology is responsible for cross-reactions presented by patients allergic to *Olea* [41, 57]. Some of the *Olea* allergens belong to different families of protein. For example, Ole e 2 is a profilin, Ole e 5 a superoxide dismutase, Ole e 3 and Ole e 8 are calcium-binding proteins, Ole e 7 is a lipid transfer protein, and Ole e 9 belongs to the 1,3-β-glucanases [60]. Recently, Oeo-Santos et al. have shown that Olea e 7, a non-specific lipid transfer protein (nsLTP), is associated with severe respiratory symptoms in areas with high exposure to *Olea* and is responsible for co-sensitization to Pru p 3, a peach nsLTP [61].

Cupressaceae

The order Pinales includes 4 allergenic families: Pinaceae, Cupressaceae, Podocarpaceae, and Taxodiaceae [62]. Cupressoideae is a subfamily from the Cupressaceae family and includes many species and genera, such as *Cupressus*, *Thuya*, and *Juniperus*, that pollinate in all seasons, although mainly in winter, with variations from species to species. The genus *Cupressus* includes several species in the Mediterranean area, Central Asia, China, and North America. In the Mediterranean basin, the most common species are *Cupressus arizonica Cup. sempervirens*, *Cup. macrocarpa*, and *Cup. lusitanica*, with *Cupressus arizonica* the most relevant and better studied (from an allergic point of view) [63] (Figure 6). These trees are responsible for winter pollinosis in Europe and other zones when no other species are flowering at that time [7]. On the other hand, in Japan, the most important member of the family Cupressaceae, from an allergy point of view, are Japanese cedar (*Cryptomeria japonica*), also called "sugi" in Japanese, belonging to the subfamily Taxodioideae and Japanese cypress (*Chamaecyparis obtuse*), included in the subfamily Cupressoideae [64].

Figure 6. *Cupressus arizonica* hedge.

With an optical microscope, it is impossible to distinguish the morphological characters of the Cupressaceae family pollen grains. These grain pollens are sized from 20 to 30 μm, usually without porus and are spherical, with a thin exine and a thick intine [63] (Figure 7).

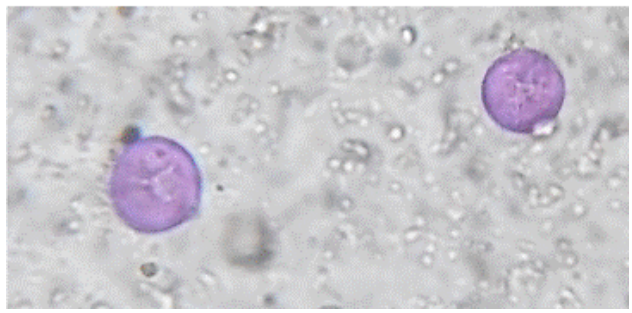

Figure 7. Cupressaceae pollen grains.

In the last decades, Cupressaceae pollen has shown an increasing trend in the number of collected grains pollen in different zones, which has been related to the increased use of these trees as ornamental trees in urban gardens and green spaces and, also, private gardens, and possibly with climate change and related global warming [65]. In this respect, a very recent study in Milan found this increasing trend in airborne Cuppressaceae pollen counts in an area where 30-years ago, cypress pollen was not considered a relevant allergen [66]. Globally, the percentage of the general population with cypress allergy is about 0.6% to 3%, depending on the geographical area of residence and the degree of pollen exposure, and about 9% to 65% of allergic outpatients could be sensitized to cypress pollen, and the trend of these percentages is also increasing [65].

In the Cupressaceae family, 4 main groups of allergens have been described (Table 4). The group 1 allergens are considered major allergens, sensitizing almost all cypress allergy patients. They belong to the pectate lyase family, with a molecular weight of around 43 kDa and show high cross-reactivity between different Cupressaceae species, although Cry j 1 is more distant, sharing a minor sequence identity. Group 2 allergens are also major glycosylated allergens from the polygalacturonase protein family with a similar molecular weight to group 1 that contributes to pollen tube growth and grain maturation. Group 3 belongs to the thaumatin-like protein family, with a molecular weight of about 23-34 kDa that sensitizes about 40-60% of the patients with cypress allergy. This family is included in the group pathogenesis-related protein class 5 (PR-5) that confers resistance against fungal infections. Finally, group 4 are minor allergens from the calcium-binding proteins family, presenting 4 binding sites for calcium. They have a molecular weight of around 17-18 kDa, with great identity between Cup a 4 and Jun o 4 from *Juniperus oxycedrus* [65].

Table 4. Main allergens group from Cupressaceae family

Allergens Group	Major/Minor	ST (%)	Protein Functions	MW (kDa)
1	Major	>90	Pectate lyase	43
2	Major	>80	Polygalacturonase	43
3	Major/minor	40-60	Thaumatin-like protein	23-34
4	Minor	10-15	Calcium-binding protein	17-18

ST: Sensitized patients; MW: Molecular weight.

The association between air pollution and Cupressaceae pollen allergy has been extensively investigated in the last 4 decades. It was demonstrated that patients allergic to cedar trees living in Japan urban areas were more affected than those who lived in rural areas [67]. A mouse

model study showed exposure to diesel exhaust particles increased reactivity to *Cryptomeria japonica* [68]. More recently, it has been described that the expression of Cup a 3 is increased after tree exposure to air pollution [69, 70].

Platanaceae

Platanaceae is a family included in the Proteales order. The family consists only of the genus *Platanus*, which includes 8 species. These trees are tall, even reaching 30 m in height, native to temperate zones of the Northern Hemisphere and being called plane trees or sycamores in some world regions. Allergenic pollens from members of this family are well-studied in different regions due to their important presence as ornamental urban trees and the potential to produce sensitization in the population [71]. These trees are characterized by rapid growth and resistance to air pollution. The flowering period is short but intense, reaching daily average very high concentrations of pollens that appear abruptly in late March or early April, reaching frequent peaks of more than 1,000 grains/m^3 in the atmosphere in different Spanish cities such as Madrid and Barcelona [72].

Although previously not described as relevant allergic pollen, in the mid-1990s, a study realized by Subiza et al. showed that *Platanus* pollen was important allergenic pollen in Madrid, sensitizing 56% of the studied outpatients with a history of rhinitis and/or bronchial asthma [73]. The European Academy of Allergology and Clinical Immunology (EAACI) has now included a skin prick test with *Platanus* in the standard battery recommended for clinical use and research in Europe [74], and a very recent study realized with an allergen nano-bead array has shown that *Platanus acerifolia* was the second most common IgE-sensitization found using different aeroallergens extract in a group of patients from Iran [75].

Three main allergens have been described in *Platanus acerifolia* pollen. The most important, Pla a 1 is an 18 kDa non-glycosylated protein belonging to the invertase inhibitory family proteins. Pla a 2, with a molecular weight of around 43 kDa, is a glycoprotein with polygalacturonase activity. Pla a 3 is a 10 kDa nsLTP that sensitizes 45% of patients and shows a 58.3% sequence homology with Pru p 3 from peach [41, 71]. Nevertheless, a recent published study in Spain has shown that 76.3% of patients were sensitized to Pla a 2, and Pla a 1 and Pla a 3 were detected only in 44.7% and 23.7% of patient´s serum, respectively. These results show a higher prevalence of Pla a 2 than Pla a 1 and Pla a 3. This study characterized the minor Pla a 4 allergen as a glutathione-S-transferase [72].

Weed Pollen

Ragweed

Ambrosia artemisiifolia, also known as short or common ragweed, belongs to the family Asteraceae, together with *Ambrosia trifida* (giant ragweed). It is an annual herbaceous plant with an origin in North America, although nowadays it is present in many geographical zones. Ragweed pollen is considered an important health problem in North America and now, also in Europe and other continents, because of its potential to cause allergic rhinoconjunctivitis and asthma in late summer and autumn. The medium diameter of the ragweed pollen grain is around 15-25 μm, and the pollen surface is echinate, with short spines [76]. This pollen is produced in enormous amounts, each plant producing millions of grains that can be transported long

distances due to its small size [7]. It has been published that exposure to as little as 10 grains/m^3 can elicit symptoms in sensitized patients [76].

Around 15% of the USA population is sensitized to ragweed [77]. This percentage is much higher in different populations suffering from rhinoconjunctivitis and/or asthma. In 2009, a multicentric European study included patients with respiratory symptoms from 16 centers in 13 European countries. The rate of ragweed sensitization was above 2% in all countries, showing Hungary, as expected, with the higher percentage (more than 40%), followed by Denmark (with a prevalence of sensitization of 19.8%). It was described that 23.7% of all *Ambrosia*-sensitized European patients suffered from asthma [78]. Later studies have shown percentages of ragweed-sensitized patients of 50% in Lombardy [79], 47% in France [80], and 18% in the North-West of Romania [81] in different areas of Europe, with an increasing rate of ragweed sensitization also described in China [82]. So, there is an increasing body of evidence about the impact of ragweed on human health and the economic costs associated, and, nowadays, some strategies and projects are being developed aiming to decrease the advance of ragweed as an invasive species in Europe as, for example, realizing a biological control using the accidentally introduced leaf beetle *Ophraella communa* [83].

So far, 11 ragweed pollen allergens have been described, of which 2 are considered major allergens and the remaining minor (Table 5). Amb a 1, an acidic non-glycosylated protein with 397 amino acids and a molecular weight of around 38 kDa, belongs to the pectate lyase protein family. Five Amb a 1 isoforms have been described with reported homologies between 63% and 86% [76]. Amb a 1 has cross-reactivity with the allergen *Helianthus annus* 6 (Hel a 6), another allergen from the pectate lyase family, sharing a sequence homology around 67% [84] and is also cross-reactive with the mugwort allergen *Artemisa vulgaris* 6 (Art v 6), showing a sequence homology of 58% [76]. Amb a 11, a cysteine protease, has been described as another major allergen and should be considered for ragweed diagnosis and treatment as a key component [85]. Around 66% of patients are sensitized to this major allergen, with a molecular weight of 37 kDa and 386 amino acids. It shares homology with other allergens from the cysteine protease family, such as Der p 1/Der f 1, Act d 1 from kiwi and Ana c 2 from pineapple [76].

Table 5. Main ragweed allergens

Allergens	Major/Minor	ST (%)	Family name	MW (kDa)
Amb a 1	Major	>95	Pectate lyase	38
Amb a 4	Minor	20-40	Defensin-like protein	13-15
Amb a 6	Minor	20	NsLTP	10
Amb a 8	Minor	35-50	Profilin	14
Amb a 11	Major	66	Cysteine protease	37

ST: Sensitized patients; Ns: Non-specific lipid transfer protein; MW: Molecular weight.

Parietaria

Parietaria is a widely found weed that can be annual or perennial, also called wall pellitory, that grows in walls and rocks. In North America, *Parietaria pensylvanica* is present from Canada into Mexico, and *Parietaria floridiana* is prevalent in southern California, Mexico, the Atlantic and the Gulf States. *Parietaria officinalis* and *Parietaria judaica* are the main species found in the Mediterranean basin and other countries like Australia [86]. *Parietaria* is included in the Urticaceae family. It is impossible to distinguish *Parietaria* pollen from the pollen of

most species of the genus Urtica, and this is the cause why these pollen grains are identified as the pollen type Urticaceae [87]. Pollen grains are small, about 13-14 μm in diameter, oblate spheroidal, and normally triporate. The intine and exine are thin, although the intine is thickened beneath the pores. The surface is microechinated [86].

The *Parietaria* pollen season is long in the Mediterranean basin, extending about 6-7 months, with the main peak in spring and a lower one in autumn, although regional variations are described. This fact is important from a clinical point of view because clinical symptoms are long-lasting, almost perennial [7, 86, 88]. *Parietaria* is one of the most relevant aeroallergens that causes rhinoconjunctivitis and asthma in patients living in the Mediterranean basin. Almost one-third of allergic patients from Southern Italy are sensitized to *Parietaria judaica*, reaching local sensitization percentages up to 60%. Nevertheless, sensitization in the non-Mediterranean patients from Europe is marginal [41]. A Spanish study described that *Parietaria judaica* pollen grains behave like a perennial aeroallergen, causing the same prevalence of asthma and more severe allergic rhinitis than house dust mites [89]. Some studies in Italy have shown that sensitization to *Parietaria* persists until late age without decreasing with age. So, symptoms can persist for years without decreasing the severity, unlike mite sensitization which significatively diminishes after the fifth decade of life [87].

Par j 1 and Par j 2 have been identified as the major, non-glycosylated allergens from *Parietaria Judaica*, with molecular weights of 15 kDa and 11.3 kDa, respectively. Both allergens belong to the family of nsLTP, consisting of 4 alpha-helices stabilized and linked using 4 disulfide bonds. Although most patients are sensitized to Par j 2, there are also patients reacting stronger to Par j 1, which is why both allergens have been recommended to be included in the extracts used for specific immunotherapy with *Parietaria* [90]. Minor *Parietaria* allergens, such as Par j 3 and Par j 4, from the profilin and calcium-binding families, have also been described [91, 92].

Conclusion

Many scientific reports describe the unquestionable relationship of grain pollens with asthma and rhinitis.

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Chapter 4

Mites and Fungal Spores Causing Asthma

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Abstract

Changing lifestyles in the 21st century have increased the significance of indoor allergens in respiratory allergies. House dust mites (HDM) are the most significant indoor allergens worldwide and HDM sensitization is the main risk factor in the development of asthma. Up to 50% of asthma patients are sensitized to dust mites, and greater exposure to HDM has been shown to increase the risk of developing asthma in patients with a genetic predisposition. *Dermatophagoides pteronyssinus* is one of the most important species among the many described. Allergens are found primarily in fecal pellets and to a lesser extent in body components, and the major allergens are groups 1, 2 and 23. Storage mites have also been implicated as important sources of occupational asthma allergens in storage facilities for cereals and cured foods.

Fungal spores represent the fourth most common cause of sensitization in allergic asthma. Certain genera of fungal allergens can act as both indoor allergens as a consequence of perennial exposure in the home, and as seasonal outdoor allergens, predominantly in the summer and autumn months, when they reach high atmospheric concentrations that vary according to geoclimatic conditions. Sensitization to fungal spores is associated with an increased risk of severe asthma, decreased lung function, increased use of rescue medication, severe asthma exacerbations, hospital admissions, and asthma deaths. *Alternaria alternata* is the most important species and the most common allergen is Alt a 1, to which up to 80% of patients with *A. alternata* allergy are sensitized.

A proper diagnosis of HDM and fungal allergy using *in vivo* skin tests and *in vitro* specific IgE studies against complete extracts and molecular components is essential to identify sensitization to responsible allergens and to decide on an appropriate therapeutic approach. In patients sensitized to mites and fungi, avoidance measures in the home are recommended, as exposure is associated with an increased risk of asthma exacerbation.

Keywords: allergens, house dust mites, Der p 1, fungal spores, Alt a 1

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Introduction

House dust mites (HDM) and fungal spores are among the most important allergens of the predispose of the development of asthma, both the exposition to high concentrations and continuous exposure to these allergens.

In this chapter a review of the involved allergens and the factors that favor the development and aggravation of asthma after sensitization is carried out.

The chapter is divided into two parts, the first one about HDM and the second about fungal spores.

Dust Mites

Introduction

House dust mites (HDM) are one of the most important sources of allergens worldwide. They can sensitize patients and induce an IgE-mediated hypersensitivity response, leading to multiple respiratory diseases [1]. They have also been associated with immediate reactions after ingestion of foods contaminated with HDM, such as stored grains [2] or cured meats and have been recognized by the WHO as a major health problem [3]. An increase in prevalence has been reported in recent decades, and an estimated 50% of asthma patients are sensitized to dust mite allergens [4]. In addition to exposure under specific environmental conditions, genetic predisposition is essential for the development of asthma in patients exposed to HDM [5].

Discovery of the Role of Dust Mites in Allergic Diseases

The importance of household dust as a source of allergic reactions was discovered in 1921 by R. Kern, who found that many allergic patients had a positive skin prick reaction to extracts of dust from their homes [6]. In 1967, Voorhorst and Spieksman identified the dust mite *Dermatophagoides pteronyssinus* as the main source of HDM allergens [7], and finally in 1981, Tovey, Chapman, and Platts-Mills determined that the fecal particles of this mite were an important source of allergens and were able to purify Der p 1 [8].

Classification of Dust Mites

Dust mites are arthropods of the arachnid class, subclass *Acari* (Figure 1). More than 50,000 species have been described and, while it is estimated that up to one million species may exist, fewer than 25 are related to allergic diseases. Mites are one of the most ancient land-dwelling insects on earth; they are widely distributed around the world and are capable of adapting to and surviving in extreme conditions all over the planet.

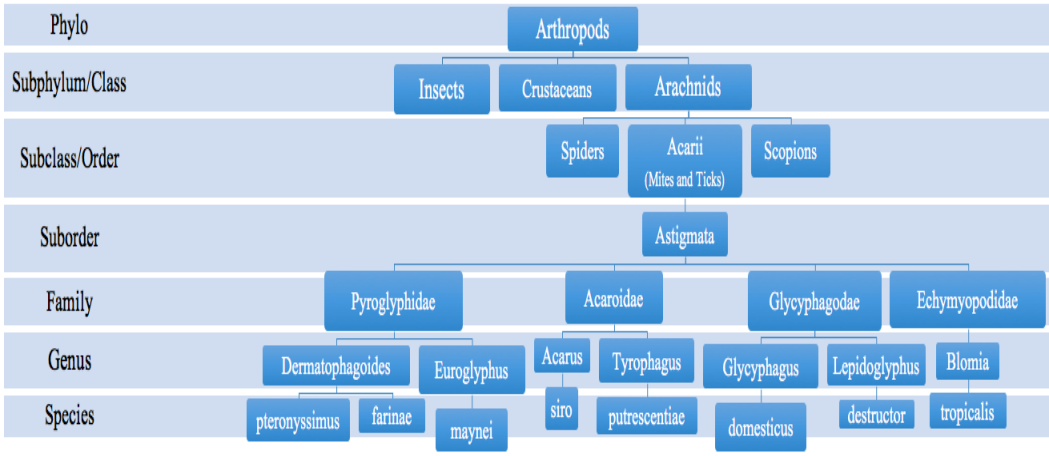
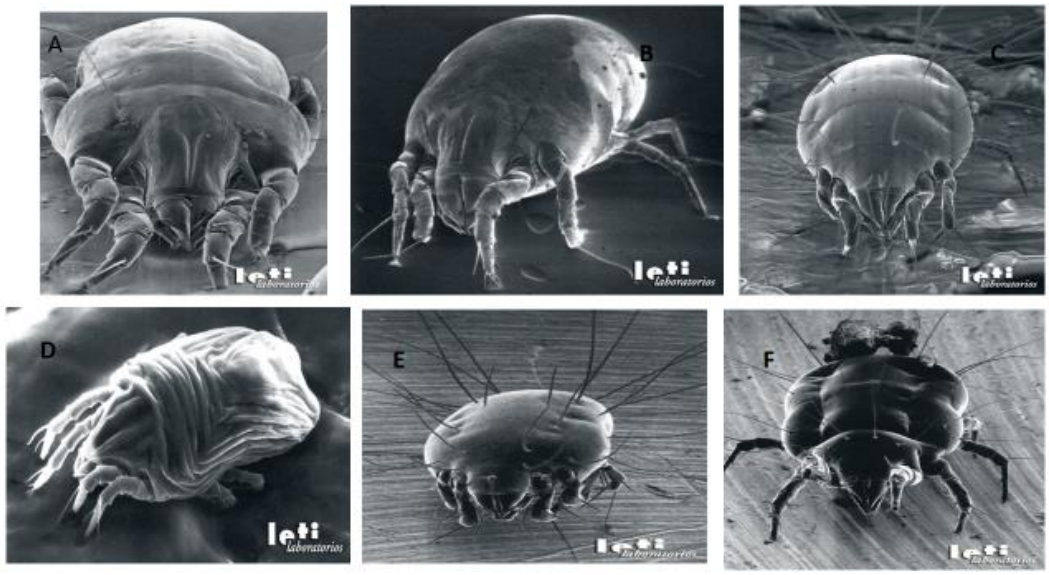


Figure 1. Taxonomy of dust mites.

Mites of interest in allergology have a pale, amber, translucent body that measures 250-350 μm in length and a life expectancy of 65 to 100 days. Females can produce between 50-80 eggs during their lifetime [9]. They mainly feed on organic residues, such as human skin scales and other micronutrients. The families that have the greatest impact on allergies in humans are included in the *Astigmata* suborder:

- *Pyroglyphoidae*: this is the most relevant family, and the species *Dermatophagoides* (*D.*) is the most important because of its common presence in the domestic environment and the frequency with which it causes sensitization. *D. pteronyssinus* predominates in Europe, where it is followed by *D. farinae*, while *D. farinae* predominates in America*; Euroglyphus maynei* is less common in domestic environments [10].
- *Acaroidae*: *Acarus siro* and *Tyrophagus putrescentiae* are the most representative members of this family. They are most prevalent in stored cereals and cured foods and play an important role in occupational asthma [11].
- *Glycyphagodae*: members of this family that produce allergic sensitization are storage mites, primarily *Lepidoglyphus destructor* and *Glycyphagus domesticus* [12].
- *Echymyopodidae*: *Blomia tropicalis* is a domestic mite that predominates in tropical and subtropical areas, and sensitization is very prevalent among asthma patients [13].

They were initially classified according to their habitat as HDM and storage mites, but they are identified in both homes and other environments, so this classification has become somewhat obsolete. They are detected in many areas of the home, with beds forming the perfect habitat, offering warmth, humidity, and food; they are also present in other places in the home, such as carpets, clothing, pet areas, etc. [14]. They are found in facilities that store cured grains and food, where they can contaminate, invade, and reproduce in processed foods that contain grains that have become damp or are stored in humid environments [15].



Images provided by Laboratorios LETI.

Figure 2. House dust mites seen under the microscope: A) *D. pteronyssinus*; (B) *D. farinae;* (C) *B. tropicalis;* (D) *E. maynei;* (E) *L. destructor;* (F) *T. putrescentiae.*

Images provided by Laboratorios LETI.

Figure 3. Dust mites seen under a magnifying glass: (A) *D. pteronyssinus;* (B) *D. farinae;* (C) *B. tropicalis;* (D) *L. destructor;* (E) *T. putrescentiae;* (F) *E. maynei.*

Dust Mite Allergens

To date, more than 30 allergens demonstrating different biological activities have been identified in dust mites (Table 1). The major HDM allergens, that is, those that cause

sensitization in more than 50% of allergic patients and have been detected in up to 95% in HDM fecal particles, are:

- Group 1 (Der p 1 and Der f 1): digestive proteolytic enzymes that are destroyed at 60ºC. These can disrupt the tight junctions between neighboring mucosal epithelial cells, damaging the epithelial barrier, facilitating the interaction of allergens with the immune system, and activating innate immunity signal transduction pathways that induce the recruitment of effector cells and promote a Th2-type immune response [16].

Table 1. Biochemical and physico-chemical characteristics of HDM allergens

Group	Allergen	Frequency of reactivity (%)	Molecular weight (kDa)	Biological activity
1	Der p 1, Der f 1, Der m 1, Der s 1; Eur m1, Blo t 1	> 90	27	Cystein-protease
2	Der p 2, Der f 2, Der s 2, Blo t 2, Eur m 2, Lep d 2, Gly d 2,Tyr p 2, Sui m 2	> 90	15	NPC2 protein family
3	Der p 3, Der f 3, Der s 3, Eur m 3, Tyr p 3	90	31	Serin-protease: trypsin
4	Der p 4, Eur m 4, Blo t 4	25-46	60	Alpha-amylase
5	Der p 5, Lep d 5, Blo t 5	9-70	14	Unknown
6	Der p 6, Der f 6, Blo t 6	39	25	Chymotrypsin
7	Der p 7, Der f 7	38-53	27-31	Unknown
8	Der p 8, Blo t 8, Lep d 8	40	27	Glutathione S-transferase
9	Der p 9	> 90	29	Serin-protease:
10	Der p 10, Der f 10, Blo t 10, Lep d 10, Tyr p 10, Cho a 10	81	36	Tropomyosin
11	Der p 11, Der f 11, Blo t 11	82	103	Paramyosin
12	Blo t 2, Lep d 12	50	14	Chitinase
13	Der f 13, Blo t 13, Lep d 13, Gly d 13, Aca s 13, Tyr p 13	11-23	15	Lipid-transfer protein
14	Der p 14, Der f 14, Eur m 14	84	177	Apolipophorin
15	Der p 15, Der f 15	95	98-109	Unknown
16	Der f 16	50-62	53	Gelsolin/villin
17	Der f 17	35	53	Calcium-binding protein
18	Der p 18, Der f 18	63	60	Chitinase
19	Blo t 19	10	7	Antimicrobial peptide homologue
20	Der p 20	0-44	40	Arginine kinase
21	Der p 21, Der f 21, Blo t 21	26	14	Unknown
22	Der f 22	Unknown	Unknown	Unknown
23	Der p 23	74	14	Peritrophin
24	Der f 24, Tyr p 21	100	13	Ubiquinol-cytochrome C reductase protein homologue/troponin C
25	Der f 25	76	34	Triosephosphate isomerase
26	Der f 26	Unknown	18	Myosin alkali light chain
27	Der f 27	Unknown	48	Serpin (tryptase inhibitor)
28	Der f 28	68	70	Heat shock protein
29	Der f 29	70-86	16	Peptidyl-prolyl cis-trans isomerase (cyclophilin)
30	Der f 30	63	16	Ferritin
31	Der f 31		15	Cofilin
32	Der f 32	Unknown	35	Secreted inorganic pyrophosphatase
33	Der f 33	Unknown	52	Alpha tubulin

- Group 2 (Der p 2 and Der f 2): epidermal secretory proteins resistant to temperatures of 100ºC that participate in lipid transport and promote Toll-like receptor 4 endotoxin binding, stimulating immune system activity and acting as adjuvants in the allergic reaction [17].
- Group 23 (Der p 23): the most recently described major allergen, homologous to chitin-binding proteins [18].

Prevalence of Dust Mite Allergies

Collof estimated that 1%-2% of the world's population (65-130 million people) are hypersensitive to dust mites [19]. The geographical distribution of patients with HDM allergy is very variable, occurring generally in coastal areas [20] and in the vicinity of rivers.

HDM are indoor allergens whose life cycle requires a series of stable climatic conditions for their survival: relative humidity of 75% to 90% and warm temperatures of 23ºC to 27ºC [9]. A study conducted in Central Europe detected HDM in up to 70% of households [21], while another study by Ulrik and Backer [22] found that in asthmatic patients, the prevalence of HDM sensitization was higher than 50%, and up to 80% in tropical areas.

Factors Involved in the Development of Asthma in Patients with HDM Allergy

Numerous factors have been identified for the development of asthma, including the genetic predisposition of the patient, environmental exposure to mite allergens and tobacco smoke, chemical irritants, air pollution, physical and nutritional factors, and repeated respiratory infections [23].

The molecular properties of HDM allergens, together with exogenous agents present in fecal pellets, make them a source of very potent allergens [17]. These granules are transformed into easily inhaled airborne particles that contain bacterial components that further stimulate the innate immune system. Sensitization occurs mainly in the respiratory tract, although a 2009 study showed that skin barrier changes, such as eczema, are also an important route of sensitization [24]. Overproduction of thymic stromal lymphopoietin (TSLP) cytokine in eczematous skin facilitates allergen penetration and promotes sensitization of the airways by HDM, facilitating the development of allergic asthma [25]. These observations would explain the progression from eczema to asthma, a phenomenon known as atopic march.

There is a close association between rhinoconjunctivitis (RC) and asthma -an estimated 29% to 46% of RC patients have asthma- and this strong correlation is often interpreted as evidence of a common underlying sensitization mechanism [26].

1. *Relationship between exposure and sensitization to dust mites*: a high correlation between exposure and sensitization to HDM and the development of asthma has been demonstrated [27]. There is evidence of a dose-response relationship between exposure and sensitization to dust mite allergens. One study showed that the highest sensitization rates occur at levels of between 3.5 and 23.4 μg/g of dust [28]. This sensitization is likely to occur when mite allergens are airborne, forming minute

particles within the respirable range (1.1 to 4.7 μm) [29]. Sensitization to HDM in early life is associated with persistent allergic asthma in childhood and with reduced lung function. It has also been found that exposure to high levels of dust mite allergens in the first year of life increases the risk of developing asthma [30], and a higher prevalence of group 1 and group 2 allergen sensitization has been reported in the pediatric and adult population, respectively. These findings suggest that group 1 may trigger sensitization, due to protease activity in the epithelium and as a nonspecific adjuvant, while group 2 may determine severity and a poorer clinical prognosis [31].

2. *Genetic predisposition*: as mentioned above, genetic predisposition plays a key role in the IgE response to mites. Multiple attempts have been made to identify genes that regulate the IgE-mediated response to HDM, focusing on the major histocompatibility complex (MHC) because of its importance in the adaptive immune response. In 1990, the involvement of the MHC was first determined in a study of pairs of twins sensitized to *D. farinae* with allergic asthma [32]. Subsequently, alleles associated with risk factors and alleles with a protective effect in the allergic response to mites were identified [33].

   Polymorphisms have also been described in genes involved in the Th2 response and their different cytokines and in genes involved in the innate immune response, suggesting that genetics play a role from the early stages of the immune response. Research has focused in recent decades on genome-wide association studies (GWAS) and gene expression analyses, confirming the role of MHC alleles [34] and revealing new chromosomal regions associated with HDM sensitization. These discoveries form the basis of personalized medicine, which may be the future foundation of specific treatments designed on the basis of genetic alterations involved in the disease.
3. *Other factors*: In children, a history of atopy, both personal and familial, is the most important risk factor for the subsequent development of asthma. Additionally, some childhood infections, such as respiratory syncytial virus and rhinoviruses, may act as a protective factor or risk factor in the development of asthma in the early years of life. Hormonal factors are also relevant, as a shift in the prevalence of allergic asthma in adulthood from men to women has been observed.

Occupational Asthma Caused by Dust Mites

Exposure to storage mites is a risk factor for the development of occupational asthma in certain work environments. Nasal symptoms usually precede the development of asthma [35]. There is a moderate level of evidence that storage mites act as etiological agents in agriculture and bakeries, as well as in other labor sectors such as the food, poultry, and ham industries [36]. They also play a role in certain work environments, such as schools, hotels, libraries, and public transport. Finally, higher concentrations of HDM allergens have been detected in some workplaces than in households [8, 37].

Diagnosis

When a rigorous patient history and physical examination suggest the involvement of mites in respiratory disease, and after confirming a diagnosis of asthma with spirometry and

bronchodilation or methacholine challenge tests, it is essential to further investigate the mites and allergens involved.

For this purpose, *in vivo* tests must be conducted first, using skin prick tests with commercial mite extracts, that, given their high sensitivity, simplicity, speed, reproducibility, and price, are considered the first-line diagnostic procedure. Commercial HDM extracts are obtained from mite bodies and excrement [14]. Their main limitation today is that only the contents of groups 1 and 2 have been quantified, while the content of other relevant groups, such as group 23 and minority groups, which may be present in small quantities or even in undetectable amounts, is unknown [38]; there is also significant variability from one pharmaceutical company to another.

The *in vitro* techniques we use include quantitative techniques to determine specific IgE against complete extracts, most commonly ImmunoCAP. Raw HDM extracts contain a mixture of allergenic and non-allergenic components, standardized for major groups 1 and 2.

Quantitative techniques for specific IgE to molecular components allow us to determine a more accurate patient sensitization profile. For this purpose, ImmunoCAP (ImmunoCAP ISAC), currently in common use, analyzes 112 molecular components, while the more recent ALEX technique determines 300 allergenic molecular components. Assessment of Der p 1 and/or Der p 2 sensitization is essential for the selection of candidates for immunotherapy [39].

Bronchial exposure to mites is a procedure restricted exclusively to the diagnosis of occupational asthma and for research purposes, as it can cause bronchial airflow obstruction, increased bronchial hyperreactivity, and inflammatory infiltrate in the airways that can persist for weeks [40, 41].

Treatment

The three pillars of treatment are:

1. *Allergen avoidance measures*: Primary prevention should be a priority, as greater exposure to mites carries a higher risk of sensitization [27]. It is, however, difficult to implement and is not always possible: sources of exposure are numerous and varied, so the measures taken in homes may be insufficient. Following sensitization to HDM, secondary prevention is controversial because there is no evidence-based information to support the effectiveness of dust mite avoidance in preventing or delaying the development of asthma among individuals sensitized to mites or those with allergic rhinitis. However, secondary prevention appears to be beneficial in controlling asthma severity in sensitized patients who are exposed to high levels of HDM allergens, in whom significant FEV1% reduction and airway hyperresponsiveness have been detected [42].

   Controlling humidity is the most important factor in avoidance measures. Humidity in the local microenvironment and the direct retention of moisture by mites are crucial for mite growth and reproduction [9, 43]. The high levels of humidity in certain parts of the world make it impossible to implement these measures, but it is very effective to reduce relative humidity in the home to 50% with the use of dehumidifiers and air conditioning [44]. The home should be regularly reviewed, problems with damp should be resolved, and adequate ventilation should be ensured. However, some direct

interventions against mites have been shown to help asthma control in sensitized patients [45]. These include removing decorative items from the home such as rugs, carpets, curtains, etc. and, in general, all objects that collect dust. Washing bed linen at least once a week at over 60ºC has also been shown to be effective, as it destroys both mites and eggs almost instantaneously [46]; clothes should be dried in direct sunlight [47] or at a high temperature, the mattress, pillow, and slatted base should be vacuumed with an HEPA filter, and a special waterproof mattress protector should be used. Clothes that have been stored for some time should be washed and the house should be cleaned regularly, using a damp cloth on surfaces to prevent dust particles from becoming suspended in the air, from which they can easily be inhaled. These measures should be extended to other sites, such as schools and places of work, where patients spend a lot of time. Finally, patient education must be prioritized.

2. *Pharmacological treatment*: Pharmacological measures provide relief of clinical symptoms. Drugs often have a rapid and predictable effect on symptom control but do not alter the natural course of the disease. Current medication guidelines recommend the use of antihistamines, topical corticosteroids, antileukotrienes, and β2-agonists to relieve respiratory symptoms [48]. Symptomatic relief, however, should not be the ultimate goal of treatment.
3. *Allergen immunotherapy*: Allergen immunotherapy (AIT) for HDM allergens constitutes the delivery of precision medicine [49]. It has become a complementary or alternative option for treating HDM allergy and provides a lasting benefit even after completion of treatment [50]. It is the only treatment capable of modifying the natural course of allergic diseases [51], reducing exacerbations and inflammatory responses in tissues. Standardized extracts are recommended, as other formulas vary in biological activity and effectiveness.

   AIT can relieve symptoms and reduce the use of medication in asthma patients sensitized to mites and although it does not improve lung function, it reduces asthma symptoms and the use of asthma medications and improves bronchial hyperresponsiveness. AIT has been shown to reduce the risk of sensitization to new allergens and prevent the development of bronchial asthma in allergic individuals [52]. It is considered more cost-effective than drug therapy, but efficacy is dependent on patient compliance. Two types of AIT with proven clinical efficacy and safety are available on the market: subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT).

Summary of House Dust Mites

HDM represent one of the most important allergenic sources for the development of asthma. It has been proven, that the exposure to high concentrations, as well as genetic predisposition and factors such as atopic, both family and personal, imply determining factors for the appearance of asthma and the degree of severity of asthma.

Fungi

Introduction

According to the *Alergológica* 2015 report, fungi are the fourth most common cause of allergic respiratory disease, after mites, pollens and epithelia. Exposure to fungal spores can occur both in open spaces and indoors, but outdoor levels are higher and, as such, will have more impact on the sensitization and development of allergic disease [53]. Of the more than 100,000 species of fungi described, over 180 have demonstrated allergen production [54], those with greatest clinical impact being the phyla *Ascomycota* and *Basidiomycota* (see Figure 4) [55].

Spores, composed of small ellipsoidal particles ranging in size from 5 to 50 μm, are the main source of fungal allergens [56]. They participate in fungal reproduction: they are easily transported by air to spread and germinate, originating new organisms. The structure varies widely from one species to another. Their concentrations are usually higher than those of pollens in outdoor spaces, because fungal spores form the largest proportion of aerobiological particles [57] at certain times of the year. Allergens have also been identified in fragmented hyphae, mycelia, and yeast forms.

Atmospheric Concentrations of Fungal Spores

Fungal spores are present in the atmosphere in fluctuating concentrations throughout the year, depending on the geoclimatic characteristics required by each fungus, although in general, concentrations are high in spring and very high in summer and autumn [58], when they behave as seasonal allergens. Atmospheric concentrations of fungal spores are dependent not only on seasonality, but also on humidity, wind, rainfall, and the organic substrate. They can be released in dry, windy conditions (*Alternaria* and *Cladosporium*) or when environmental humidity is high (*Ascomycota* and *Basidiomycota*).

Indoor factors favoring sporulation are relative humidity, temperature [59], darkness, accumulation of dust and organic materials, and poor ventilation: under these conditions, spores are released continuously and behave as perennial allergens [60]. Different fungi are identified in the different areas of the home [61].

Involvement of Fungi in Allergic Diseases

Sensitization to fungal allergens is caused by exposure to fragmented spores and hyphae, and can trigger hypersensitivity reactions that cause different diseases, IgE-mediated sensitization being the most common. It is associated with allergic respiratory disease, but other diseases such as pneumonitis, bronchopulmonary mycosis, and skin infections are also involved [62].

The most relevant fungal species are *A. alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum* and *Penicillium notatum* [63] (Figure 5), and while most species are cross-reactive, some allergens, such as Asp f 1, Alt a 1, Cop c 1 y Mala s 1, are species-specific [64]. Air pollutants can modify sporulation and total antigen production [65].

Figure 4. Taxonomy of fungi.

Images provided by Laboratorios Leti.

Figure 5. Fungal spores under the macroscopic vision: (A) *A. alternata.* (B) *A. fumigatus*. (C) *C. herbarum*.

The most allergenic outdoor fungi are *Alternaria* and *Cladosporium*. Sensitization to these species is associated with the development of asthma [66] and rarely with infection. *Penicillium* and *Aspergillus*, on the other hand, are the principal sources of indoor allergies [67]. *Aspergillus* can colonize the bronchi in asthmatic patients and cystic fibrosis patients and cause allergic bronchopulmonary aspergillosis (ABPA) [66], while *Malassezia* has been associated with atopic dermatitis [68].

1. *Alternaria* (Figure 6): The genus *Alternaria* is involved in the decomposition of organic matter and, as a pathogen, can cause infections, toxicosis, and allergic diseases [69]. It is composed of more than 350 species that have a great capacity to survive in extreme environments with low nutrient levels, temperatures ranging from -3ºC to 35ºC [70] and relative humidity greater than 84%, permitting universal distribution and development. It is considered both an outdoor fungus, with levels of up to 7500 spores/m^3 in the atmosphere at certain times of the year, and an indoor mold, reaching levels of up to 280 spores/m^3 [71]. It is often associated with the so-called "sick building syndrome," characterized by factors including high humidity, poor ventilation, and the presence of cockroaches or cat epithelium [72].
   Exposure to *Alternaria* in the range of 80 to 300 spores/m^3 indoor is thought to favor the development of allergic symptoms [73] and its spores are considered to be the most allergenic [68]. *A. alternata* and its major allergen Alt a 1 are the most important and widely studied in fungal allergic disease. Only two species of *Alternaria* have been identified as inducers of allergic respiratory diseases: *A. alternata* and *A. chartarum* [70]. *A. alternata* is the most allergenic species and constitutes the most potent source of fungal aerosensitization [74], producing 60% of positive skin tests in patients sensitized to fungi [75].

To date, 17 allergens have been described (Table 2), most of which have a high structural homology with other genera of the *Pleosporaceae* family. Alt a 1, an acidic glycoprotein, is the major allergen of *A. alternata*. It is recognized by between 80% and 98% of allergic patients [76] and is considered a primary marker of sensitization [77]. Its biological activity is unknown: studies have suggested that it intervenes in pathogenicity [78] or in the development of plant-fungal interactions [77], since it is found in the cell wall of spores and as a component of the mycelia. It can induce airway inflammation by activating the epithelium, leading to release of alarmins (TSLP, IL-25 and IL-33) and other inflammatory cytokines [70]. It has also been linked to increased eosinophils in the airways and high IgE production [79]. It has been associated with chronic asthma and is a risk factor for severe, life-threatening asthma [76] and increased asthma exacerbations, especially in children [72]. This allergen has also been identified in other *Pleosporaceae* families with a high degree of cross-reactivity [80]. Other allergens described are minor, with a recognition rate between 2% and 42% among sensitized individuals [81].

2. *Cladosporium* (Figure 7) is highly ubiquitous and can develop in extreme habitats. In allergenic terms, it is not the most aggressive fungus in humans. *Cladosporium herbarum* is the dominant species in dry inland regions of Spain. It colonizes plant substrates.
3. *Aspergillus* (Figure 8) is the most important fungal allergen after *Alternaria* and proliferates under extreme conditions. *Aspergillus fumigatus* is the most important strain and can produce highly allergenic mycotoxins.

Table 2. Biochemical and physico-chemical characteristics of *A. alternata* allergens

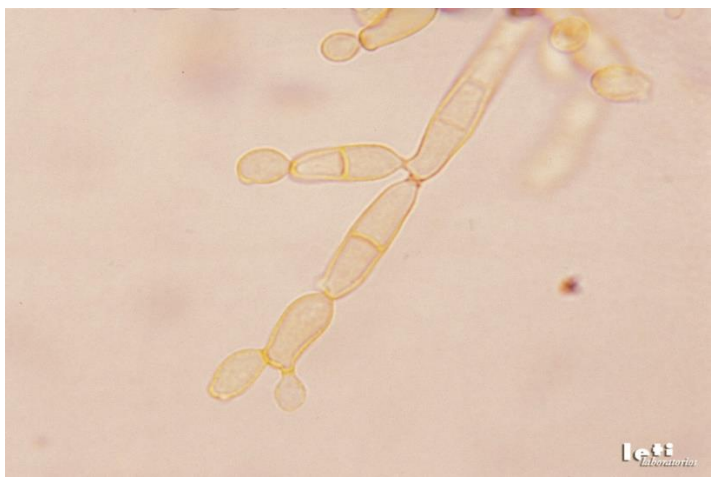
Allergens	Frequency of reactivity	Molecular weight (kDa)	Biological function
Alt a 1	>80%	14	Unknown
Alt a 2	0-61	20	EIF-2alpha kinase
Alt a 3	5	70	HSP70
Alt a 4	42	57	Protein disulfide isomerase
Alt a 5	8	11	Ribosomal protein P2; Cla h 4 homologues
Alt a 6	50	45	Enolase
Alt a 7	7	22	1,4-benzoquinone reductase; Cla H5 homologue
Alt a 8	41	29	Mannitol dehydrogenase
Alt a 10	2	54	Aldehyde dehydrogenase; Cla h 3 homologue
Alt a 12	Unknown	11	Acidic ribosomal protein P1
Alt a 13	82	26	Glutathione S-transferase

Prevalence

The prevalence of fungal spore sensitization varies from one study to another and is estimated to be around 3%-10% [77], with wide geographical variability. In the United States, a prevalence of fungal sensitization of 80% has been reported in asthma patients, affecting 12.9% of the population [82], while in Europe it has been estimated to affect between 5% and 9% of the population [83]. According to the *Alergológica* 2015 report, 20% of Spanish allergic asthma patients are sensitized to fungal spores, while the prevalence has doubled in recent years, with a clear predominance of *A. alternata* sensitization in inland regions, making it the most prevalent in Spain [84].

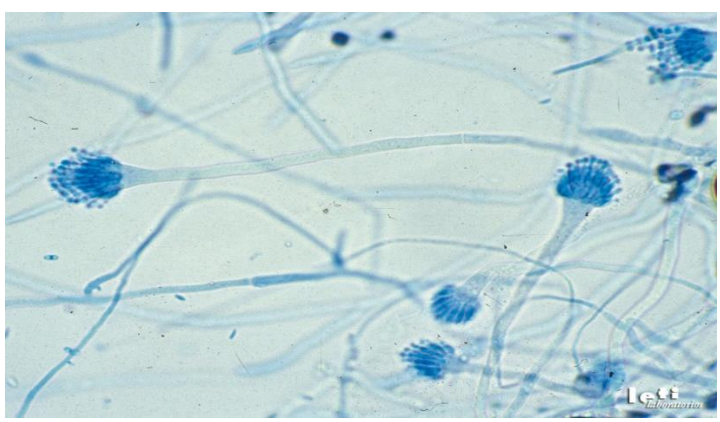
Images provided by Laboratorios Leti.

Figure 6. *Alternaria alternata* microscopic vision.



Images provided by Laboratorios Leti.

Figure 7. *Cladosporium herbarum* microscopic view.



Images provided by Laboratorios Leti.

Figure 8. *Aspergillus fumigatus* microscopic view.

Association between Asthma and Fungal Allergy

Fungal allergens are among the most important in inducing asthma. As mentioned above, at certain times of the year, under specific weather conditions, very high atmospheric concentrations of very small fungal spores are detected, favoring access to the airways and inducing asthma [85] in genetically predisposed patients [86].

A study conducted in Germany (1997-2018) showed that the percentage of fungal sensitization with clinical impact doubled in the second decade of life compared to the first decade [87]. Fungal sensitization, as already mentioned, is associated with more severe allergic lower respiratory tract disease than that caused by other allergens [88], and also with partially controlled episodic asthma in children, according to GEMA 5.1.

Monosensitization to fungi is exceptional [89], since fungal sensitization is often associated with sensitization to other allergens, and if several fungi are involved, the outcome can be poorly controlled asthma [90]. Fungal allergens have also been described as having the ability to activate the immune system, inducing an increase in the inflammatory response caused by other allergens such as grass pollen [91].

The relationship between asthma and exposure to indoor fungi is not entirely clear [72], although there is some evidence that humidity levels and exposure to fungal spores in homes are decisive factors in developing asthma [92].

Fungal sensitization is a risk factor for the development of severe asthma, decreased lung function, and increased use of asthma control medications [54], and is associated with severe asthma attacks, hospital admissions [93], and asthma deaths [94]. Finally, it is involved in the development of early-onset atopic eosinophilic asthma.

Occupational Fungal Asthma

High exposure to fungal allergens in workers employed in farms, gardens, mills, sawmills, and bakeries is a risk factor for developing allergic reactions and infections in the workplace. Young people with a personal history of respiratory disease are at greater risk of developing *A. alternata* sensitization than older people [95]. Various genera of fungi have been implicated, with *Alternaria*, *Cladosporium* and *Aspergillus* being associated with severe asthma in this context [96].

Diagnosis

Aerobiological monitoring, using sensors to quantify atmospheric levels of fungal spores, is essential for determining the sporulation period of the different populations [58], and can help establish a diagnostic suspicion in the context of a suggestive clinical history. Results vary depending on geography, climate type, and predominant species [97]. A study conducted in Barcelona in 2021 showed the presence of spores throughout the year, with higher concentrations in summer and autumn and interannual variations [58]. *Alternaria* and other fungi of the *Pleosporales* order were predominant. Exposure to fungi in the home may be constant, if the above-mentioned set of requirements are met.

The quality of the fungal allergen extracts for the skin prick test varies from species to species, so these tests are not always effective, as the extracts may have low allergenic activity and high lot-to-lot variability [98] due to the poor standardization of most fungal extracts available. Of the 180 fungal allergens identified to date, only 8 are available for molecular diagnosis to help define clinical patterns associated with sensitizations (Table 3).

Table 3. Allergens available for molecular diagnostics

Allergen	Utility	Biological function
Alt a 1	Major allergen of *Alternaria:* most relevant	Unknown
Alt a 6		Enolase
Asp f 1	Progression to allergy and ABPA	Ribonuclease
Asp f 2		Homology with *Candida albicans* filaggrin-binding protein
Asp f 3		Peroxisome membrane protein
Asp f 4		Homology with ATP-binding cassette transporters
Asp f 6		Manganese superoxide dismutase
Cla h 8	Major allergen of *Cladosporium herbarum*	NADP-dependent mannitol dehydrogenase

Treatment

Atmospheric spore monitoring is a very useful tool for alerting and preventing exposure of sensitized patients during periods of higher concentration [58]. Measures to reduce exposure to fungal spores are of great importance and are summarized below:

1. *Indoors*: control humidity, maintaining a relative humidity < 60%; use antifungal paints; keep the most humid areas of the home dry; avoid storing damp clothing or footwear in cupboards or poorly ventilated areas; avoid the accumulation of dust in the house; do not leave food outside the refrigerator for much time; make sure house plants are in good condition; and avoid the use of room humidifiers. Use dehumidifiers and properly maintained air conditioners, if possible, ensuring they are not a source of contamination.
2. *Outdoors*: avoid contact with dead or decomposing vegetation; avoid the growth of dense vegetation near the home; avoid going out in sunny, windy weather.

If AIT is proposed in patients with *Alternaria* respiratory allergy, IgE-mediated sensitization to Alt a 1 must first be confirmed [96]. Immunotherapy in these patients has been shown to reduce the intensity of symptoms and the use of rescue medication and, in up to 20% of individuals, progressive desensitization at 2 years of treatment can be achieved [81].

Summary of Fungal Spores

Fungal spores are among the most relevant allergens implicated in inducing more severe asthma compare with the produced by other allergens. This could be explained by its high atmospheric concentrations at certain times of the year as well as its small size which will favor access to the lower airways.

Conclusion

House dust mites and fungal spores are aeroallergens of great relevance in the development of asthma.

In recent decades, there has been an increase in the prevalence of respiratory diseases due to sensitization to HDM and fungal allergens, precipitated by lifestyle changes in the 21st century. Exposure to these allergens in the early years of life is related to the development of asthma. Greater exposure confers a greater risk of developing asthma and presenting asthma exacerbations.

Avoidance measures may be a strategy in the prevention of exacerbations, but the treatment of choice in candidate patients that can modify the clinical course of the disease is allergen-specific immunotherapy.

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Chapter 5

Diagnosis of Asthma: Clinical Symptoms and Tests

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Abstract

Asthma is considered a syndrome, that is a chronic inflammatory disease with diverse clinical manifestations and signs with plenty of variations throughout its course. The diagnosis of asthma is a challenge itself, even that current guidelines have aimed to standardize and protocolize its assessment. In this chapter we review several novel aspects of current procedures and tests in order to serve as a guide to a precise approach towards patients with compatible symptoms, and also invite to follow a proposed algorithm which could facilitate its diagnosis.

Keywords: asthma, diagnosis, guidelines

Introduction

Asthma is a chronic inflammatory disease with various clinical manifestations and many variations (phenotypes) [1], showing 2 key defining features:

1. History of respiratory symptoms: Wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity.
2. Variable expiratory airflow limitation.

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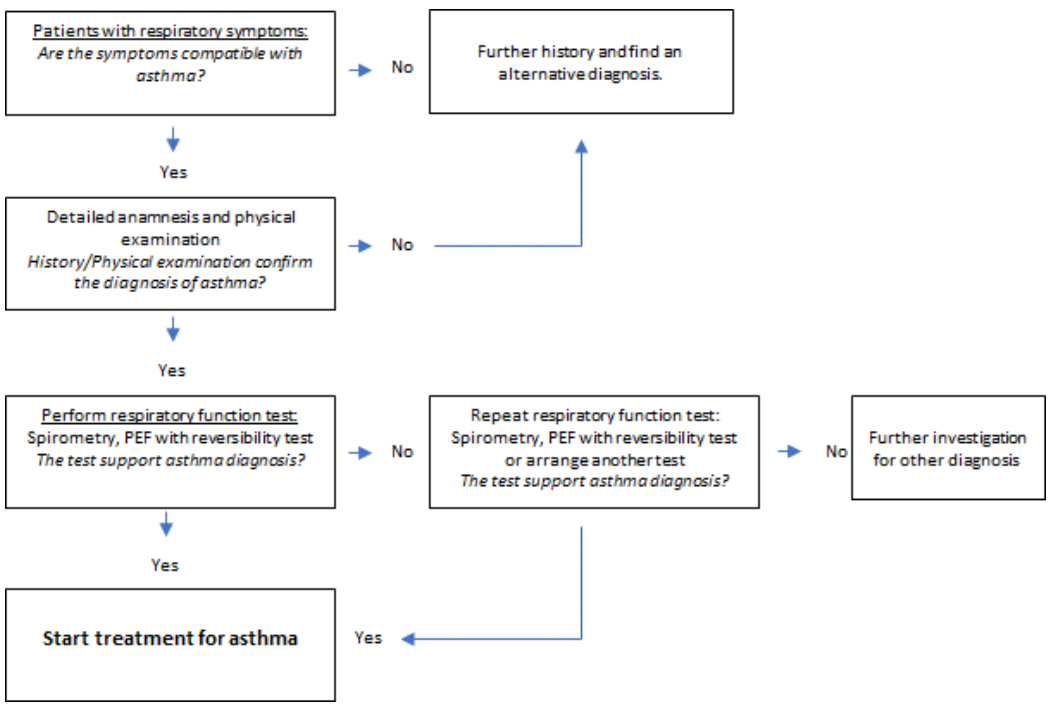
Respiratory function tests are diverse and not available in all centers. Herein we review from basic and commonly used techniques, to advanced respiratory function tests which are limited, in general, to specialized centers.

Clinical Approach

The diagnosis of asthma should be considered in the presence of suspected clinical signs and symptoms, such as wheezing (the most characteristic), dyspnea or shortness of breath, cough, and chest tightness [1, 2]. These are the so-called guiding symptoms, which are typically variable in time and intensity, predominantly at night or in the early morning and could be caused by different triggers such as: viral infections, allergens, tobacco smoke, pollution, allergens, exercise, emotions, etc. We propose an algorithm for the diagnosis for asthma in clinical practice (Figure 1).



Adapted from Global Initiative for Asthma (GINA) 2022 Guidelines [1].

Figure 1. Diagnosis for asthma in clinical practice.

Anamnesis [1, 2]: The patient's anamnesis should also consider: the onset of symptoms and the presence of comorbidities such as:

1. Chronic rhinosinusitis with or without polyps.
2. Atopic diseases: dermatitis, rhinitis and other atopic diseases.
3. Family history of asthma or atopy.

All above increase the likelihood of a diagnosis of asthma [1, 2]. Important differential diagnoses shall be considered as COPD (Table 1) and others (Table 2).

Table 1. Differences between asthma and COPD [2]*

Characteristics	Asthma	COPD
Age of onset	Any age	After the age of 40
Smoking	Indifferent	Always
Comorbid Atopic Disease	Frequent	Infrequent
Family background	Yes	Indifferent
Reversibility of bronchial obstruction	Significant	Less significant
Symptom variability	Yes	No
Glucocorticoid response	Almost always, very good	Undetermined or variable

* COPD: Chronic Obstructive Pulmonary Disease.

Table 2. Differential diagnosis [2]

Age of onset	Disease	Symptoms
Between 15 and 40 years old	Inducible laryngeal obstruction	Dyspnea, inspiratory stridor
	Hyperventilation	Dizziness, paresthesia
	Foreign body inhaled	Sudden onset symptoms
	Cystic fibrosis	Coughing and excessive mucus
	Bronchiectasis	Recurrent infections
	Congenital heart disease	Heart murmurs
	Pulmonary embolism	Chest pain, tachycardia, sudden onset dyspnea
Over 40 years old	Inducible laryngeal obstruction	Dyspnea, inspiratory stridor
	Hyperventilation	Dizziness, paresthesia
	Bronchiectasis	Recurrent infections
	Parenchymal lung disease	Exertional dyspnea, dry cough
	Heart failure	Exertional dyspnea, night symptoms
	Pulmonary embolism	Chest pain, tachycardia, sudden onset dyspnea

Key Questions for the Identification of Patients with Suspected Asthma [1, 2]

- Have you ever had “wheezing” in your chest?
- Have you had a cough or any other symptom, especially at night or on your waking?
- Have you had coughing, wheezing, shortness of breath at certain times of the year or in at certain times of the year, or in contact with animals, plants, tobacco or at work?
- Have you had coughing, wheezing, shortness of breath after moderate or strenuous exercise?
- Have you ever had colds that “go down in your chest”?
- Have you used inhaled medicines that relieve or reduce these symptoms?
- Do you have any allergies? Do you have any family members with asthma or allergies?
- Do your symptoms occur variably over time or and vary in intensity?

Ask About [1, 2]

- Work environment (exposure to toxic substances).
- Exposure to domestic animals.
- Home conditions (exposure to plants, humidity's).
- Sportive activities.

Physical Examination [1]

Physical examination in asthmatic patients is often normal.
Most frequent finding: wheezing on auscultation, especially on forced expiration.
Anterior rhinoscopy: Nasal obstruction, search for nasal polyps.
However, a normal physical examination does not rule out the diagnosis of asthma.

Respiratory Function Tests

Spirometry [3]

Expiratory airflow is generally assessed by spirometry, with the most important indices being the forced expiratory volume in 1 second and forced vital capacity (FEV1, FVC), and the FEV1/FVC ratio [3].

Maximal airflow is generally assessed spirometrically and may be limited by different diseases that lead to different outcomes [3]:

- Impaired expiratory muscle function (weakness or poor effort-neuromuscular ventilatory impairment), reduced elastic recoil or reduced chest wall expansion which reduce peak expiratory flow (PEF), FEV1 and FVC, with a variable FEV1/FVC ratio.
- Physical obstruction of a central airway (outside of lung parenchyma), which can affect the trachea/major bronchi and leads to a disproportionate reduction in PEF compared to FEV1 with variable FEV1/FVC ratio.
- Intra-pulmonary airflow obstruction produced by premature airway collapse, bronchoconstriction or airway inflammation/wall thickening/oedema leading to airway narrowing. These obstructed airways reduce PEF and FEV1 to a much greater extent than any reduction in FVC so the FEV1/FVC is characteristically low.

While we recognize the normal physiologic events involved in expiratory "airflow limitation" we use the term "airflow obstruction" to refer to pathological reduction in airflow from the lungs that leads to a reduced FEV1/FVC ratio.

An obstructive ventilatory impairment is defined by FEV1/FVC below the lower limit of normal (LLN), which is defined as the 5th percentile of a normal population, however this contrasts with the definitions suggested by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and the ATS/ERS guidelines on COPD which use a fixed FEV1/FVC value of 0.7 to define an obstructive ventilatory impairment.

The widely used cut-offs of 80% predicted for FEV1 (% predicted = Observed*100/ Predicted) and the 0.70 cut-off for FEV1/FVC are not recommended. Percent of predicted does not consider the observed age-related changes in measurement variability.

Is important to remember that in people with early manifestations of lung disease, and especially in children, spirometry values can be normal even in those with confirmed disease.

Examination of the expiratory flow-volume loop can be very helpful in assessing an upper airway obstruction. When a forced expiratory effort is acceptable, the repeatable pattern of a plateau of forced inspiratory flow in the presence of relatively normal expiratory flow suggests variable, extrathoracic upper airway obstruction. Conversely, the pattern of a repeatable plateau in forced expiratory flow with relatively normal inspiratory flow suggests variable, intrathoracic central airway obstruction. The pattern of a repeatable plateau in both forced inspiratory and expiratory flows suggests fixed central or upper airway obstruction [3].

Bronchodilator Response (BDR)

BDR test assesses the change in respiratory function in response to bronchodilator administration. The BDR result reflects the integrated physiological response of airway epithelium, nerves, mediators, and airway smooth muscle, along with structural and geometric factors that affect airflow in the conducting airways [3, 4].

The choice of bronchodilator, dose, and mode of delivery is a clinical decision. The relative merits of different protocols are unclear. The 2022 Spanish Guideline for Asthma recommended that 4 successive inhalations of 100 μg salbutamol, or equivalent, should be administered via a pressurized inhaler with a spacer chamber and spirometry shall be repeated after 15 minutes [2]. A positive response (or significant bronchodilation) is an increase in FEV1 ≥12% and ≥200 ml from baseline [2, 5]. An alternative bronchodilation criterion is an increase in peak expiratory flow >20% [2, 6]. Reversibility can also be identified by an improvement in FEV1 or PEF after 2 weeks of treatment with systemic glucocorticoids (40 mg/day prednisone or equivalent) or 2-8 weeks of inhaled glucocorticoids (1,500-2,000 μg/day fluticasone propionate or equivalent) [2].

However, changes in FEV1 and FVC following bronchodilator responsiveness testing should be expressed as the percent change relative to the individual's predicted value. A change >10% of the predicted value should be considered as a positive response [4].

Severity of Lung Function Impairment

A three-level system to assess the severity of lung function impairment using z-score.

Values should be used [3, 4]:

- z-score >-1.645: *normal*.
- z-scores between -1.65 and -2.5: *mild*.
- z-scores between -2.5 and -4: *moderate*.
- z-scores <-4: *severe*.

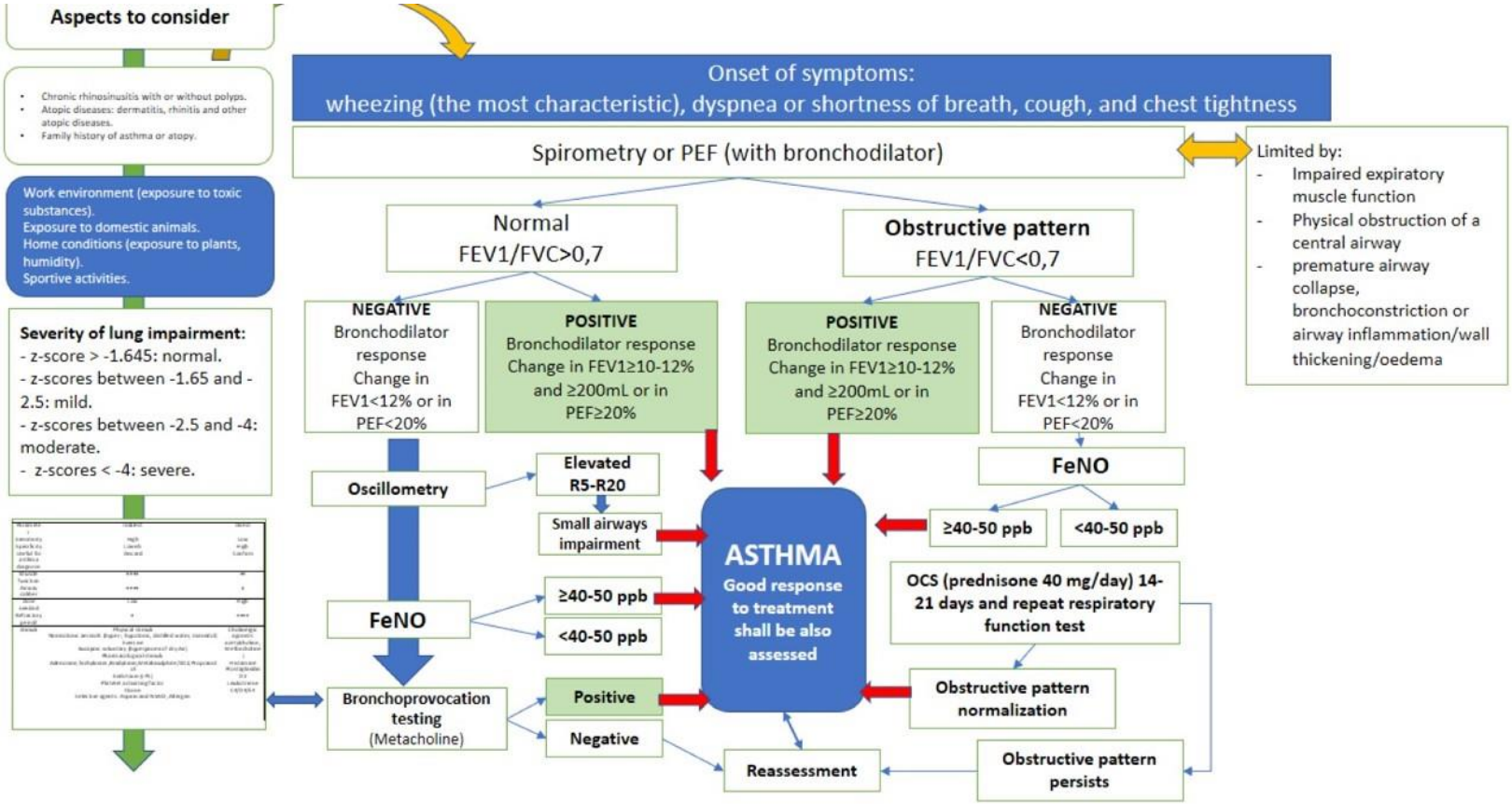


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Figure 2. Algorithm for the assessment of asthma Extracted from [4].

Z-scores express how far an observed lung function value is from the predicted value after accounting for sex, age, height, and ancestral grouping, expressed in standard deviations. This is the method recommended for determining the limit of normality and for stating the degree of lung function impairment (Figure 2). Percentile values are easily derived from z-scores and explicitly indicate the probability a healthy individual would have a result below this level and where the individual's result lies in relation to the healthy population. Percentile values are useful in assessing results around the normal range but are less useful for extreme values.

FeNO

The fractional concentration of exhaled nitric oxide (FeNO) is modestly associated with levels of sputum and blood eosinophils. FeNO has not been established as useful for ruling in or ruling out a diagnosis of asthma. FeNO is higher in asthma that is characterized by type 2 (T2) airway inflammation, but it is also elevated in non-asthma conditions (e.g., eosinophilic bronchitis, atopy, allergic rhinitis, eczema), and it is not elevated in some asthma phenotypes (e.g., neutrophilic asthma). FeNO is lower in smokers and during bronchoconstriction and in the early phases of allergic response; it may be increased or decreased during viral respiratory infections [1, 2].

Results of FeNO measurement at a single point in time should be interpreted with caution. In several studies of FeNO-guided treatment, problems with the design of the intervention and/or control algorithms make comparisons and conclusions difficult.

In comparation with COPD, asthma have a high level of FENO > 50 parts per billion (ppb) in non-smokers and is moderately associated with eosinophilic airway inflammation.

In children, FeNO-guided treatment significantly reduces exacerbation rates compared with guidelines-based treatment (evidence A). However, further studies are needed to identify the populations most likely to benefit from sputum-guided or FeNO-guided treatment, and the optimal frequency of FeNO monitoring.

Does FeNO Help in Deciding Whether to Commence Inhaled Corticosteroids (ICS)?

In studies mainly limited to non-smoking patients, FeNO > 50 parts per billion (ppb) has been associated with a good short-term response to ICS. However, these studies did not examine the longer-term risk of exacerbations. Such evidence therefore does not mean that it is safe with regard to exacerbations to withhold ICS in patients with low initial FeNO. Consequently, in patients with a diagnosis or suspected diagnosis of asthma, measurement of FeNO can support the decision to start ICS but cannot be used to decide against treatment with ICS. Based on past and current evidence, GINA recommends treatment with daily low dose ICS or as-needed low dose ICS-formoterol for all patients with mild asthma, to reduce the risk of serious exacerbations.

Oscillometry

Expiratory airflow is generally assessed by spirometry, with the most important indices being the FEV1, FVC, and the FEV1/FVC ratio. The earliest changes associated with respiratory

diseases that produce airflow obstruction are thought to occur in the smaller, more distal airways. In people with early manifestations of lung disease, and especially in children, spirometry values can be normal even in those with confirmed disease [3, 4].

A number of attempts have been made to quantify this small airway impairment, especially when the FEV1 and the FEV1/FVC ratio are normal ("isolated small airway dysfunction"). A common approach is to measure the average flow between 25% and 75% of exhaled FVC (FEF 25-75%). Other tests such as oscillometry, multiple breath washout, and imaging, may also provide evidence of airflow obstruction when FEV1/FVC is normal [3, 4].

Higher frequency sounds (>20 Hz) remain in the larger airways, while lower frequency sounds (<15 Hz) travel to the small airways and lung parenchyma.

The differential effect of airway size on the transmission of sound waves of differing frequencies enables the resistances of small airways to be distinguished from that of large airways. The resistance at 5 Hz (R5) is taken to represent the total airway resistance, while the resistance at 20 Hz (R20) gives the resistance of only the large airways. Consequently, the difference (R5-R20) gives the resistance due to small distal airways. R5-20 has been shown to correlate well with FEF 25-75 in terms of small airways impairment [7].

The measurements of respiratory system resistance by the non-invasive techniques of oscillometry, which require only tidal breathing, may be useful in individuals who are unable to perform a maximal forced expiratory maneuver, including very young children.

Bronchodilator challenge after impulse oscillometry is a matter of debate, as it is not fully validated for routine clinical practice, but it could be useful after further research.

Bronchoprovocation Tests

Several types of bronchoprovocation testing are available to assess airway responsiveness (Table 3), both direct and indirect methods, including pharmacologic challenge, exercise challenge, eucapnic voluntary hyperpnea, food additive challenge and antigen challenge [4, 8, 9].

All in all, there are differences between the methods of assessing airway hyperresponsiveness, specified in Table 3 and their indications [6, 10, 11] and contraindications and precautions [1, 2, 4, 11] are comprised in Table 4 [10].

Measurement of airway responsiveness by bronchoprovocation testing is potentially useful for several reasons:

- Failure to show airway hyperresponsiveness argues against the diagnosis of asthma.
- Airway hyperresponsiveness may be the sole objective evidence of airway dysfunction.
- Airway hyperresponsiveness is quantitatively associated with the presence and severity of disease.
- Suppression of airway responsiveness is one of the outcomes that can be used to assess new asthma therapies.
- The occurrence of airway hyperresponsiveness in an asymptomatic person may help predict the future development of asthma.

Table 3. Direct and indirect methods of bronchoprovocation testing

Parameter	Indirect	Direct
Sensitivity	High	Low
Specificity	Lowish	High
Useful for asthma diagnosis	Discard	Confirm
Muscle function	++++	++
Airway caliber	++++	±
Dose needed	Low	High
Refractory period	+	++++
Stimuli	Physical stimuli: Nonisotonic aerosols (hyper-, hypotonic, distilled water, mannitol) Exercise Eucapnic voluntary (hyperpnoea of dry air) Pharmacological stimuli: Adenosine, Tachykinins, Bradykinin, Metabisulphite/SO_2, Propranolol Endotoxin (LPS) Platelet activating factor: Ozone Selective agents: Aspirin and NSAID, Allergen	Cholinergic agonists Acetylcholine, Methacholine Histamine Prostaglandin D2 Leukotriene C4/D4/E4

Table 4. Indications and contraindications of bronchoprovocation tests

Indications	Contraindications
• Diagnosis of asthma • Symptoms compatible with asthma • Normal pulmonary function test • No response to bronchodilator • Atypical symptoms of bronchospasm • Non-specific asthma symptoms • Occupational asthma suspicion, reactive airways dysfunction syndrome or irritant induced asthma • Professional mandatory screening test for asthma (scuba divers, military personnel, etc.) • Assessment of therapy response (non-specific bronchoprovocation challenge) • Evaluation of specific asthma triggers: in case of assessing reactivity to specific food additives, occupational or environmental antigens	• Unstable cardiac disease, a myocardial infarction or stroke within the past three months. • In the absence of signs or symptoms of significant airflow obstruction. In patients with significant baseline impairment in the FEV1 a bronchodilator reversibility study is usually indicated instead of bronchoprovocation. • Patients with an FEV1 < 60% predicted (adults or children) or an FEV1 < 1.5 liters (adults).

Pharmacologic Challenge

A number of provocative agents are administered via a nebulizer device (methacholine, histamine, adenosine) [3, 4, 10, 12].

Methacholine

Methacholine, a derivative of acetylcholine, is the agent most commonly used for bronchoprovocation and is an acceptable form of bronchoprovocation for assessing asthma in Olympic athletes.

After baseline spirometry, the diluent is administered via nebulizer during tidal breathing for at least one minute. After inhalation of the aerosol, the FEV1 is measured at 30 and 90 seconds. The dose or concentration of methacholine is sequentially increased one step at a time until a decrease in FEV1 greater than 20 percent or a 35 to 40 percent decrease in specific airways conductance (SGaw) is observed.

Typically, when there is a positive test, the FEV1 decreases more than 20 percent, so the dose of the inhaled agent is referred to as the provocative dose or PD20. Generally, a methacholine PD20 of 200 μg or a PC20 of 8 mg/ml (100 μg or 4 mg/ml for SGaw) or less is considered a positive test. A PD20 greater than 400 μg (PC20 greater than 16 mg/ml) is considered a negative test.

Mannitol

Mannitol dry powder is rapidly inhaled in progressively increasing doses. The FEV1 is measured at baseline and repeated at 1 minute after each dose, the highest of two repeatable values is used. A 15% fall in FEV1 (from baseline) at a total cumulative dose of <635 mg (known as the provocative dose or PD15) is considered a positive response, or if the FEV1 decreases by 10% from the previous dose. Mannitol challenge appears to be safe, however, cough is a common side effect.

Interpretation [3, 4, 12]

To interpret a bronchoprovocation challenge test, a graph is drawn plotting the fall in the FEV1 versus the dose or concentration of the provocative agent. This dose of the provocative agent (provocative dose or concentration of methacholine for a 20% fall in the FEV1 or provocative dose of mannitol for a 15% fall in FEV1) is used to interpret the test. Through experience with each agent, doses and concentrations in the normal and the asthmatic ranges have been ascertained.

As noted above, the PD20-FEV1 for methacholine in patients with asthma is usually 200 microg (PC20 8 mg/mL) or less. A graded system of borderline (100 to 400 μg, 4 to 16 mg/mL), mild (25 to 100 μg, 1 to 4 mg/mL), moderate (6-25 μg, 0.25 to 1 mg/mL) and marked (<6 μg, <0.25 mg/mL) airway hyperresponsiveness has been proposed, although its clinical utility has not been fully determined.

False positive results may be seen in patients with allergic rhinitis, cystic fibrosis, heart failure, chronic obstructive pulmonary disease and bronchitis. The negative predictive value is the most useful aspect of methacholine challenge.

Difficult Interpretations

- *Asymptomatic patient, positive test*: Approximately 1% to 7% of the population have reactive airways (up to 26% if smokers are included) but are otherwise normal or asymptomatic. These individuals may have asthma but do not perceive any symptoms.
- *History suggestive of asthma, negative test*: A few examples of this are:
  - The inhalation of an antigen with a subsequent late asthmatic response, as seen in certain occupational exposures. Many patients are hyperresponsive for weeks, but gradually return to normal.
  - Paradoxical vocal cord motion (vocal cord dysfunction) may result in symptoms suggestive of asthma but a negative challenge test result if the only endpoint is the change in FEV1.
  - Central airway obstruction by a tumor, polyp, or foreign body can also mimic asthma symptomatically, but results in a negative methacholine challenge.
- *History suggestive of asthma, atypical spirometry pattern*: Upper airway responses to various challenge procedures are common and can lead to confusing results.

Exercise Challenge

Exercise may be the only trigger for bronchoconstriction in patients with asthma. Guidelines from the American Thoracic Society (ATS) state that symptoms alone are inadequate to diagnose exercise-induced bronchoconstriction (EIB), and a challenge test with serial lung function measurements is necessary. Bronchodilator therapy is withheld prior to testing as bronchodilators can block a bronchospastic response to exercise testing, causing a false negative result.

The presence of EIB is determined using the following general protocol:

- Spirometry is performed prior to exercise, and at 5, 10, 15, 20 and 30 minutes thereafter. Bronchoconstriction usually occurs 10 to 15 minutes after the end of the exercise.
- Monitoring during test (electrocardiogram, blood pressure, pulse oximetry and minute ventilation).
- Control of the temperature and humidity of the inhaled air are needed to ensure test reliability.
- The preferred modes of exercise are either a motor-driven treadmill or the electromagnetically braked cycle ergometer.
- The exercise protocol is selected to allow the patient to achieve 80% to 90% of predicted maximum heart rate in the first 2 minutes of exercise and maintain it for the remaining 8 minutes of the test.
- The test is positive if the FEV1 decreases by 10%, although a fall of 15% is more diagnostic.

Quality of Life Assessment [1, 2]

The level of asthma control is the extent to which the manifestations of asthma can be observed in the patient or have been reduced or removed by treatment. Asthma control should be described in terms of both symptom control and future risk domains. Both should always be assessed. Poor symptom control is also strongly associated with an increased risk of asthma exacerbations.

Lung function is an important part of the assessment of future risk; it should be measured at the start of treatment, after 3-6 months of treatment (to identify the patient's personal best), and periodically thereafter for ongoing risk assessment.

To assess symptom control, ask about the following in the past 4 weeks: frequency of asthma symptoms (days per week), any night waking due to asthma or limitation of activity, and frequency of short acting beta agonist (SABA) reliever use for relief of symptoms.

When different systems are used for assessing asthma symptom control, the results correlate broadly with each other, but are not identical. Respiratory symptoms may be non-specific so, when assessing changes in symptom control, it is important to clarify that symptoms are due to asthma.

Numerical 'asthma control' tools: these tools provide scores and cut points to distinguish different levels of symptoms control, validated against health care provider assessment. Numerical asthma control tools are more sensitive to change in symptoms control than categorical tools.

Examples of numerical asthma control tools for assessing symptoms control are:

Asthma Control Questionnaire (ACQ): Scores range from 0-6 (higher is worse). The ACQ score is the average of 5, 6 or 7 items: all versions include 5 symptom questions; ACQ-6 includes SABA reliever use; and ACQ-7, pre bronchodilator FEV1. The authors stated that ACQ $\leq$ 0.75 indicates a high probability that asthma is well-controlled; 0.75-1.5 as a 'grey zone'; and $\geq$1.5 a high probability that asthma is poorly controlled, based on concepts of asthma control at the time. GINA prefers ACQ-5 over ACQ-6 or 7 because ACQ has not been validated with ICS-formoterol as the reliever, and if ACQ is used in adjustment of treatment, inclusion of FEV1 in the composite score could lead to repeated step-up in ICS dose for patients with persistent airflow limitation.

Asthma Control Test (ACT): Scores range from 5-25 (higher is better). Scores of 20-25 are classified as well controlled; 16-19 as not well-controlled; and 5-15 as very poorly controlled asthma. The ACT has 4 symptoms/reliever questions plus patient self-assessed control.

In children, as in adults, assessment of asthma symptom control is based on symptoms, limitation of activities and use of rescue medication. Careful review of the impact of asthma on a child's daily activities, including sports, play and social life, and on school absenteeism, is important. Many children with poorly controlled asthma avoid strenuous exercise so their asthma may appear to be well controlled. This may lead to poor fitness and a higher risk of obesity.

Children vary considerably in the degree of airflow limitation observed before they complain of dyspnea or use their reliever therapy, and marked reduction in lung function is often seen before it is recognized by the parents. Parents may report irritability, tiredness, and changes in mood in their child as the main problems when the child's asthma is not controlled. Parents have a longer recall period than children, who may recall only the last few days;

therefore, it is important to include both the parent's and child's information when the level of symptom control is being assessed.

Assessment of QoL of Asthma in Children

Several numeric asthma control scores have been developed for children. As this is not the objective of the chapter, we will merely enunciate the scores. For further information, see references. These include:

- Childhood Asthma Control Test (c-ACT) with separate sections for parent and child to complete
- Asthma Control Questionnaire (ACQ)

Some asthma control scores for children include exacerbations with symptoms. These include:

- Test for Respiratory and Asthma Control in Kids (TRACK)
- Composite Asthma Severity Index (CASI)

Diagnosis of Asthma in Special Conditions [1]

Occupational Asthma and Work-Exacerbated Asthma

Every patient with a suspect diagnosis of adult asthma should be asked about occupational exposures, and whether their asthma is better when they are away from work or worsen during workdays. It requires confirming the diagnosis of asthma and demonstrating its relationship to the work environment.

The methacholine test for diagnostic test has a high negative predictive value, due to its high sensitivity (87%-95%), especially if the patient has had recent exposure, but has a low specificity (36%-40%). The gold standard is a bronchial provocation test with the suspected causal agent. For further information see chapter number 6.

Pregnant Women

Between 2%-13% of pregnant women suffer from asthma, which is the most common respiratory disorder in pregnancy. Asthma control changes during the pregnancy: one-third of women's asthma symptoms worsen; in one-third they improve and in one-third they remain unchanged [1, 13].

Diagnosing asthma in the pregnant patient is the same as for the nonpregnant patients with 2 important exceptions: Methacholine and allergen skin testing are not performed [1, 13].

As physicians it is important to ask all pregnant women and those planning pregnancy whether they have asthma and advise them about the importance of taking asthma controller treatment for the health of mother and baby [1, 2, 13].

Patients with Persistent Cough as the Only Symptom [1, 2]

This may be due chronic airway cough syndrome derived from post-nasal drip, chronic sinusitis, gastroesophageal reflux disease (GERD), inducible laryngeal obstruction also known as vocal cord dysfunction, eosinophilic bronchitis, or cough variant asthma.

Exercised-Induced Asthma

It is defined as a transient and reversible obstruction of the lower airways triggered by strenuous exercise. Exercise-induced bronchoconstriction most commonly occurs in patients diagnosed with asthma, but it also may occur in patients without asthma. Prevalence is higher in athletes, children, and adolescents [1, 2, 4]. Symptoms such as cough and dyspnea with wheezing, usually occur during or after exercise, with a refractory period of 2-3 days, after exercise [1, 2, 4]. Self-defined symptoms are not diagnostic. A fall in FEV1 above 10% from the previous value, measured 30 minutes after exercise and compared to the previous FEV1 is the diagnostic test [14, 15].

Aspirin-Exacerbated Respiratory Disease (AERD)

A history of exacerbation followed by aspirin ingestion or other NSAIDs is highly suggested of AERD. Aspirin challenge is the gold standard for the diagnosis: oral, bronchial or nasal [16]. For further information see corresponding chapter number 14.

Asthma and COVID-19 [1, 2]

People with asthma do not appear to be at increased risk of acquiring COVID-19. For further information see Chapter 11.

Conclusion

Asthma is a challenge due to the plethora of symptoms, features, and differential aspects that vary in severity and frequency. A structured diagnosis shall be attempted in order to better characterize the patient with asthma to implement the best possible treatment following a patient-centered strategy and always taking into account the patient as a whole, including the potential atopic history, past treatments and socioeconomic aspects.

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Chapter 6

Occupational Allergic Asthma

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Abstract

Occupational asthma (OA) is defined as a type of asthma caused by the workplace. OA is usually due to an allergic response to high or low molecular weight agents, either through the interaction with specific IgE antibodies or by other immune mechanisms which lead to chronic and acute airway inflammation. About 500 agents encountered in the workplace have been reported to induce OA in susceptible individuals.

Allergic OA, which appears after a latency period necessary for the worker to acquire sensitization to the causal agent, is the most common type of OA, accounting for more than 90% of cases. The diagnosis of OA should be performed using objective methods. Complete cessation of exposure to the offending agent, which usually implies removal of the affected person from work, is the mainstay in the treatment of allergic OA. Thus, proper management of a patient in whom OA is suspected depends on the establishment of a definite diagnosis.

Keywords: occupational asthma, allergic asthma, workplace agents

Introduction

Work-related asthma (WRA) is any type of asthma that worsens at work. It is a highly prevalent lung disorder that is associated with undesirable effects on psychological status, quality of life, workplace activity and socioeconomic status [1, 2]. WRA encompasses 2 different entities: occupational asthma (OA) and work-exacerbated asthma. OA is defined as a type of asthma caused by workplace exposures, whereas work-exacerbated asthma refers to asthma triggered by various work-related factors, such as irritants or exercise, in workers who are known to have concurrent or pre-existing asthma [3]. OA is usually due to an allergic response to high-molecular-weight (HMW, ≥5 kDa) or low-molecular-weight (LMW, <5 kDa) agents, either

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through the interaction with specific IgE antibodies or by other immune mechanisms. These allergic events lead to chronic and acute airway inflammation, which is similar when OA is caused by either type of agent [4]. Allergic OA is the most common type of OA, and represents more than 90% of cases [1, 3]. Less commonly, OA can result from high level irritant exposures at work. The different types of WRA are shown in Figure 1.

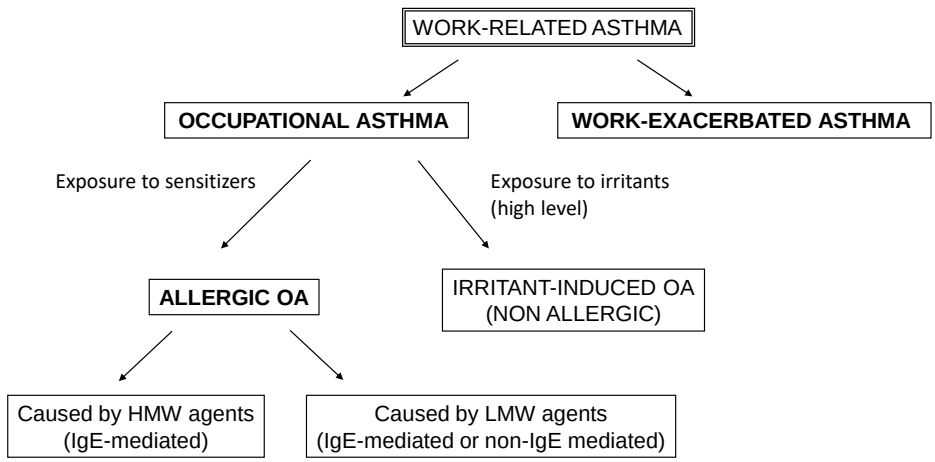

Figure 1. Classification of work-related asthma.

Epidemiology

WRA is the commonest occupational lung disease of short latency industrialized countries and those undergoing rapid economic development [5]. An international prospective population-based study (ECRHS-II) showed that the population-attributable risk for adult asthma due to occupational exposures ranged from 10% to 25%, equivalent to an incidence of new-onset OA of 250-300 cases per million people per year [6]. The frequency of OA, however, varies among types of industries, and it is dependent on physicochemical properties of the inhaled agent, level and duration of exposure, host factors and industrial hygiene practices.

A recent American Thoracic Society (ATS)/European Respiratory Society (ERS) statement on the occupational burden of nonmalignant respiratory diseases, using pooled data from 9 longitudinal studies, reported a population attributable fraction for the occupational contribution to incident asthma of 16% [95% confidence interval (CI), 10-22%] [7].

The proportion of women in the labour market has increased significantly in the past 25 years, and sex issues in occupational health are becoming more relevant. Moscato et al. [8] published a systematic review on sex differences in occupational respiratory and cutaneous allergic diseases, including asthma. The prevalence of OA among males and females varies significantly depending on the occupations, workplace exposures, industries and job categories. Overall, the sex distribution of WRA varies across countries without clear global difference. Notwithstanding, occupational rhinitis and contact dermatitis are higher in women, although it is unclear if this is due to a sex effect or to differences in work exposure.

Causal Agents

Occupational exposures are significant health hazards and have been associated with several allergic and non-allergic conditions, both as inducers and aggravating factors. About 500 agents encountered in the workplace have been reported to induce OA in susceptible individuals. These agents can be divided into 3 major categories based on their pathogenesis: HMW agents, LMW agents and irritants [1, 3]. New cases of OA caused by HMW and LMW agents are continually being reported [9], and it is important for physicians to stay alert and suspect OA in workers exposed to these substances.

The Association of Occupational and Environmental Clinics (AOEC) has elaborated a web based listing of agents associated with new onset WRA[1][10], and based in their review, substances were designated either as a sensitizing agent or an irritant. A total of 327 substances were identified as causative agents of OA; 173 (52.9%) were coded as sensitizers, 35 (10.7%) as generally recognized as an asthma causing agent, 4 (1.2%) as irritants, 2 (0.6%) as both a sensitizer and an irritant and 113 (34.6%) agents were pending of revision.

In an evidence-based study, Baur et al. [11] found that the strongest evidence of association with an individual agent, profession or worksite was found to be the co-exposure to various laboratory animals. An association with moderate evidence level was obtained for α-amylase from *Aspergillus oryzae*, various enzymes from *Bacillus subtilis*, papain, bakery (flour, amylase, storage mites), western red cedar, latex, psyllium, farming (animals, cereal, hay, straw and storage mites), storage mites, rat, carmine, egg proteins, atlantic salmon, fishmeal, norway lobster, prawn, snow crab, seafood, trout and turbot, reactive dyes, toluene diisocyanates and platinum salts. Table 1 shows the causal agents and jobs more commonly associated with allergic OA.

Table 1. Common specific agents and jobs associated with allergic OA

Causative agents	Selected jobs or industries
High-molecular-weight	
Cereal flours/grain dust	Bakers and pastry makers, grain handlers
Animal epithelia, hairs, secretions	Farmers, livestock workers, veterinarians
Seafood and other food-derived proteins	Food processors, cooks, butchers
Latex proteins	Healthcare and social workers
Enzymes (from bacterial, fungal and plant origin)	Detergent industry workers, researchers, bakers, food technology, livestock farmers
Vegetal gums	Printing, food industry, carpet manufacture
Insects, mites	Farmers, greenhouse workers, researchers
Low-molecular-weight	
Isocyanates	Spray painters, lacquerers, foam workers
Metals (e.g., platinum, nickel sulfate)	Alloy and refinery workers, electroplating
Persulfate salts	Hairdressers
Acrylates (methacrylate, cyanoacrylate)	Glue handlers, dentists, artificial nail workers
Aldehydes (e.g., glutaraldehyde)	Hospital and laboratory workers
Acid anhydrides (e.g., trymellitic anh.)	Plastics industry, epoxy resins workers
Amines (e.g., ethanolamine)	Metal workers (cutting fluids), various
Soldering flux (colophony)	Welders
Mixed or uncertain relevant compounds	
Wood dust (red cedar, iroko, obeche, etc.)	Woodworkers, carpenters, sawmill workers

[1] http://www.aoecdata.org/ExpCodeLookup.aspx.

Evaluation of the worker with suspected OA should consider the type of exposure (HMW allergens, LMW chemical sensitizers, irritants) since exposure to these substances usually determines the type of asthma. As will be discussed in this chapter, the clinical phenotype of OA from a HMW sensitizer, associated with specific IgE antibodies, is very similar to that of non-occupational allergic asthma, for which it can serve as a model, and pathology is typically eosinophilic. In contrast, although chemical exposures are common outside the workplace setting, recognized asthma due to LMW sensitizing agents may or may not be associated with eosinophilic inflammation, has generally been limited to occupational cases and the contribution of chemical sensitizers to asthma outside the workplace is currently unknown [12].

Clinical Manifestations and Phenotypes

OA should be suspected in every adult with new-onset asthma, and the suspicion is increased if the patient reports worsening of asthma symptoms on working days compared with weekends or holidays. This worsening may occur within minutes of the onset of exposure at work (early asthmatic responses) or after several hours (late asthmatic responses). The patient may also exhibit both immediate and late (dual or biphasic) asthmatic responses. The clinical history has been found to have high sensitivity but low specificity for the diagnosis of OA caused by several agents [3].

It is usually considered that the patient with OA is symptomatic at work and shows a marked improvement during weekends and holidays. However, this well-defined pattern occurs mainly at the onset of the disease. After prolonged exposure, the disease tends to show a more insidious course. In this situation, the patient may have predominant nocturnal symptoms, may react not only to the occupational agents but also to nonspecific irritants found outside the workplace, and at a final stage, the patient may lose the pattern of reversibility away from work. Then, the suspicion of the work-relatedness of asthma may be much more difficult. A common misconception is the assumption that if asthma symptoms do not improve away from work, the asthma is not work-related. Exposure of an asthmatic patient to an agent that is known to cause OA should be considered sufficient grounds to investigate the possible implication of that agent in the asthmatic symptoms.

Over the last few years, efforts are being made to better identify the different phenotypes of work-related asthma and specifically of OA [12, 13]. The traditional classification of OA in allergic or sensitizer-induced and irritant-induced (nonimmunologic) subtypes is helpful for diagnostic purposes and to implement the most adequate management and prevention strategies. A recent article [12] discusses the main OA clinical phenotypes: OA caused by HMW or LMW agents, irritant-induced asthma, and occupational asthma/chronic obstructive pulmonary disease (COPD) overlap. This clinical classification, which is based on pathophysiological mechanisms, is practical, easy to perform and with relevant implications for management and prevention. The assessment of the inflammatory profile (eosinophilic and noneosinophilic) within the airways is useful to define the inflammatory phenotypes of OA, as well as to confirm the diagnosis and to prescribe antiinflammatory and/or biological therapy [13]. Given the marked overlap between the inflammatory and the aforementioned clinical phenotypes of OA, both classification approaches are necessary for a better characterization of the patients.

Vandenplas et al. [14] have studied a large European multicenter cohort (E-PHOCAS) of 1,180 individuals with OA diagnosis based on a positive-specific inhalation challenge (SIC) result. LMW agents were involved in 635 (53.8%) and HMW agents in 544 (46.1%) of the SICs, while in 13 individuals the causal agent was not identified. Individuals with OA caused by HMW agents, as compared with LMW agents, were slightly younger, more often atopic, never smokers and more frequently had work-related rhinitis, conjunctivitis and wheezing at work, but less often experienced chest tightness and sputum production. Individuals with OA caused by HMW agents more frequently showed peripheral blood eosinophilia (>300 cells/ml), and a higher increase in post-SIC fractional exhaled nitric oxide (FeNO) levels, than individuals with OA due to LMW agents. However, no differences in sputum eosinophil counts were found between both groups at baseline nor post-SIC. Individuals with OA caused by LMW agents had a significantly higher rate (26%) of severe asthma exacerbations while exposed at work than individuals sensitized to HMW agents (19%), but the latter individuals showed a higher risk of airway inflammation.

This study found phenotypic differences between OA induced by HMW and LMW agents in their clinical, inflammatory and functional characteristics, supporting the need to correctly identify the OA phenotype, which should lead to a more personalized management approach. In the same cohort, Vandenplas et al. [15] found that 16.2% (95% CI, 14.0-18.7%) individuals had severe OA defined by ERS/ATS criteria [16]. Multivariable logistic regression analysis revealed that severe OA was associated with persistent exposure to the causal agent at work; a longer duration of the disease; a low level of education; childhood asthma; and sputum production. This study indicates that a substantial proportion of individuals with occupational asthma manifests with severe asthma and identifies potentially modifiable risk factors for severe occupational asthma that should be targeted to reduce the adverse impacts of the disease.

Precision medicine should also be used in the evaluation of patients with suspected WRA and can help in the identification of clinical phenotypes [17].

Allergic Occupational Asthma Caused by High-Molecular Weight Agents

The phenotype of OA caused by a HMW agent is induced by a workplace sensitizer, usually a protein or glycoprotein with the ability to act as a complete antigen and stimulate the adaptive immune system causing allergic sensitization, which is very similar to the pathogenesis of common allergic asthma. There are more than 500 identified causative agents of OA, and at least 372 HMW allergens have been identified as causative agents of OA by evidence-based medicine [11]. The pathophysiology usually involves an IgE-dependent mechanism and activation of the type 2 immunoinflammatory pathway in the airways, with involvement of dendritic cells, mast cells, CD4+ Th2 lymphocytes, B lymphocytes/plasma cells and eosinophils [12]. Specific IgE antibodies bind to high affinity receptors in mast cells and basophils and, upon exposure to the sensitizing agent, cause the release of potent proinflammatory mediators and chemoattractant molecules, that result in a dense inflammatory infiltrate of the airways with a predominance of eosinophils. Once this allergic mechanism is initiated, subsequent exposures to very low concentrations of the offending agent can elicit asthma symptoms, usually accompanied, and often preceded, by rhinoconjunctivitis.

Diagnosis of IgE-mediated sensitization to HMW agents is made by skin prick testing or by measuring circulating specific IgE antibodies against workplace allergens. However, these

tests are limited by the lack of commercially available and standardized reagents, and in many cases, it is necessary to use homemade allergen extracts following strict procedures. The quality of the occupational allergen extracts used for SPT is very heterogeneous and the sensitivity of several SPT solutions is low [18]. Another limitation is that demonstration of IgE-mediated sensitization does not imply clinical relevance, since this phenomenon can be due to cross-reactivity to panallergens or epitopes from unrelated sources, or in some cases, due to latent sensitization without clinical expression [19]. A systematic review and meta-analysis on 62 studies of OA confirmed by SIC and/or peak expiratory flow (PEF) monitoring showed that specific IgE tests had a sensitivity of 0.74 and specificity of 0.71 for HMW allergens [20].

There is limited information of the specific allergenic components that are causally involved in OA caused by HMW, with the exception of natural rubber latex and wheat flour, or in the cases when the allergen source is an isolated protein itself (e.g., enzymes such as alpha-amylase, papain, and subtilisin). The identification of the relevant allergenic molecules and the introduction of molecular diagnosis could be an important advance to improve the diagnosis of OA [20, 21].

The diagnosis of OA is most definitively confirmed by SIC, a positive challenge with a HMW agent is commonly manifest by early or dual asthmatic responses, and an increase in FeNO [14]. However, since this SIC is available in only a few specialized centers around the world, the combination of sensitization to a workplace agent and bronchial hyper-responsiveness to methacholine has been used as surrogate to establish the diagnosis. A recent non-SIC-based probability model for OA induced by HMW agents has been suggested, especially when referral to a tertiary centre is not possible. This score combines age ≤40 years, work-related rhinoconjunctivitis, inhaled corticosteroid use, agent type, bronchial hyperresponsiveness, and work-specific sensitization, with an area under the receiver operating characteristics curve (AUC) of 0.91 [22].

Allergic Occupational Asthma Caused by Low-Molecular Weight Agents

LMW sensitizing agents (<5 kDa) can give rise to OA, most likely after combining with a carrier protein. The diagnosis has been based on both subjective evidence from the clinical history (asthma worsening at work and improving during periods off work), as well as objective evidence, most convincingly from SIC with use of non-irritating exposure concentrations of the suspected agent [12]. In some cases, the diagnosis may be supported from changes in serial PEF recordings, repeated methacholine challenges, and serial measures of induced sputum cytology, comparing results performed during work periods with exposure and periods off work or out of exposure [3].

These investigations have identified the presence of OA from many LMW sensitizers, and the mechanism is presumed to be from specific immunologic responses. However, this remains a presumption for many agents since there are only a few for which specific IgE antibodies can be identified. In contrast to the sensitivity of 0.74 and specificity of 0.71 for HMW allergens, a recent systematic review found the sensitivity 0.28 and specificity 0.89 for LMW agents [20]. Immunologic support has been provided by demonstration of specific IgE antibodies for some chemical respiratory sensitizers such as acid anhydrides (e.g., phthalic anhydride, and maleic anhydride) when conjugated with human serum albumin, complex platinum salts, nickel and chrome salts. The most common LMW sensitizers in many clinical series have been

diisocyanates (e.g., toluene diisocyanate, diphenylmethane diisocyanate, and hexamethylene diisocyanate). Serum specific IgE antibodies have been demonstrated in a substantial subset (55%) of patients with diisocyanate-induced OA, directed against a conjugate with albumin after exposure to the vapor of the diisocyanate [23]. For those patients with diisocyanate-induced OA and no demonstrable specific IgE, there may be IgE antibodies to a substrate that has not been identified, or there may be other mechanisms.

Most LMW sensitizers are very reactive chemicals, usually with at least two double-bonds. These features have led to the development of quantitative structural activity relationship models and other *in vitro* methods as predictive strategies to identify potential sensitizing chemicals [24, 25] for which further validation is required.

The phenotype of OA from most LMW sensitizers has some differences from the allergic phenotype associated with the HMW agents although there can be overlap. Suojalehto *et al.* [26] assessed the characteristics of acrylate-induced OA in the E-PHOCAS European multicenter cohort study. A total of 55 individuals with OA induced by acrylates confirmed by SIC were recruited and compared with individuals with OA due to other LMW agents and isocyanates. When compared with OA caused by other LMW agents, acrylate-induced OA was associated with female sex, younger age, lower body mass index (BMI), more frequent work-related urticaria and lower treatment level. Work-related rhinitis was more frequent in acrylate-induced than isocyanate-induced OA and the increase in post-SIC FeNO was greater than in OA induced by other LMW agents or isocyanates. All individuals with acrylate-induced OA showed sputum eosinophilia post-SIC, while this inflammatory pattern was less frequently observed in OA caused by other LMW agents (61%) or isocyanates (67%). These phenotypic features of acrylate-induced OA resembles more OA caused by HMW agents, suggesting that acrylates may induce OA through immunological mechanisms that are different from other LMW agents.

Diagnosis

Diagnosis of OA among adult asthmatics is based on a full history, physical examination, and objective confirmation of the diagnosis of asthma. Then, the primary objective of the diagnosis of OA is to prove the causal relationship between the asthma symptoms and the workplace-specific exposure. A stepwise approach can be followed for the diagnosis (Figure 2), based on a combination of medical history (worsening of symptoms at work and improvement away from work), physical examination, positive bronchodilator response or methacholine challenge result, determination of IgE-mediated sensitization especially in the case of HMW allergens by skin prick testing and/or serum specific IgE measurements [1-3].

The anamnesis includes the onset or worsening of asthma symptoms at work with exposure to common allergens and/or irritants, which improve at off-work with avoidance. It is also needed to obtain exposure information. Physical examination and asthma diagnosis are similar to cases with non-occupational asthma including the presence of sputum eosinophilia and/or neutrophilia, bronchial hyperresponsiveness to methacholine and the degree of airway obstruction with reversibility measured by spirometry. In addition, changes in inflammatory cells within sputum, serial PEF recordings and bronchial hyperresponsiveness with work-shifts will be helpful in confirming WRA and its causative agents [12, 27]. In cases of allergic OA,

allergy skin testing and detection of serum specific IgE antibodies to sensitizers (or sensitizer-carrier protein conjugates) are supportive to confirm the causative agent [28].

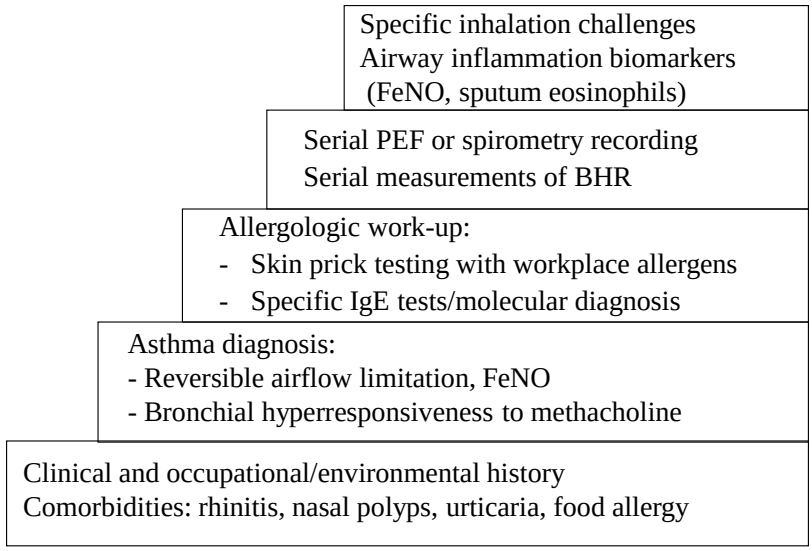

Figure 2. Diagnosis of allergic occupational asthma.

SIC testing is the reference standard that is recommended for confirmative diagnosis; however, it should be performed in a specialized center with close supervision and to include a control challenge and gradual increases in exposure to the suspected causative agent [29]. A positive response is defined when FEV_1 (%) falls greater than 15% from baseline for at least 6 hours after exposure to the suspected agent. However, it has relatively commonly been performed in some countries such as France, Italy, Spain, Poland, and Asian countries but has seldom been performed in North America. Since there are hundreds of sensitizers and new causes added each year, absence of a known sensitizer at work does not exclude OA. The accuracy of the diagnosis can be improved by the measurement of sputum eosinophils before and after challenge [30] or by measurement of FeNO, because an increase of FeNO after SIC is highly predictive of OA [31]. If the patient is working, serial PEF monitoring at work and away from work is also a useful option [2, 3]. If SIC in the laboratory and/or PEF monitoring at work are not possible and OA is strongly suspected from history, a combination of objective evidence of asthma plus a positive skin test or the verification of specific IgE to the suspected agent has a high predictive value for OA [32].

Exposure Assessment

The assessment of environmental exposures begins with a focused occupational and environmental history. This may be sufficient in many instances to identify the substance that is causing the asthma symptoms. Sometimes, however, this history may have to be complemented by a work site visit and air sampling. A walk-through visit to the workplace may be of great help to understand the type, characteristics and extent of the exposure. Additional

information can be obtained by requesting patients to provide labels from substances present at work and material safety data sheets for chemical in the workplace, which may help to clarify the presence of work sensitizers or irritants [1-3]. Industrial hygiene data, if available, usually includes a process review and exposure assessment and air monitoring data and can be extremely useful in identifying relevant exposures.

The physicochemical properties of the inhaled substance, duration and intensity of exposure, and conditions of use are important elements in the development of respiratory sensitization. Several studies have been able to demonstrate the presence of exposure-sensitization and exposure-symptoms relationships to several occupational allergens [3]. The intensity of exposure necessary for initial sensitization is probably higher than that required to provoke symptoms in the sensitized subject.

Air sampling may be useful in selected cases of OA. The measurements of specific occupational agents should focus on relevant substances in the work environment that are suspected of causing asthma. Bioaerosol monitoring should target the identification of specific allergens in the work environment that may cause or exacerbate asthma. This type of monitoring is especially indicated when the etiology is unclear and may provide objective data that can help in the diagnosis of OA. Collection of aerosols can be performed by either high-volume or personal sampling pumps. Unfortunately, few health-based exposure standards have been developed for exposure to aeroallergens in the air [10, 11]. The air samples can be used to culture microorganisms, determine the concentration of endotoxins, assay for suspected chemicals, and to perform immunoassays (ImmunoCAP, ELISA) for specific allergens.

Management

Patients with OA due to a workplace sensitizer should be completely prevented from further exposure to achieve the best outcome [33]. In addition, patients with WRA should be managed as any other asthmatics with regard to asthma education, control of exposure to environmental triggers and appropriate pharmacotherapy following the treatment guidelines (Table 2). Inhaled corticosteroids usually combined with inhaled long-acting beta-2 agonists are the mainstay of treatment, and should be used to control asthma symptoms and to prevent asthma exacerbations. Monitoring of asthma control, lung function parameters as well as inflammatory biomarkers such as FeNO and sputum eosinophilia should be performed during follow-up. When asthma symptoms are not well controlled, biologics such as anti-IgE monoclonal antibodies (omalizumab) can be beneficial for patients with severe allergic OA [34, 35]. Other biologics such as anti-IL-5 (mepolizumab, benralizumab), anti-IL-4/IL-13 (dupilumab) and anti-TSLP (tezepelumab) should be evaluated in terms of their effectiveness in the management of patients with OA.

The best prognosis has been associated with an early diagnosis and early removal, with milder asthma at the time of diagnosis [36]. Therefore, if OA or work-exacerbated asthma is suspected, it is recommended to refer patients to a specialty clinic to confirm their diagnosis [2]. If diagnosis is delayed, asthma severity can be rapidly progressive, resulting in lung function decline. However, in some workers, when absolute avoidance is not possible due to significant socioeconomic impact, changing workplace products and procedures and close monitoring is essential.

Table 2. Main steps in management of allergic OA

1. Asthma treatment according to asthma guidelines • Assessment of asthma control and severity • Optimal pharmacotherapy (including biologics) • Avoidance of asthma triggers, environmental control • Patient's education
2. Work exposure • Avoid any further exposure to causative agents. If this is not possible, then reduce exposure as low as possible
3. Assist patient with relevant compensation claim and rehabilitation
4. Consider other co-workers affected and notify public health and company

Prevention

There have been some studies to recommend changing ventilation facilities and to applying respiratory protective devices; however, these measures have been demonstrated not to be as effective as complete removal of exposure [33]. To detect OA patients earlier, there have been many studies investigating different biomarkers [37].

Primary prevention measures are most effective when feasible. Ideally, sensitizers would be removed from workplaces and substituted with non-sensitizing agents. A good example is the removal of powdered latex gloves and use of non-latex gloves. When latex gloves cannot be replaced, then a change to powder-free, low-protein latex gloves has also been effective, and these measures have essentially prevented OA from natural rubber latex [38]. Encapsulation of powdered enzymes in the detergent industry also has been an effective preventive measure, and risks of sensitization for animal care workers have been reduced by measures to reduce allergen exposure [12]. For LMW agents similarly, measures include removal and replacement, when possible.

Reducing exposure by occupational hygiene measures such as improved ventilation, containment, and finally by use of respiratory protective equipment are less effective but can also be helpful in reducing risk of sensitization [39]. Worker-education is an important component to understand risks [40, 41] and improve adherence to occupational hygiene measures.

Secondary prevention for OA from a sensitizer can be effectively added to primary measures, as in enzyme manufacturing industries [42] and diisocyanate workers [39]. This includes surveillance measures, such as air monitoring to ensure exposure levels are not exceeded, and medical surveillance to detect OA early, by pre-placement and regular respiratory questionnaires, specific IgE testing for early detection of specific sensitization and potential relocation of sensitized workers, and spirometry [43, 44]. Education of workers is also an important component of secondary prevention for all phenotypes of OA, which may lead to earlier diagnosis and prompt recognition that symptoms may be caused by work.

Tertiary prevention consists of early diagnosis and appropriate management of the patient with OA. Again, this differs based on the phenotype of OA. For those with OA from a sensitizer early diagnosis and complete removal from the sensitizing agent provide a better medical outcome [45] but can be associated with significantly reduced socioeconomic effects [46]. Other factors associated with more severe sensitizer-induced OA outcome are lower education

level and ethnicity [15]. Additional management as for non-occupational asthma includes appropriate environmental changes and pharmacologic management, as previously discussed. Support should be provided for a workers' compensation claim as appropriate for all patients with OA, and the diagnosis should be recognized as a "sentinel event" requiring notification of those who may intervene in the workplace to identify other affected workers and reduce risks for co-workers [12, 47].

Conclusion

OA represents a significant burden for the working population. Our understanding of this complex disease, which includes various phenotypes and endotypes, with allergic OA being the most prominent, has improved markedly in recent years. For agents that act through an IgE-mediated mechanism, skin testing or serologic measurement of specific IgE antibodies can be used to assess sensitization, provided a suitable preparation of allergen is available. Specific inhalation challenges are the reference standard in the diagnosis of OA. These tests are indicated in studying new causes of OA and in determining the precise etiologic agent, as well as for research on mechanisms of OA. Sophisticated equipment and trained personnel are necessary to perform these challenge tests.

A critical reassessment of the clinical aspects, etiological agents and diagnostic tests of OA, as well as the optimal preventive and therapeutic strategies, is necessary to achieve better results in its management and prevention. Current research is aimed at establishing the immunological pathways that determine not only the phenotypes and endotypes of the disease, but also the presence of risk factors and biomarkers that should allow a more personalized management approach [17].

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Chapter 7

Severe Asthma

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Abstract

Severe asthma is a heterogenous condition with several distinct phenotypes and endotypes. The diagnosis and treatment of severe asthma is time consuming and requires special experience. There is a need for competent treatment centers, continuing medical education, and research on the prevalence of severe asthma. To achieve and maintain control in severe asthma high dose inhaled corticosteroids with a second and a third controller are usually

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needed. Before any further treatments are evaluated, differential diagnoses of asthma should be ruled out, comorbidities should be treated, persistent triggers should be eliminated, and patient adherence should be optimized. Targeted treatment with biologicals and small molecules has revolutionized the management of severe asthma.

Keywords: biomarkers, endotypes, phenotypes, severe asthma

Introduction

A minority of patients with asthma have severe or difficult to control asthma despite intensive treatment. These patients present a special challenge because of the extensive diagnostic evaluation that they need and their high consumption of healthcare resources. This chapter tackles severe asthma as a heterogenous condition (hence problematic to define and treat in an uniform manner) highlighting the need for a better understanding of its pathogenetic mechanisms as a starting point for the stratified approach in its management.

Definition

The goal of definitions in medicine is to provide a standardized language for medical professionals to facilitate communication and recordkeeping. When definitions are discussed and agreed upon, they give the required advice to most properly identify, characterize, and make suitable decisions. As severe asthma (SA) is a heterogeneous disease well-structured definitions are necessary to clarify misunderstandings and to achieve the most optimal management outcomes.

At present, there is no common internationally agreed definition of SA, the terms applied are not standardized and are often interchangeable. Several definitions have been proposed (Table 1).

The most accepted and used definition of SA is provided by The Global Initiative for Asthma (GINA). According to the GINA 2022 report SA is defined as: "*asthma that is uncontrolled despite adherence with maximal optimized high dose inhaled corticosteroids (ICS)-long-acting beta$_2$-agonist (LABA) treatment and management of contributory factors, or that worsens when high dose treatment is decreased*" [1].

In 2014, the European Respiratory Society (ERS)/American Thoracic Society (ATS) Task Force on Severe Asthma published their consensus agreement on a definition of SA that offers a pragmatic definition based on the level of treatment necessary to maintain asthma control. This definition introduces the term "high dose ICS" [2].

Based on The World Health Organization (WHO) 2010 definition, SA should be categorized into 3 groups: 1) Untreated asthma; 2) Difficult-to-treat asthma; 3) Therapy-resistant asthma [3].

In conclusion, the international terminology and the concepts of severity and control have moderate agreements. Further standardization is essential to facilitate epidemiologic and health economics studies and disease registries.

Table 1. Severe asthma definition comparison (GINA, ERS/ATS, WHO)

GINA 2022[1]		ERS/ATS 2014[2]		WHO 2010[3]		
Asthma is uncontrolled despite		Asthma which requires treatment with		Uncontrolled asthma which can result in:		
a) adherence with maximal optimized high dose ICS-LABA treatment	b) and management of contributory factors	a) guideline suggested medications for GINA steps 4-5 asthma (high dose ICS and LABA or leukotriene modifier/theophyl line) *for the previous year*	b) or systemic CS for ≥50% *of the previous year*	a) risk of frequent severe exacerbations (or death)	b) and/or adverse reactions to medications	c) and/or chronic morbidity (including impaired lung functions or reduced lung growth in children)
		to prevent it from becoming uncontrolled*				
B) Asthma worsens when high dose treatment is decreased		B) Asthma which remains uncontrolled** despite this therapy				
At present, therefore, "severe asthma" is a retrospective label. It is sometimes called "severe refractory asthma" since it is defined by being relatively refractory to high dose inhaled therapy. However, with the advent of biologic therapies, the word "refractory" is no longer appropriate. Asthma is not classified as severe if it markedly improves when contributory factors such as inhaler technique and adherence are addressed.		* Controlled asthma that worsens on tapering of these high doses of ICS or systemic CS (or additional biologics). ** Uncontrolled asthma defined as at least one of the following: 1) Poor symptom control; 2) Frequent severe exacerbations; 3) Serious exacerbations; 4) Airflow limitation.		There are three categories which may overlap, that are included in the definition: 1) Untreated severe asthma 2) Difficult-to-treat severe asthma 3) Treatment-resistant severe asthma – this group includes: a) Asthma for which control is not achieved despite the highest level of recommended treatment: refractory asthma and CS-resistant asthma. b) Asthma for which control can be maintained only with the highest level of recommended treatment.		

Epidemiology and Burden

A key issue in estimating the exact prevalence is the definition of SA. The exact prevalence is unknown because of the heterogeneous diagnosis criteria and the lack of a standard and clear definition of severe asthma [4]. In the adult asthmatic population, SA has been estimated worldwide to account for only 3% to 10% of the total cases [4, 5]. However, there is a notable lack of studies regarding the prevalence of SA, especially in the pediatric population. A recent study estimated that the prevalence of 2.1% for SA among 12-year-old children [6].

Severe asthma is associated with high morbidity and excessive mortality rates. Furthermore, there is a significant link between asthma severity and economic burden for the community [7]. The costs for mild asthma of USD$ 2,646 increases to USD$ 4,530 for moderate asthma and to USD$ 12,813 for SA [8, 9]. The analysis of a national administrative claims database showed disproportionate costs for persistent SA, with USD$ 5,112 per year or 2.9-fold greater as compared to mild persistent asthma [10].

Severe or difficult-to-treat asthma is associated with high rates of depression, anxiety and school or work absenteeism [11, 12].

Risk Factors

Several risk factors for SA in adult patients are well-established, including type 2 (T2) inflammation and eosinophilia, late-onset asthma (LOA), smoking, sensitization to fungi and *Staphylococcus aureus* enterotoxins, chronic rhinosinusitis with nasal polyps (CRSwNP) and non-steroidal antiinflammatory drugs exacerbated respiratory disease (NERD) [13] (Figure 1). The link between low socio-economic status and severe asthma is controversial [14]. In addition, atopy, late-onset wheeze and/or bronchial hyperresponsiveness (BHR) may lead to SA in paediatric patients [15, 16].

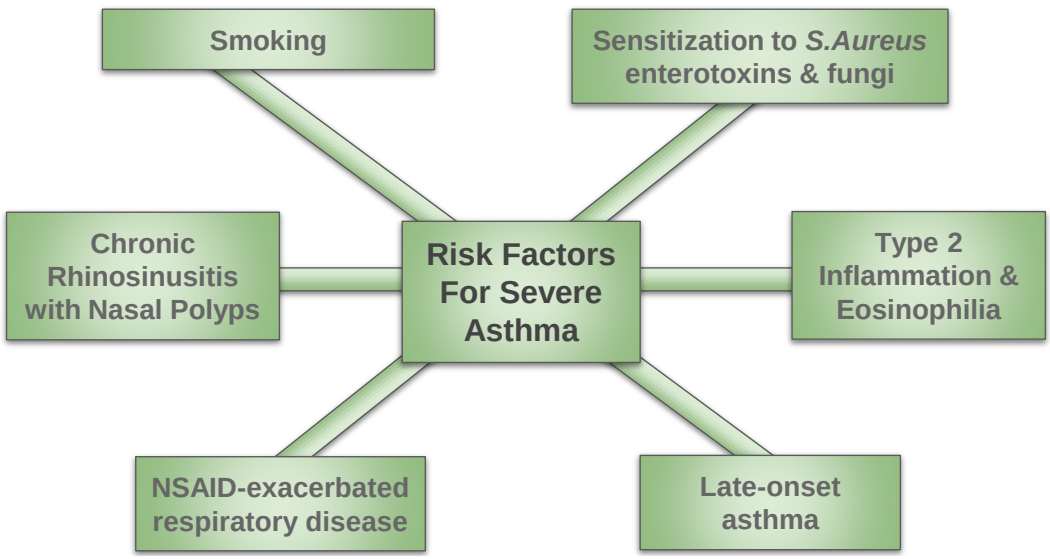

Figure 1. Risk factors for severe asthma in adults.

T2 inflammation involves both the innate and adaptive immune systems, underlying the complicated pathophysiology of chronic inflammation in SA [17, 18, 19, 20, 21, 22]. Immunoglobulin (Ig) E production, high blood and sputum eosinophil count and elevated T2 biomarkers (such as exhaled nitric oxide) are currently used as biomarkers for the T2 severe asthma endotype [17, 18, 23, 24]. Eosinophils are the most common inflammatory cells in the asthmatic lungs, and they play a significant role in two key events: airway remodelling and BHR which lead to constant damage of the airways promoting SA [25].

Asthma usually has its onset during childhood, however, when the first asthma symptoms appear in adulthood, a more severe evolution is noted. Several risk factors may contribute to LOA increased severity, such as female sex and obesity. The direct mechanisms behind aging and asthma severity are yet unclear [22, 26].

Substantial data suggests that current smoking and exposure to environmental tobacco smoke are important risk factors for increased asthma severity, lower quality of life (QoL), rapid decline of lung function, poor asthma control, higher asthma healthcare utilization including hospital admissions and increased risk of mortality [16, 27, 28].

Staphylococcus aureus (*S. aureus*) is a major bacterial human pathogen responsible for a wide variety of clinical features, characterized by disease-modifying properties in upper and

lower airway diseases. Its enterotoxins may cause polyclonal IgE production, which has been linked to allergy multimorbidity [29]. Previous findings showed a significant association between sensitization to *S. aureus* enterotoxins and the SA [20, 30]. The first longitudinal study confirmed this sensitisation as a substantial risk factor for SA asthma [31]. Isolation of *Aspergillus fumigatus* from the SA airways is very common [32].

CRSwNP was reported in 42% of SA patients in a cross-sectional analysis of the registry of Severe Asthma Network in Italy [33]. SA with associated CRSwNP is more likely associated with long term oral corticosteroids (OCS) users, therefore being at a higher risk of developing corticosteroids side effects [34].

NERD is a distinct asthma and CRSwNP phenotype characterized by severe chronic eosinophilic inflammation of upper and lower airways, with symptoms aggravated by non-steroidal antiinflammatory drugs [35]. NERD reported prevalence is 7% in adult asthmatics, but it is twice as high in SA [36].

Mechanisms

T2 Severe Asthma

Both the adaptive and the innate immune response are involved in the pathogenesis of T2 asthma [17, 18, 21, 37]. In response to allergen, pollutants, viral infections, or other triggers damaged airway epithelial cells secrete IL-33, IL-25, and thymic stromal lymphopoietin (TSLP), which activate group 2 innate lymphoid cells ILC2s to produce T2 cytokines such as interleukin (IL)-5, IL-13, and IL-9 [19, 37]. ILC2s are the predominant population of ILCs in the asthmatic lungs. ILC2s are increasingly being recognised as key controllers of T2 inflammation, and are highly elevated in allergic rhinitis, CRSwNP, and asthma [38, 39]. ILC2 facilitate the polarisation of naive CD4-positive T cells into T helper (Th) 2 cells, partly through releasing cytokines such as IL-13 and possibly acting as antigen-presenting cells [40]. The ILC2-related T2 pathway is known to be corticosteroid-resistant in nature, suggesting that it may be implicated in SA [41]. Paediatric SA is characterised as well by high ILC2s, reduced by systemic corticosteroids but not maintenance ICS [42]. Basophils and mast cells (MCs) are also major innate immune cellular sources of T2 cytokines in chronic asthma. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) binds to PGD2. CRTH2 is expressed on various cell types including eosinophils, MCs, and basophils. Thus, CRTH2 and PGD2 are involved in allergic inflammation and eosinophil activation.

CD4+ Th2 lymphocytes and their associated cytokines (IL-4, IL-5 and IL-13) are hallmark features of T2 asthma. Classical T2 cytokines (IL-4, IL-5 and IL-13) together with CCL11 (eotaxin-1) regulate critical aspects of eosinophil recruitment, T2 inflammation and BHR. T2 cytokines could operate in parallel, but their signals are usually integrated through crosstalk with resident and migrated inflammatory cells. These cytokines also had distinct temporal roles in the development of inflammation and BHR (e.g., IL-4 promoted the early phase of inflammation and IL-13 and IL-5 acted as late-stage effector molecules) [43, 44]. Bidirectional signalling between eosinophils and Th2 cells regulates T cell cytokine production [45, 46]. IL-18 derived from eosinophils is a factor regulating IL-13 production. In addition, eosinophils

and ILC2 could act as antigen presenting cells and directly promote allergic inflammation by activating Th2 cells in an antigen-specific manner [47].

T2 asthma was described as a complex endotype where several pathways (the IL-5, the IL-4/IL-13 and the IgE pathways) have a dynamic non-linear interaction [21].

T regulatory cells (Tregs) have been shown to be critical in hampering the T2 inflammation [48]. Both cell-cell contact (through membrane bound transforming growth factor (TGF)-beta or via suppressive molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) and soluble cytokines (TGF-beta and IL-10) dependent mechanisms have been shown to contribute to the ability of Tregs to operate effectively. A novel Tregs subpopulation, defined by CD103 expression, programmed to prevent exuberant T2 inflammation and keep homeostasis in the respiratory tract under control, was recently described [49]. IL-10-producing regulatory B cells (Bregs) were widely ascribed with potent immune regulatory functions [50]. A recent study reported on phenotypic and functional alterations of Breg subsets in adult allergic asthma patients showing that OCS significantly affects the frequency as well as their ability to express IL-10 [51].

Non-T2 Severe Asthma

Several recent studies in SA identified non-T2 asthma as a distinct endotype with relevant features such as increased severity and remodelling and poorer response to antiinflammatory treatment [52]. The understanding of non-T2 SA mechanisms lags far behind T2-high SA. Several pathways were evaluated, such as the dysregulated innate immune response, including neutrophil intrinsic abnormalities, the inflammasome pathway and the activation of the IL-17 pathway [17, 18, 20, 53].

Many facets of SA are mechanistically associated with Th17 cell-derived cytokines and other immune factors that mediate neutrophilic influx to the airways. Th17-secreted IL-17A is an independent risk factor for SA that impacts remodelling [54, 55]. TGF-β1 is a pivotal mediator involved in airway remodelling that correlates with enhanced Th17 activity and is essential for Th17 differentiation and IL-17A production. IL-17A can reciprocally enhance activation of TGF-β1 signalling pathways, whereas combined Th1/Th17 or Th2/Th17 immune responses additively impact severity [56, 57]. A subset of SA patients with exaggerated neutrophilic responses have increased vital NETosis. The increased levels of cytoplasts seen in these patients correlated significantly with IL-17 levels in broncho-alveolar lavage (BAL) fluid [58].

Shared Pathogenetic Pathways

Severe asthma is a polygenic trait. Twin studies estimate that approximately 25% of the phenotypic variability in asthma severity is determined by genetic factors, with the remainder driven by environmental and psychosocial factors, behavioural traits, and co-morbidities. Most genetic association studies of asthma severity performed to date are underpowered and not designed to clearly distinguish severity from susceptibility variants. Future research should explore the role of rare genetic variation and gene-by-environment interaction in SA [17, 18].

Results of numerous studies have indicated that epigenetics plays a major pathogenetic role in the development of SA through interactions with and between various susceptibility genes, immunologic influences, and environmental factors. Epigenetics holds an important position to unravelling the complex associations between SA phenotypes and endotypes [17, 18].

Chronic inflamed airways can lose tolerance over time to antigens released following frequent eosinophil degranulation. In the sputum of patients with prednisone-dependent eosinophilic inflammation and/or recurrent pulmonary infections there was evidence of anti-EPX and anti-nuclear antibodies of the IgG subtype. Extensive cytokine profiling of sputum revealed increased levels of signalling molecules linked to ectopic lymphoid structures. Immunoprecipitated sputum immunoglobulins from these patients triggered eosinophil degranulation *in vitro*, with release of extensive histone-rich extracellular traps, an event possibly contributing to corticosteroid unresponsiveness [59].

Less in known of neural mechanisms in SA. Transient receptor potential (TRP) channel TRPV1 is significantly higher expressed in the epithelial cells in SA and its blockage in murine models alleviated BHR, inflammation, and remodelling [60, 61]. Cholinergic mechanisms and non-adrenergic non-cholinergic mechanisms, both inhibitory and excitatory, may be especially important in certain phenotypes of SA. Dysfunction of M2 muscarinic receptors induced by a range of stimuli including allergen, viral infection, ozone, eosinophil products and cytokines may lead to excessive bronchoconstriction and mucus secretion. Various studies suggest that substance P is increased in asthma [17, 18].

Tissue remodelling is an important process occurring in response to repetitive lung injuries and characterised by profound changes and reorganizations at the cellular and molecular levels of the lung resident cells. It is of particular importance to understand the mechanisms involved in airway remodelling, as this is strongly associated with SA [62]. Barrier disruption and an excessive immune response of the epithelium contribute to the pathophysiology of SA [17, 18, 19, 63]. IL-13 released from ILC2 directly disrupts the tight junctions between epithelial cells and induces epithelial leakiness [63]. The viscous mucus layer produced by goblet cells is a physiological defence mechanism. An aberrant mucin (MUC) expression is responsible for airway obstruction due to its high viscous characteristics. TGF-β stimulates mucus hyper-secretion in asthma by inducing MUC5AC hyper-expression [64]. Mucus plugs score on computed tomography (CT) was associated with a marked increase in sputum eosinophils and EPO [65].

Increased mass and phenotypic modifications of the airway smooth muscle cells (ASM) are hallmark feature of remodelling occurring in asthma independent or induced by the inflammatory process. Increased ASM mass may be collectively due to airway infiltration with myofibroblasts, neighbouring ASM cells in the bundle, or circulating hemopoietic progenitor cells [66]. ASM contraction is additionally altered by the extracellular matrix stiffness by regulating cell-cell contacts [67]. ASM migration is mediated through a variety of cytokines including IL-13 (through the IL-4 receptor alpha), TNF-α, Th-17-secreted cytokines (IL-17A, IL-17F, IL-22), and the epithelial derived cytokines. The myofibroblast trans-differentiation pathway plays a key role in the remodelling process. TGFβ induces fibroblast to myofibroblast trans-differentiation associated with glucocorticoid receptor (GR) expression and a preferential localisation of GR in the nucleus. Furthermore, the non-functional GR isoform GRβ is increased, thus supporting the link between remodelling and corticosteroid resistance in severe asthma [68].

Evaluation

Pulmonary Function Testing

Pulmonary function tests (PFT) are important for an accurate diagnosis of SA, for assessing its functional impairment, and for monitoring control and differential diagnosis.

Expert groups recommend the initial use of spirometry to assess airway obstruction [76]. If airway obstruction is present and forced expiratory volume in 1 second (FEV_1) < 80% predicted, a bronchodilator test is required, to assess reversible airway obstruction, a key feature of asthma. In patients with relatively normal FEV_1% predicted, further tests should be performed: inflammometry, peak expiratory flow (PEF) variability or bronchial challenge test. SA may present chronic airflow limitation less responsive to bronchodilators or corticosteroids and air trapping that may lead to impaired ventilation of small airways and severe exacerbations.

Body-plethysmography may provide additional information regarding total lung capacity, functional residual capacity, residual volume, and specific airway resistance and conductance. Body-plethysmography is useful to assess air trapping and hyperdistention induced by small airway disease (SAD) in patients with preserved FEV_1/FVC ratio [76].

Impulse oscillometry (IOS) is a non-invasive technique for SAD, frequently associated with uncontrolled asthma. In patients with SA intrabreath oscillometry may be better evaluate disease control and future risk of exacerbations [77].

Imaging

In SA the new non-invasive imaging techniques provide significant structural and functional insights. This approach aims to define ‘imaging biomarkers’ in order to enable clinicians to better characterize SA phenotypes [78, 79, 80]. The imaging techniques currently used in SA are Computed Tomography (CT) - the most sensitive method for assessing morphological and functional changes associated with asthma; and magnetic resonance imaging - for assessing small airway impairment, lung microstructure, and ventilation/perfusion ratio, and gas exchanges. Other modern imaging techniques are Position Emitted Computed Tomography, Single Photon Emitted Computed Tomography, Endobronchial Ultrasound, and Optical Coherence Tomography (Table 2).

Table 2. Imaging techniques used in severe asthma

Imaging method	Structural Assessment	Functional Assessment	Clinical relevance
Computed Tomography	Airway morphology Vasculature Lung parenchyma	Ventilation Perfusion	Measures air trapping and the mosaic perfusion, correlated with SAD Precise assessment of the bronchial wall thickening and mucus plugging associated with obstruction, ground-glass opacities and eosinophilia Biomarker for therapeutic response Excludes comorbidities and other pathologies

Imaging method	Structural Assessment	Functional Assessment	Clinical relevance
Magnetic Resonance Imaging	Lung microstructure Detailed structural evaluation (combined with CT)	Ventilation Perfusion Complementary to CT, MRI quantifies functional changes by using hyperpolarized helium and xenon gas	Identification of SAD Biomarker for therapeutic response Excludes comorbidities and other pathologies
Position Emitted Computed Tomography	Detailed structural evaluation (combined with CT)	Ventilation Perfusion Pulmonary inflammation	Identify and target lung inflammation Drug delivery evaluation Response to antiinflammatory therapy
Single Photon Emitted Computed Tomography	None	Ventilation Perfusion Pulmonary inflammation	Visualization of ventilation defects at the sub-segmental level Analyses ventilation distribution through the lung parenchyma, detecting the areas of bronchoconstriction Response to inhaled therapy
Endobronchial Ultrasound	Airways >4 mm with visualisation of multiple layers of the bronchial wall	None	Enables access to the different layers of the small airways wall Monitor serial airways modifications
Optical Coherence Tomography	2D-images of the airway wall (spatial resolution = 1-15 μm)	None	Monitor serial airways modifications Bronchial wall evaluation Airway distension

Inflammometry

Airway inflammation represents a major feature of SA, and its assessment represents an important step in the management of asthmatic patients toward the stratified approach. There are several approaches and biomarkers for assessing airway inflammation, which involve sputum, BAL, and exhaled breath.

Sputum collection represents a non-invasive diagnostic method that allows the analysis of cells and mediators from the lower airways. Since spontaneous production may lead to samples of poor quality this issue was overcome by sputum induction, a procedure that involves inhalation of a nebulized hypertonic saline solution. Induced sputum improves the diagnostic work-up in SA as several phenotypes of inflammation were established based on the granulocytic airway content, with prognostic and therapeutic implications: eosinophilic, neutrophilic, mixed granulocytic, and pauci-granulocytic [81, 82]. Levels of sputum eosinophils >2-3% are associated with a T2-high asthma endotype while high levels of sputum neutrophils suggest non-T2 asthma [84]. Besides asthma phenotypes differentiation, evaluating airway cell content may provide valuable information regarding clinical outcomes: sputum eosinophilia predicts a good response to ICS or OCS, while the neutrophilic asthma phenotype is usually associated with resistance to ICS [76, 82, 83, 84]. Another sputum inflammation biomarker is periostin, associated with persistent airflow limitation in asthmatics with airway eosinophilia, despite treatment with high-dose ICS [85].

The analysis of the BAL fluid includes cell count, specific staining, and microbiological investigations. Similar to sputum analysis, counting the cells and measuring the inflammatory

mediators from BAL fluid allows a better characterization of the asthma phenotype [86, 87, 88].

Airway inflammation levels can also be monitored by non-invasive methods, such as exhaled breath condensate (EBC) and measurement of fractional exhaled nitric oxide (FeNO). The most frequently analyzed parameters from EBC are pH and exhaled markers of inflammation (e.g., cytokines, leukotrienes) and oxidative stress (e.g., H_2O_2, 8-isoprostane). A low EBC pH was was suggested as a marker for severe or uncontrolled disease [89]. FeNO correlates with allergic airway inflammation and with the extent of airway eosinophilic inflammation. FeNO > 25 ppb is highly supportive for T2 asthma, however it is not a specific for asthma, as it can increase in eosinophilic chronic bronchitis, allergic rhinitis or eczema. Furthermore, FeNO can be used as a biomarker for monitoring treatment responses or adherence to corticosteroids, and, potentially for biologics targeting the IL-4/IL-13 pathway [90].

Bronchoscopy, combined with biopsy, lavage, or both, is invasive, thus its indication for SA diagnosis, accurate phenotyping and therapeutical approach remains controversial. The diagnostic workup in patients with SA may include a bronchoscopic procedure for those with "atypical" features (cortico-dependent or with a background of autoimmune disease), or for unclear differential diagnosis [91]. In SA refractory to treatment, bronchial thermoplasty (BT), a bronchoscopic technique based on thermal energy, reduces exacerbations and improves QoL with sustained clinical benefit 10 years or more and with an acceptable safety profile [92].

Management

Severe asthma is best managed in a multidisciplinary environment centred on the patient and his/her needs [93]. Skilled asthma physicians are required to confirm the diagnosis and provide treatment plans, co-morbidities are managed jointly with other disciplines, specialized nurses can perform lung function tests, oversee the delivery of treatments, and provide education, pharmacists can offer instruction on using inhalers and offer guidance on using concurrent drugs. The coordination of the interdisciplinary team is crucial if consistent and comprehensive treatment is to be provided. It is especially important that asthmatic patients participate in the shared decision-making as part of their asthma management plan [1, 2, 24]. Patient-centred asthma treatment cannot be applied in the era of personalized medicine without considering a patient's values and preferences in addition to the phenotypic and endotypic biomarkers. Patients should receive support to enable them to cope with the tremendous physical, emotional, social, and financial burden of SA.

The main objectives of SA management are achieving and maintaining control, decreasing exacerbation risk, slowing lung function decline and minimization of medication side effects [1, 2]. The four key elements of SA management are patient education, asthma triggers control, monitoring for changes in lung function or symptoms, and pharmacologic therapy.

Severe asthma is difficult to control due to gaps in its diagnosis and in the coordination, integration, and resources. Therefore, a framework for understanding the underlying pathological mechanisms and selecting the optimal management option is essential.

The GINA guideline offers an integrated strategy and decision tree for difficult-to-treat and SA with add-on medications, such as long-acting muscarinic antagonists (LAMA), macrolides,

or biologicals guided by the inflammatory phenotype, recommended in Step 5 [1]. Similar recommendations are provided by the joint guideline of the ERS/ATS [94]. The evaluation of the response to the add-on medication is done after 3-6 months in order to discontinue ineffective treatments and consider alternatives. Re-evaluation should be performed every 3-4 months [1, 24, 94].

Patients with SA may benefit greatly from the use of biologicals that target the IgE, IL-4, IL-5 and, most recently, the epithelial derived cytokines TSLP and IL-33 and its receptor ST2 [1, 24, 94, 95, 96]. The main benefits of add-on biologicals are a significant decrease in severe exacerbations and a decreased need for OCS. There are also benefits in improving lung function and in patient-reported outcomes. Unfortunately, at the moment, there are no head-to-head trials between these biologicals so recommendations are formulated based on expert-opinion consensus. In addition, some patients with T2 asthma show suboptimal response or lose control of their asthma over time. In order to select the appropriate biological therapy, GINA recommends an initial assessment of the SA phenotype, as well as an evaluation of factors that may contribute to symptoms, exacerbations and QoL. For eosinophilic asthma, GINA recommends monoclonal antibodies targeted against the interleukin (IL)-4/13 (dupilumab) or IL-5 (mepolizumab, reslizumab, benralizumab) pathways, while for allergic asthma, it recommends anti-IgE monoclonal antibodies (omalizumab) [1]. The European Academy of Allergy and Clinical Immunology guidelines on the use of biologicals in SA recommend a triple pillar decision based on visible properties, biomarkers and outcomes combined with the shared-decision process with the patient in the selection of the biological and in defining the threshold for response [24]. Tezepelumab, a TSLP inhibitor was approved for patients above 12 years old with SA, regardless of phenotype [97].

For non-T2 SA alternatives such as LAMA, macrolides, or BT is selected cases may be considered.

Educational support has traditionally been seen as a crucial component in the management of chronic diseases. The goal of education in asthma is to assist the patient in acquiring the knowledge and skills necessary to avoid triggers, recognize when their asthma is getting worse, take appropriate action, and follow their treatment plan. Asthma education has been shown to stall the progression of airway remodelling due to inflammation, decrease exacerbations and risk of death from asthma, improve inhalation technique and QoL, and lower costs [98]. The two most crucial elements of asthma education are the detailed asthma action plan and the shared-decision process between the patient and its healthcare providers. One challenge in asthma education is that several factors need to be tackled while creating the personalised management framework such as age, health beliefs, health and digital literacy, language and other barriers to communication and access to care.

Pulmonary rehabilitation is another cornerstone of SA management, provided by a multidisciplinary team of physicians, respiratory therapists, nurses, psychologists, and dieticians. A preliminary evaluation is usually carried out, in which the patient is subjected to a thorough examination by a specialist healthcare provider in order to customize each intervention to the needs of the individual patient. Pulmonary rehabilitation usually consists of exercise training, breathing retraining, and psychological support. Exercise training might help SA patients perform better during exercise. It is not completely clear how exercise increases endurance. Walking, cycling, stairs, or rowing strengthens peripheral muscles and induces biochemical and physiological changes that decrease respiratory rate and dynamic lung hyperinflation in chronic pulmonary diseases. Younger individuals, patients with a history of

smoking, or with a lower baseline exercise tolerance appear to benefit more from pulmonary rehabilitation. Pulmonary rehabilitation also decreases BHR, systemic inflammation, asthma exacerbations and improves the QoL. Patients with more severe symptoms seem to benefit more from these effects [99, 100].

Comorbidities

Comorbidities are often a treatable component of SA, so their recognition and treatment are key to achieve and maintain asthma control. However, this requires careful clinical assessment, special investigations and a multidisciplinary approach [69].

Allergic rhinitis is associated with the early onset of SA and is an independent risk factor for long-term lung function decline [70]. CRSwNP is associated with late-onset eosinophilic SA and is an independent predictor of exacerbations [70, 71].

Sleep apnoea alters thoracic mechanics, and generates local and systemic inflammation due to repetitive upper airway obstruction and hypoxia [72, 73].

Obesity worsens asthma by adding a different type of inflammation, and by reducing the therapeutic response, including biologicals. Obesity-associated late-onset asthma is a distinct phenotype of SA, with female predominance [74].

Severe asthmatics, especially those steroid-dependent, experience more psychological distress, cognitive dysfunction, and anxiety than those with moderate asthma [75].

Conclusion

Severe asthma is challenging in its correct diagnosis and management. Inclusion of as many patients as possible in severe asthma registries, better coverage of the diagnosis and treatment of severe asthma in physicians' training and assessment of patients with severe asthma in specialized asthma centers, is warranted to improve the clinical management pathways. The unbiased endotype-driven approach holds the promise of discovery of new therapeutic targets, especially for non-T2 asthma.

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Chapter 8

An Update on Thunderstorm Allergy and Asthma

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Abstract

The thunderstorm-asthma outbreaks, described in several cities in all the World, are characterized, at the beginning of thunderstorms in pollen seasons, by a rapid increase of visits for asthma in general practitioners or hospital emergency departments. Pollen grains can be carried by thunderstorm at ground level, with release of allergenic biological aerosols of paucimicronic size, derived from the cytoplasm of pollens ruptured or not, and which can penetrate deep into lower airways.

Subjects without asthma symptoms, but affected by seasonal rhinitis can experience an asthma attack. The event of November 21, 2016 in Melbourne, with the involvement of more than 10,000 persons and 10 deaths, added a new vision about these epidemics. If there is severe acute asthma or near fatal asthma or a sudden death in asthma subjects it is evident that during a thunderstorm in a pollen season there is in atmosphere a high level of

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allergenic activity released by pollen grains ruptured by heavy rain and able to penetrate deeply in the airways, inducing an inflammation sometimes also of severe degree.

In these cases, as evidenced in the outbreak which happened in Naples in 2004, only a very quick intervention with intubation and infusion of high concentration of drug such as corticosteroids (CS), theophylline and magnesium sulphate are able to save patients while in other cases of near fatal asthma there was need of intravenous high dosage of corticosteroids, teophylline and inhaled Beta-2 adrenergic agonists bronchodilators. However, it is important to inform all patients with pollinosis and allergic asthma about the importance of having spray of bronchodilators with inhaled corticosteroids (ICS) and not only of salbutamol.

Keywords: thunderstorm-asthma, pollen allergy, mould allergy, allergic rhinitis, allergic asthma, acute asthma attacks, near fatal asthma

Introduction

There are descriptions of asthma epidemics associated with thunderstorms, in several cities in the world, in Europe (Birmingham and London in the UK and Napoli in Italy) and in other areas of the World. In Australia and in particular in Melbourne several times have been observed outbreaks of Thunderstorm Asthma (TA) and Melbourne in November (the month on the top of the Spring Australia season) appears to be the city with higher risk of TA in the World [1-10].

Box 1. Thunderstorms and asthma epidemics

The thunderstorm-asthma epidemics are characterized, at the beginning of thunderstorms, by a rapid increase of visits for asthma in general practitioner or hospital emergency departments. Subjects without asthma symptoms, but affected by seasonal rhinitis can experience an asthma attack. Thunderstorms have been linked to asthma epidemics, especially during the pollen seasons, and there are descriptions of asthma outbreaks associated with thunderstorms, which occurred in several cities, in Europe (Birmingham and London in the UK and Naples in Italy) and in Australia, several times in Melbourne, which is the city with higher risk of TA in the World in Australian spring season (in particular in November) [1-10]. The thunderstorm-asthma epidemics are characterized, at the beginning of thunderstorms, by a rapid increase of visits for asthma in general practitioner or hospital emergency departments. Subjects without asthma symptoms but affected by seasonal rhinitis can experience an asthma attack.

During the first phase of a thunderstorm patients suffering from pollen allergy may inhale a high concentration of the allergenic material, like biological antigenic aerosols dispersed in atmosphere, which can induce equally severe asthmatic reactions in pollinosis patients [1-10]. Even though thunderstorms can induce severe asthma attacks they are neither frequent nor responsible for high amount of disease exacerbations. Yet, the mechanisms involved in the release of allergens from pollens during thunderstorm and associated risk should be known by physicians, not only allergists but also general practitioners (GPs) and pollen allergic patients for a prevention [11, 12]. In addition, there is a potential risk of thunderstorm-related relapse

of asthma attacks in some patients [13]. This constitute a huge concern as the possibility of TA outbreaks becomes of dramatic actuality nowadays because the frequency of thunderstorms is recently increased significantly in some geographical areas, particularly in temperate and subtropical climate due to the climate change [14].

Over the last few decades, incidences of respiratory admissions have risen due to the increased atmospheric concentration of airborne allergens. The fragmentation and dispersion of these allergens is aided by environmental factors like rainfall, temperature, and interactions with atmospheric aerosols [15]. Extreme weather parameters, which continue to become more frequent due to the impacts of climate change, have greatly fluctuated allergen concentrations and led to epidemic TA events which have left hundreds, if not thousands, struggling to breathe [5, 9, 16-18]. While a link exists between airborne allergens, weather, and respiratory admissions, the underlying factors that influence these epidemics remain unknown [19]. It is important that we understand the potential threat these events pose on our susceptible populations and we must ensure that our health infrastructure is prepared for the next epidemic [20]. After the event of London 1994 [6] the most lethal TA event occurred in Melbourne, Australia, in 2016.

Studies on the affected individuals found TA to be associated with allergic rhinitis, ryegrass pollen sensitization, pre-existing asthma, poor adherence to ICS preventive therapy, hospital admission for asthma in the previous year and outdoor location at the time of the storm. Patients without a prior history of asthma were also affected [8, 10, 21, 22]. These factors are important in extending our understanding of the etiology of TA and associated clinical indicators as well as possible biomarkers which may aid in predicting who are the subjects at risk and thus the ones who should be targeted in prevention campaigns [23]. Education on the importance of recognizing asthma symptoms, adherence to asthma treatment and controlling seasonal allergic rhinitis is vital in preventing TA. Consideration of allergen immunotherapy in selected patients may also mitigate risk of future TA. Epidemic TA events are predicted to be increased in frequency and severity with climate change, and identifying susceptible patients and preventing poor outcomes is a key research and public health policy priority [24].

Associations between thunderstorms and asthma attacks have been identified in multiple locations around the world [22, 25, 26]. The TA outbreaks are characterized, at the beginning of thunderstorms by a rapid increase of visits for asthma in GPs or hospital emergency departments [12, 21]. Subjects without asthma symptoms but affected by seasonal rhinitis can experience an asthma attack [5, 9, 16].

No unusual levels of air pollution were noted at the time of the epidemics, but there was a strong association with high atmospheric concentrations of pollen grains such as grass or other allergenic plant species. However, subjects affected by pollen allergy should be informed about a possible risk of asthma attack at the beginning of a thunderstorm during pollen season [27-31]. Thunderstorm-related asthma outbreaks have been described in various geographical zones (Table 1) [1-43].

TA can affect people living in metropolitan, regional or rural areas. It can affect people who have never been diagnosed with asthma. Those at increased risk of thunderstorm asthma include people with a history of asthma, people with undiagnosed asthma and people with hay fever (particularly seasonal hay fever) or allergy to grass pollen [32].

On November 21, 2016 in Melbourne there was a dramatic event with 10 deaths and about 10,000 patients who needed medical treatments in emergency departments of Hospital for asthma attacks [1, 2, 4, 21, 22].

This in Melbourne has been the worst event of TA. In Melbourne there was an extraordinary association of environmental factors with a very unusual weather occurrence with wind and torrential rain combined with a high pollen count (grass pollen airborne count of more than 100 pollens/m^3), sending high quantity of pollens and allergenic submicronic particles derived from pollens across the city. Asthma epidemics with thunderstorms are an increasingly significant public health challenge worldwide. Australia has experienced 10 of 22 TA epidemics recorded so far, the most recent in Melbourne in 2016. In just 12 hours, a single storm put unprecedented pressure on available health resources, resulting in the death of 10 patients and 10,000 cases related to asthma in health services [1, 2, 21, 22].

Box 2. Characteristics of the event of thunderstorm-asthma in Melbourne on November 21, 2016

Description of Event of TA registered on Nov 21, 2016, in Melbourne (VIC, Australia). It was experienced the peak of an unprecedented spring heatwave. Temperatures that day climbed to 35°C, the hottest recording since March that year, and the pollen count was extremely high, with airborne ryegrass pollen concentrations of more than 100 grains/m3 of air.

Around 14:00 h, the Australian Bureau of Meteorology issued warnings across most of the state for severe thunderstorms with damaging winds. Between 17:00 h and 18:30 h, the temperature suddenly dropped from 35°C to the low 20ºC and thunderstorms started to erupt, sending severe gust front winds over Melbourne's metropolitan area. Within an hour, in a nightmarish scenario, the emergency medical services started to receive hundreds of calls for acute respiratory distress and breathing difficulties throughout the state. By midnight they had received calls for 1,326 cases, a caseload so extreme that they ran out of ambulances after attending to 500. The call volume remained above normal levels until 07:00 h the next day.

Within 30 hours of the storm breaking, there were 3,365 excess respiratory-related presentations to emergency departments (ie, 672% above the average), and 476 excess asthma-related admissions to hospital (992% above the average). Additionally, there was a substantially increased number of severe asthma attacks. In total, around 10,000 people needed treatment in hospital emergency departments for asthma attacks shortly after the thunderstorm and 10 people died, 6 within a week of the storm.

The challenge faced by emergency response services indicates that it is time to examine the role of all emergency healthcare providers in providing the solutions needed to manage outbreak events of this type [33]. It is an important alert for physicians, paramedics and emergency facilities to be prepared to respond to any large-scale storm asthma event in the future [12].

During TA epidemics, GPs experience an increase in demand for services, although GPs express willingness to help, few structures exist to liaise, support and provide information to GPs during emergency events [20].

In Melbourne ambulance and hospitals experienced high scale of involvement in such a reduced time period of a few hours on November 21 with grass allergens dispersed over a very large geographical area of Victoria. The rapid onset of the medical emergency and its consequences were unprecedented in the scale of the intensity in comparison with previous

events and it tested the capacity of health system to be ready for this type of medical emergency [7].

However, demand management strategies were insufficient to manage such a widespread and rapid onset event, with ambulance resources quickly depleted and using police officers to conduct welfare checks [8]. The event of Melbourne greatly surpassed the previous epidemic of London of June 1994 [28, 34] that was the largest documented outbreak before the Victoria epidemic.

Thunderstorms have been linked to asthma epidemics, especially during the pollen seasons, and there are descriptions of asthma outbreaks associated with thunderstorms, which occurred in several cities in Europe, Birmingham and London in the UK [6] and Naples in Italy [1, 2] and Australia (Melbourne and Wagga Wagga) [3, 4-7]. There are observations that thunderstorms occurring during pollen season can induce asthma attacks sometimes severe and near fatal asthma in patients affected by pollinosis [1, 25, 35].

According to current climate change scenarios, there will be an increase in intensity and frequency of heavy rainfall episodes, including thunderstorms, over the next few decades, which can be expected to be associated with an increase in the number and severity of asthma attacks both in adults and in children [1, 2, 26, 36].

Table 1. Examples of thunderstorm-associated asthma outbreaks [1-43]

Year	Country	Observations
1983	UK	26 sudden cases of asthma attacks in relation to thunderstorms.
1992	Australia	Late spring thunderstorms in Melbourne can trigger epidemics of asthma attacks (5 to 10-fold rise).
1994	UK	Asthma or other airways disease hospital visits. 640 cases who attended during a 30-h period on June 1994, nearly 10 times expected number.
1992-2000	Canada	Hospital ED asthma visits among children 2-15 years of age. Summer thunderstorm activity was associated with an OR of 1.35 (95% CI 1.02-1.77) relative to summer periods with no activity.
1993-2004	USA	Asthma ED visits; the visits occurred on days following thunderstorms. Significant association between daily counts of asthma ED visits and thunderstorm occurrence. Asthma visits were 3% higher on days following thunderstorms.
2000	Australia	Asthma visits during thunderstorms. History of hay fever and allergy to ryegrass are strong predictors for asthma exacerbation during thunderstorms in spring.
2001	Australia	The incidence of excess hospital attendances for asthma during late spring and summer was strongly linked to the occurrence of thunderstorm outflows
2002	UK	A case-control study of 26 patients presenting to Cambridge University Hospital with asthma after the thunderstorm *Alternaria alternata* sensitivity is a compelling predictor of epidemic asthma in patients with seasonal asthma and grass pollen allergy and is likely to be the important factor in thunderstorm-related asthma.
2004	Italy	Six cases of thunderstorm-related asthma because of pollen (*Parietaria*).
2010	Italy	20 cases of thunderstorm-related asthma because of pollen (olive tree).
2010	Australia	Epidemics of 'thunderstorm asthma' that occurred in Melbourne during spring 2010. The approach of spring, together with high winter rainfall in and around Melbourne that heralds another severe pollen season, raises the risk of allergic rhinitis and asthma in pollen-sensitive individuals.
2016	Australia	Epidemics of thunderstorm asthma in Melbourne with 10 deaths and 10,000 in emergency department.

One of the first observations regarding thunderstorms and asthma outbreaks was provided by Packe and Ayres [37] at the East Birmingham Hospital (Birmingham, UK) on July 6 and 7, 1983. These authors described a remarkable increase in the number of asthma emergency

department admissions during the hours of a thunderstorm. In a 36-h period, 26 asthma cases were treated in the emergency department, compared with a daily average of 2 or 3 cases in the days preceding the outbreak.

Another asthma outbreak occurred in London, UK, coinciding with a heavy thunderstorm on June 24, 1994, when a large increase in the number of visits for asthma at the emergency departments of London and the southwest of England was observed. Several patients who were examined, who were not known to be asthmatics or were affected only by seasonal rhinitis, experienced an asthma attack. During a 30-h period from 6 p.m. on June 24, 1994, 640 patients with asthma or other airways disease (283 of whom were not known to be asthmatic and 403 were affected only by seasonal rhinitis) attended several emergency departments, nearly 10 times the expected number of 66 patients. In total, 104 patients were admitted (including 5 to an intensive care unit) (574 patients attributable to the thunderstorm) [28].

Other asthma outbreaks during thunderstorms have been described in Australia. In Melbourne, other than the dramatic outbreak of November 21, 2016, two large asthma outbreaks (rapid increase in hospital or general practitioner visits for asthma) coincided with thunderstorms. In Wagga Wagga, 215 asthmatic subjects attended the local emergency department, 41 of whom required admission to hospital [38].

Box 3. Subjects with pollen allergy without asthma symptoms but affected by seasonal rhinitis can experience an asthma attack during a thunderstorm in pollen season

In Melbourne, south eastern Australia, also before the event of 2016, was observed that the incidence of excess hospital attendances for asthma during late spring and summer was strongly linked to the occurrence of thunderstorm outflows and demonstrated that the arrival of a thunderstorm outflow was accompanied by a large increase in the concentration of ruptured pollen grains in ambient air.

Grass pollen in the storm outflow exceeded the caliber of any previously documented pollen counts. Increased PM10, high relative humidity, decreased temperature and low ozone concentrations observed in the storm outflow were significantly correlated with increased levels of ruptured grass pollen. Interpretation current seasonal air monitoring would be improved by including levels of ruptured grass pollen and small fungal spores.

TA was observed in Naples, Italy, on June 3, 2004 [1, 2], when 5 adults and one child received treatment in emergency departments. One patient was admitted to an intensive care unit for a very severe bronchial obstruction and acute respiratory insufficiency following a sudden thunderstorm. All individuals were outdoors when the thunderstorm struck. In one severe case, a female sensitized only to *Parietaria* (Urticacea) pollen allergens o solo Parietaria (Urticacea) pollen, soon began to show symptoms of intense dyspnoea, which gradually worsened. She was taken to hospital where she was intubated and given high intravenous doses of CS. She was discharged a few days later [2]. This patient had previously suffered from seasonal asthma but had been asthma-free for the past few years and did not need continuous therapy. None of the other involved subjects took anti-allergic and/or anti-asthma drugs regularly. All 6 patients were sensitized with allergic respiratory symptoms upon exposure to *Parietaria* pollen but were not sensitized to grasses. *Parietaria* is an Urticacea that is widespread in the Naples area of Italy with a spring and summer pollen season that is, in part,

coexistent with the one of grasses. During the thunderstorm, the concentration of airborne *Parietaria* pollen grains was particularly high, with a peak of 144 grains/m^3 being recorded on June 3, 2004. Air pollution levels for both gaseous and particulate components based on the hourly concentrations of nitric dioxide, ozone and respirable particulate matter were not particularly high in Naples on June 3 and 4, 2004. Subjects with sensitization to *Parietaria* who were indoors in Naples with the windows closed during the night between June 3 and 4, 2004, did not experience asthma attacks. No moulds or viruses were involved in the Naples epidemics [1, 2].

Other outbreaks and/or case reports have been described in Atlanta (USA) [39], in Canada [25], in Barletta (Italy) [27] and in several other cities in the world.

A similar phenomenon of Thunderstorm asthma was suggested for moulds and in particular for *Alternaria* species during the season of increased presence in atmosphere of this mould in summertime [40].

Although much remains to be discovered about the relationship between an increase in the number of asthma attacks and thunderstorms, reasonable evidence exists in favour of a causal link between them in patients suffering from pollen allergy.

Box 4. About thunderstorm asthma

- Thunderstorm asthma is a form of asthma that is triggered by an uncommon combination of high pollen (usually during late Spring to early Summer) and a certain kind of thunderstorm.
- Anyone can be affected, even if you don't have a history of asthma.
- People at increased risk have a history of asthma, have unrecognised asthma, have hay fever (allergic rhinitis), particularly seasonal hay fever, or are allergic to grass pollen.
- People experiencing asthma symptoms even if for the first time should not ignore it and should seek medical advice as soon as possible.
- An asthma flare up can vary in severity and can be life threatening. If there are signs that a person's condition is deteriorating, urgent care should be sought.
- Be aware of forecast thunderstorms in the pollen season particularly on days with a HIGH or EXTREME pollen count.

Wherever possible, stay indoors with doors and windows closed until the storm front has passed.

The most prominent hypotheses for TA are linked with bioaerosols and involve the role of rainwater in promoting the release of allergenic microparticulate matter [15]. Pollen grains can be carried by thunderstorm at ground level, with increased pollen rupture with subsequent release of allergenic biological aerosols of paucimicronic size, derived from the cytoplasm of ruptured pollen grain. This microparticulate can penetrate deeply into lower airways.

In particular, there is evidence that under wet conditions or during thunderstorms, pollen grains may, under wet conditions and rupture by osmotic shock, release into the atmosphere respirable, allergen-carrying cytoplasmic starch granules (0.5-2.5 um) or other paucimicronic

components that can reach lower airways inducing allergic asthmatic reactions in pollinosis patients with severe symptoms [15, 17].

Grass pollen starch granules are the most likely cause of associations between thunderstorms and asthma. Suphioglu et al. [29] showed that ryegrass pollen grains contain a large amount of starch granules coated with allergens. After being ruptured in rainwater by osmotic shock, each pollen grain can release more than 700 starch granules, small enough to penetrate the airways and trigger asthma attacks in pollinosis subjects, also in those patients previously affected only by allergic seasonal rhinitis.

Taylor et al. [42] hypothesized that the turbulent front of the advancing outflow releases more pollen from flowering grasses and grass pollen may release large amounts of paucimicronic allergenic particles, that is cytoplasmatic starch granules containing grass allergens (allergen bearing starch granules), after rupture by osmotic shock during thunderstorms.

Even though thunderstorms can induce severe asthma attacks or exacerbations, they are neither frequent nor responsible for a high amount of disease exacerbation. This constitutes a major concern nowadays as the possibility of TA outbreaks have become of dramatic actuality due to the "highly likely" increase in frequency of heavy precipitation events, including thunderstorms, projected by the climate change scenarios for the future decades. In summary, the occurrence of these epidemics is closely linked to thunderstorm, and they are limited to late spring and summer when there are high levels of airborne pollen grains. There is a close temporal association between the arrival of the thunderstorm, a major rise in the concentration of pollen grains and the onset of epidemics. As a consequence, subjects affected by pollen allergy should be alerted to the danger of being outdoors during a thunderstorm in the pollen season [41, 42].

Box 5. Aspects of epidemics of thunderstorm-associated allergic asthma in the world

- There is a link between storms and asthma epidemics in patients with pollen allergy during pollen seasons with appearance of symptoms during the first 20-30 minutes of a storm.
- Thunderstorm-related epidemics are limited to late spring and summer (in Europe, USA and Canada from April to end of June and in Australia from October to December), when pollen and/or mold counts are high.
- There aren't descriptions of allergic symptoms in individuals with allergy to pollens and molds but who are indoors with the window closed during a storm.
- The role of sudden cold and/or electric charges as contributor trigger factors of asthma attack in allergic subjects is possible.
- Individuals with allergic rhinitis only and no previous asthma can experience bronchoconstriction sometimes also severe during thunderstorms.
- Subjects with pollen allergy need be informed about a possible risk of asthma attack at the beginning of a thunderstorm during pollen season.
- Individuals who experience rhinitis and asthma during a storm are not usually taking suitable anti-inflammatory treatment, while it is important to have a correct antiasthma treatment by using bronchodilators and corticosteroids inhalers at increasing dosage if there is a need.

- The world's worst recorded thunderstorm asthma attack was on 21 November 2016 in Melbourne, where 10 subjects died and more than 8,500 were hospitalized in Victoria. It caused many people, including those who had no history of asthma or respiratory issues, to experience mild to severe breathing difficulties and near fatal asthma.
- Any serious asthma attack during a thunderstorm can be life-threatening and can induce also tragic consequences of near fatal asthma and of death.
- The health consequences of thunderstorm asthma may be prevented with adequate measures by meteorological forecast and by correct use of patients of adequate antiallergic and antiasthma therapy and avoiding to be outdoor at the start of a storm during pollen season.

Idrose et al. [43] in a recent systematic review evaluated the associations of grass pollen and fungi in TA events, noting that of the 20 studies included in the analysis, 15 showed some relationship, 9 demonstrated effects within four days of increased pollen concentration associated with increased risk of stormy asthma. Of the 10 studies that looked at fungi, 9 showed a positive relationship with storm asthma. The rates of fungi involved varied depending on whether measurements were taken before, during or after the storm.

Xu et al. [26], reported in the city of Yulin (China) that children with mugwort allergy are susceptible to stormy asthma, with a preponderance of males. Among children hospitalized during the event, 56% of them never had attacks or were diagnosed with asthma, 25% had a medical diagnosis of asthma, 67% had a history of allergic rhinitis, 76% moderate asthma, 94% had positive IgE against mugwort pollen and 78% were monosensitized to pollen and other were with polisensitization.

Identifying at-risk individuals is the most prophylactic approach that can be taken to mitigate the deadly consequences of TA [14]. The main risk factor appears to be a history of allergic rhinitis or sensitization to a particular allergen, circulating load of triggering aeroallergens, and age between 20-50 years. People exposed to the external environment are also more likely to suffer from stormy asthma and men are more likely to be affected than women [32].

Elderly people or those with common chronic respiratory diseases, such as asthma or chronic obstructive pulmonary disease (COPD), are more susceptible to negative health effects resulting from these phenomena. Zou et al. [36] report that emergency visits for acute respiratory illnesses increased significantly during the days before major storms among Medicare beneficiaries across the continental United States, particularly those with asthma and/or COPD.

Thunderstorm was considered unlikely to occur in New Zealand (NZ) due to its local weather patterns, but events on 2 December 2017 led to an increase in asthma hospitalizations at Waikato Hospital in Hamilton, with similar presentations to international descriptions of asthma by storm [44].

Ali et al. [35], reported cases of near-fatal and fatal asthma caused by storms in Kuwait on December 1, 2016; 17 patients were admitted with near-fatal asthma, 93.8% had a previous history of asthma, with an average duration of 9 years, 33.3% reported receiving ICS from their physician and 93.8% relying only on a short-acting β2-agonist to control their asthma. 68.8% reported being outdoors during the storm and 11 patients were diagnosed with fatal asthma.

Foo et al. [45] reported, in individuals affected by TA, evidence of continuous loss of asthma control in those with previously well-controlled asthma and persistence of symptoms suggestive of asthma in those without a history or symptoms suggestive of previous asthma, even after 36 months of initial TA.

Hew et al. [21] report on patients with asthma caused by epidemic storms who present to the emergency room, greater chances of hospitalization among patients with diagnosed asthma, highlighting the vulnerability conferred by the suboptimal control of the disease. The odds of hospital admission were lower in overseas-born Asian patients, but higher in locally-born Asian patients than in non-Asian patients; these observations suggest that susceptibility to severe asthma from storms may be increased by gene-environment interactions.

Emergency planners should not assume that most disaster victims require hospitalization or even emergency department evaluation. Screening and treatment can be carried out effectively in services attended by GPs, thus avoiding the overload of hospital facilities when a large number of patients need medical care in emergency departments. However, to determine the extent and severity of possible future events and to plan the prevention long-term follow-up is required [45].

Conclusion

There is evidence that, during pollen season, thunderstorms can induce allergic asthma outbreaks, sometimes also severe asthma crisis and sometimes deaths in patients suffering from pollen allergy. It has been observed that changes in the weather such as rain or humidity may induce hydration of pollen grains during pollen seasons and sometimes also their fragmentation which generates atmospheric biological aerosols carrying allergens. Asthma attacks are induced for the high concentration at ground level of pollen grains which may release allergenic particles of respirable size after rupture by osmotic shock.

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Chapter 9

Asthma and Climatic Change

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Abstract

Several retrospective studies based on historical data have shown that there is a global warming of the atmosphere, with an estimated average increase in the temperature of the earth's surface of 1.1°C during the last century. In fact, climate change affects the start, duration and intensity of the pollen season, which, together with pollution and respiratory infections, produces a synergistic effect on rhinoconjunctivitis and asthma exacerbation in patients with pollinosis. This data is very important, because seasonal allergies and asthma represent a significant burden for health systems, since it is estimated that between 10% and 30% of the world population suffers from allergic rhinitis due to pollens, accompanied by asthma by 40% of them.

CO_2, the main gas responsible for the greenhouse effect and global warming, is also a source of carbon needed to produce sugars during photosynthesis. When plants are exposed to higher temperatures and higher CO_2 levels, they grow more vigorously, produce more pollen, and pollen is more allergenic. It has been estimated that due to rising CO_2, the US pollen season could be brought forward 40 days by the end of the century. Global warming is favoring the spread of Ragweed throughout Europe. In just 25 years, *Ambrosia* pollinosis will have doubled in Europe from 33 to 77 million people.

Keywords: climatic change, allergenic pollen, pollinosis

Introduction

It is widely accepted that the Earth's temperature is increasing. This fact is confirmed by the warming of the oceans, the rise in sea level, melting glaciers, declining sea ice in the Arctic, and declining snow coverage in the Northern Hemisphere.

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On the other hand, changes are also manifesting themselves in the amount, intensity, frequency and type of precipitation, as well as an increase in extreme phenomena, such as heat waves, droughts, floods and hurricanes.

Several retrospective studies based on historical data, have shown that a global warming of the atmosphere exists, with an estimated average increase in the temperature of the Earth's surface of 1.1°C during the last century [1].

Climatic Change and Pollen Allergy

Both climate change and human impact on vegetation, may modify the timing and intensity of the pollen season. The severity of pollen-induced symptoms depends on the number of pollen grains and their allergenicity, and these variables are related to pollution and local climate [2-6]. Therefore, climate change could potentially change pollen exposure, sensitization and rhinoconjunctivitis and/or asthma symptoms [7-10].

Physicians who treat respiratory tract allergic diseases are already seeing an increase in symptoms attributed to climate change [11]. The World Allergy Organization, made-up of 97 different medical societies from around the world, released a statement in which, in their opinion, the climate change does indeed affect the onset, duration and intensity of the pollen season, which together with pollution and respiratory infections, produce a synergistic effect upon rhinoconjunctivitis and asthma exacerbation in patients with pollinosis [7].

Increase in Patients with Pollinosis

Seasonal allergies and asthma place a significant burden on healthcare systems, with between 10%-30% of the world population estimated to be affected by allergic rhinitis due to seasonal pollen rhinoconjunctivitis. In addition, 40% of patients with seasonal allergic rhinitis also suffer from bronchial asthma [7]. Data trends suggest that the prevalence of asthma is increasing, including those cases caused by pollen, fungi and other allergenic substances. Childhood asthma rates in the United States, for example, doubled between 1980 to 1995, before decreasing to a more gradual, although ongoing, increase [12]. Furthermore, there is evidence to suggest that the prevalence of pollinosis is increasing in many parts of the world, particularly in urban areas [12-14].

Increase in Pollen Counts and Early Onset of the Pollen Season

A long-term analysis of birch pollen seasons across Europe over a 30-year period, indicated earlier start dates, estimating that start dates will occur up to 6 days earlier within 10 years [15].

In Europe, a growing trend in the total annual concentration of pollen for most taxa has been observed [16], being more pronounced in urban areas than rural ones (1977-2009 - 32 years) using 97 pollen samplers [17].

Climatologists at the University of Michigan studied more than 14 different plant pollens in the United States and used computer simulations to calculate how much seasonal pathology would worsen by the year 2100. The new study found that the period of seasonal allergy would

extend even further, and total atmospheric pollen concentrations would rise steeply. It was estimated that with moderate reductions in greenhouse gas emissions, the pollen season would start 20 days earlier by the end of the century. In the most extreme warming scenario, it would start 40 days earlier [18].

Role of CO_2 in Pollen Production

Carbon dioxide (CO_2), the main gas responsible for the greenhouse effect, not only facilitates an increase in temperature, but also aids the growth of plants and their production of pollen.

Ziska and colleagues, conducted studies in the 1990's to explore the possible links between pollen production, rising CO_2 levels, and rising temperatures, by growing *Ambrosia* in chambers containing 280 ppm (which corresponds to the ambient CO_2 concentration that existed in 1890), 370 ppm (in 2000) and 600 ppm (the concentration in the atmosphere that the Intergovernmental Panel on Climate Change predicts for the year 2050, assuming no change in current emissions). The current concentration level in the atmosphere is just over 400 ppm. Ziska found that both the size of the ragweed plants and their pollen production, increased along with the increase in CO_2, specifically, pollen production per plant increased by 131% and 320%, respectively [19].

CO_2, the main gas responsible for the greenhouse effect and global warming, is also a source of carbon which is required to produce sugars during photosynthesis. When plants are exposed to higher temperatures and higher CO_2 levels, they grow more vigorously, produce more pollen, and the pollen is more allergenic [20-22].

Role of CO_2 on Pollen Allergenicity

Ben D. Singe et al., used the enzyme-linked immunosorbent assay (ELISA), to quantify Amb a 1 in protein extracted from *Ambrosia artemisiifolia* pollen grown at different CO_2 values. The concentrations used approximated pre-industrial, i.e., late 19th century atmospheric conditions, current conditions, and projected mid-21st century CO_2 concentrations (280, 370 and 600 μmol mol^{-1} CO_2, respectively). Although total pollen protein was unchanged, significant increases in the Amb a 1 allergen were observed between pre-industrial and projected future CO_2 levels, and between current and projected future CO_2 levels (1.8 and 1.6 times, respectively) [23].

Another investigation carried out with *Quercus acutissima*, in experimental conditions of elevated CO_2 at concentrations of 560 and 720 ppm, indicated substantial and significant increases in pollen production per tree, as well as increases in the concentration of allergenic proteins [24].

Role of Climate Change in the Spread of Ragweed in Europe

Global warming is favouring the spread of *Ambrosia* across Europe. A study has recently been published to predict the increase in *Ambrosia* pollinosis in Europe due to climate change [25]. It is a multidisciplinary study that used different models in different scenarios in the presence

of gases responsible for the greenhouse effect. They ran models to predict how ragweed pollen counts will increase and how these will affect the prevalence of pollinosis. Currently, the *Ambrosia* season only begins in July in Hungary and the northern area of the Balkans, and in the future, it will be brought forward in France and north-west Italy due to the increase in temperatures. At present, concentrations are only very high in central Europe (monthly totals >5,000 grains), but by 2040, they will also be very high in almost all of Europe, with the exception of the Scandinavian Peninsula, the Baltic countries, Ireland and the majority of the Iberian Peninsula, although there will be a slight increase in the region of Catalonia. Today, the season only extends to October in central Europe, but by 2040 that will be the norm for almost all of Europe, due to higher temperatures in autumn and a delay in the arrival of frost.

Currently, a sensitization greater than 20% (i.e., a very high prevalence in the population), only occurs in Hungary and the northern area of the Balkans (especially in Serbia). By 2040, this prevalence will have become widespread across most of Europe, affecting the rest of the Balkan Peninsula, Germany, Poland, France and northern Italy. To the contrary, the Scandinavian Peninsula, the Baltic countries, Ireland, Spain, and Portugal will be saved. In just 25 years, *Ambrosia* pollinosis will have doubled in Europe from 33 to 77 million people [25].

Climatic Change, Pollen Counts and Pollinosis in Madrid

Up to 88% of pollinosis patients in Madrid are polysensitized, usually to Poaceae, *Olea europaea*, *Platanus acerifolia*, *Cupressus arizonica* [26] and, in recent years, also to *Quercus ilex*, thereby increasing the duration of symptoms throughout the year [27, 28]. The objective of a study recently published by our group, was to verify if any increase in temperature has affected the aerobiological and clinical behaviour of the main allergenic pollens in Madrid over the last 40 years [29].

Pollen counting was carried out from 1979 to 2018 using Hirst-type volumetric collectors. Meteorological data from the Madrid-Barajas station located at 9 km from the clinic, were used. The beginning of the season was considered as the first of three consecutive days with >10 grains/m^3 in the air, and the end, the last of three consecutive days with >10 grains/m^3 in the air [16].

The prevalence of Positive Skin Tests or PST, (Inmunotek Laboratory, Madrid, Spain), was studied among pollinosis patients born and living in and around Madrid in 1979 (n = 100), 1994 (n = 316) and 2019 (n = 100), making a total of 516 patients aged 4 to 77 years old (average age 27 years), of which 98% suffered from rhinoconjunctivitis and 41% from asthma. An increase of 1.3ºC in the 5-year mean temperature records over 40 years in Madrid, was observed ($r_s = 0.81$, $p = 0.014$). The Cupressaceae, *Platanus* and *Quercus* 5-year mean total pollen concentrations increased dramatically and correlated significantly to the increase in temperature ($r_s = 0.7$; $p = 0.037$), ($r_s = 0.9$; $p = 0.002$) and ($r_s = 0.7$ and $p = 0.047$), respectively (Figure 1).

Furthermore, it is remarkable how most of the peak days were concentrated within the last 5-year period (Table 1). The duration of the corresponding pollen season increased by 13.4 and 7 days respectively, with a decrease of Poaceae, -3 days and *Olea* -1 day.

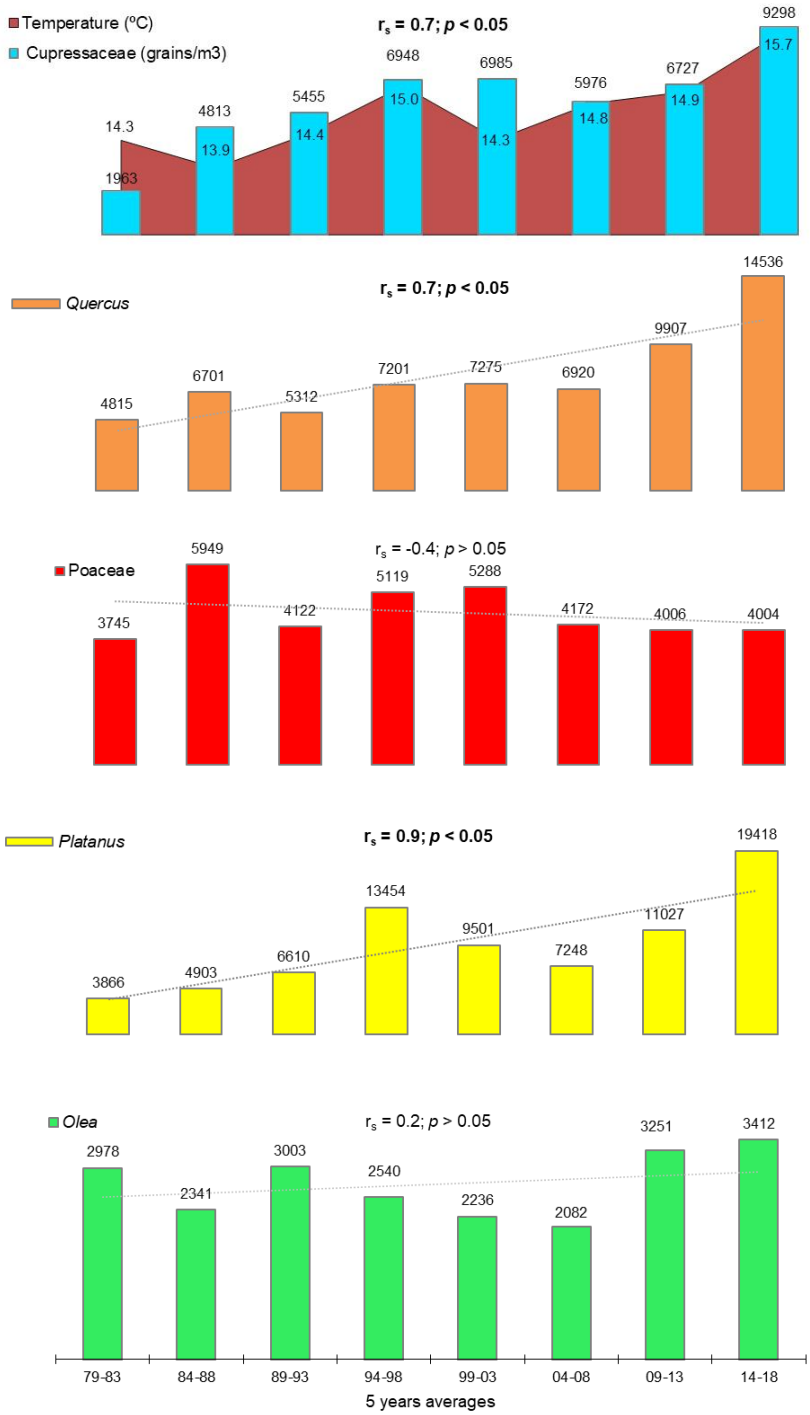

Figure 1. Temperature (ºC) and pollen counts expressed in grains/m^3 of air, total year, and 5-year averages. Note the increase in temperature and Cupressaceae, *Platanus* and *Quercus* concentrations. Also note the close significant correlation between both variables. In the linear regression analysis, a one-degree annual mean increase in temperature has produced an annual increase of approximately 3,000, 9,000 and 5,000 pollen grains/m^3, respectively. Adapted from Subiza et al., [29].

Table 1. Peak day in Madrid, using a Hirst-type volumetric collector

	Cupressaceae			*Platanus*			*Quercus*			Poaceae			*Olea*		
Period	Day	Year	Grains/m^3	Day	Year	Grains/m^3	Day	Year	Grains/m^3	Day	Year	Grains/m^3	Day	Year	Grains/m^3
1979-1983	07 Jan	1983	470	02 Apr	1981	1037	16 May	1979	841	22 May	1979	281	25 May	1982	718
1984-1988	15 Feb	1988	1086	26 Mar	1988	1734	18 May	1986	1382	29 May	1988	546	13 Jun	1985	340
1989-1993	21 Feb	1991	3306*	13 Apr	1991	1464	19 May	1989	492	18 May	1990	306	21 May	1989	535
1994-1998	15 Dec	1994	2376	13 Mar	1997	4265	16 Apr	1997	1303	01 Jun	1996	552	04 May	1997	574
1999-2003	31 Jan	2002	1180	04 Apr	1999	2830	28 Apr	2002	1128	21 May	2002	545	27 May	1999	424
2004-2008	09 Jan	2004	834	06 Apr	2005	1151	29 Apr	2005	1080	21 May	2006	395	26 May	2005	692
2009-2013	27 Jan	2013	1150	31 Mar	2011	2958	18 May	2012	1200	26 May	2012	351	05 Jun	2013	779
2014-2018	26 Jan	2014	2031	30 Mar	2015	5297*	13 May	2015	1880*	05 May	2016	958*	09 May	2017	780*

* It is remarkable how most of the peak days were concentrated within the last 5-year period.

The pollen season began early for Cupressaceae, *Platanus*, *Quercus*, Poaceae and *Olea* (-31, -6, -13, -4 and -7 days, respectively) (Figure 2 and Table 2), and also ended early for Poaceae and *Olea* (-7 and -8 days, respectively), (Figure 3 and Table 2).

Table 2. Pollen season, start and end date. Adapted from Subiza et al., [29]

	T	Cupressaceae		*Platanus*		*Quercus*		Poaceae		*Olea*	
5 years averages		Start	End	Start	End	Start	End	Start	End	Start	End
79-83	14.3	28 Jan	25 Apr	28 Mar	20 Apr	24 Apr	25 Jun	13 May	17 Jul	23 May	28 Jun
84-88	13.9	2 Jan	28 Mar	29 Mar	1 May	19 Apr	19 Jun	10 May	15 Jul	25 May	1 Jul
89-93	14.4	11 Jan	21 Mar	22 Mar	30 Apr	19 Apr	22 Jun	8 May	11 jul	17 May	26 Jun
94-98	15.0	7 Dec	8 Apr	14 Mar	20 Apr	29 Mar	10 Jun	8 May	10 Jul	6 May	14 Jun
99-03	14.3	29 Dec	19 Mar	16 Mar	21 Apr	5 Apr	11 Jun	8 May	8 Jul	14 May	14 Jun
04-08	14.8	13 Dec	15 Apr	21 Mar	27 Apr	11 Apr	22 Jun	6 May	7 Jul	13 May	16 Jun
09-13	14.9	31 Dec	8 May	25 Mar	25 Apr	1 Apr	19 Jun	6 May	8 Jul	11 May	14 Jun
14-18	15.7	10 Dec	29 Mar	25 Mar	27 Apr	8 Apr	24 Jun	8 May	3 Jul	15 May	17 Jun

T = temperature in ºC. The beginning of the season was considered as the first of three consecutive days with >10 grains/m3 in the air, and the end, the last of three consecutive days with >10 grains/m3 in the air. The season starts and ends earlier for most of the pollen types studied, although especially for Cupressaceae.

Likewise, PST also acutely increased, but only for *Cupressus arizonica* (0%, 20%, 59%), *Platanus acerifolia* (2%, 52%, 56%) and *Quercus ilex* (0%, 14%, 22%). The fact that this finding only occurred with the types of pollen that had an increased presence in the atmosphere of Madrid is noteworthy, and sensitization rates were stable for Poaceae, whose concentrations did not increase. This suggests that the main factor responsible for this phenomenon was the increase in pollens in the atmosphere due to increasingly warm winters, and hot and sunny springs, caused by the change in climate over the last 40 years [29]. These data concur with previous research published in both the USA and Europe, that emphasize the early onset of the season and the increase in pollen concentrations due to global warming [8, 15-17, 21, 30-33].

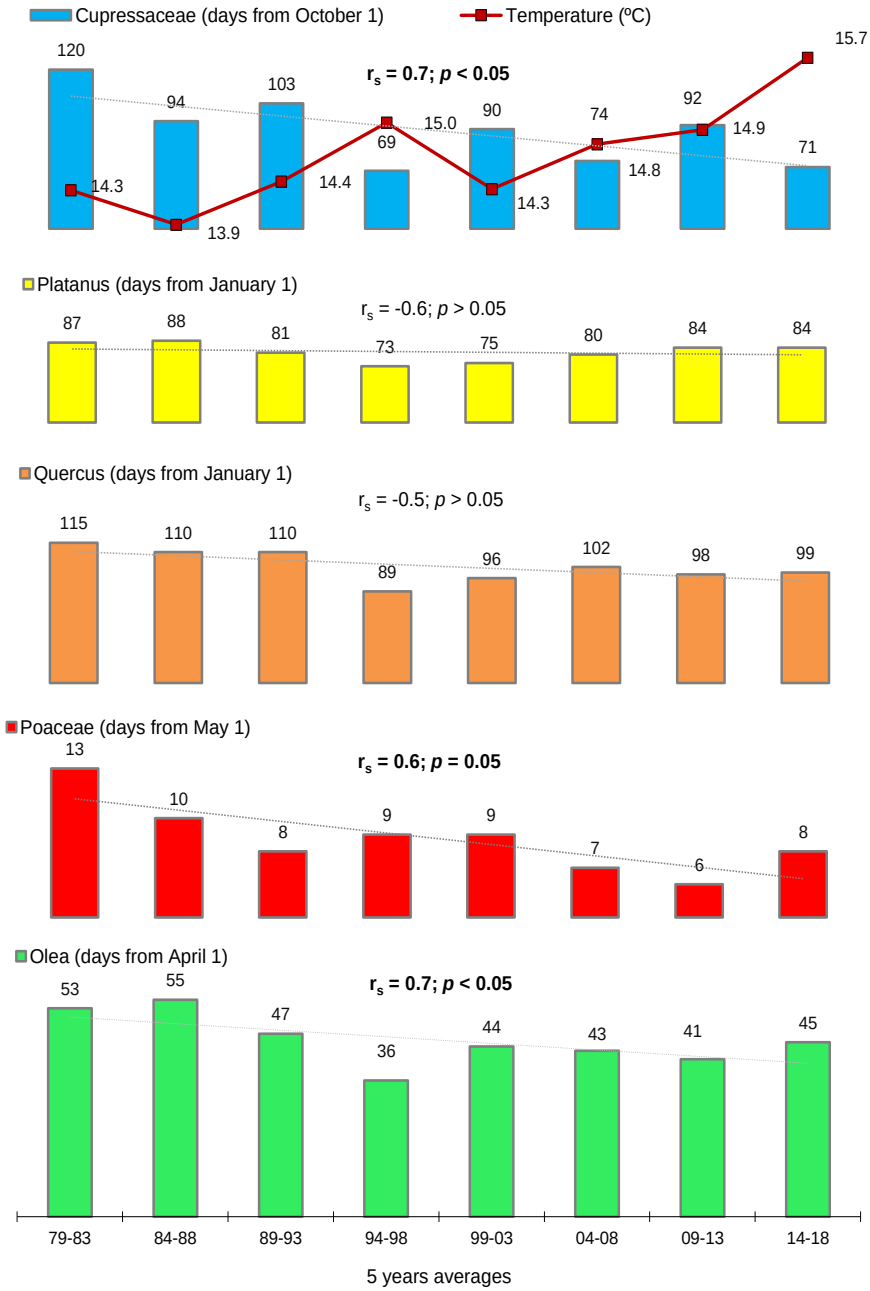

Figure 2. Pollen season, start. Early onset was observed for Cupressaceae, -31 days, which correlates significantly with the 5-year mean temperature (r_s = -0.76 p = 0.18), *Olea*, -7 days, (r_s = -0.71, p = 0.047) and almost significant in the case of Poaceae, -4 days, (r_s = -0.690, p = 0.058). A non-significant early onset was observed for *Quercus*, -13 days, and *Platanus*, -6 days.

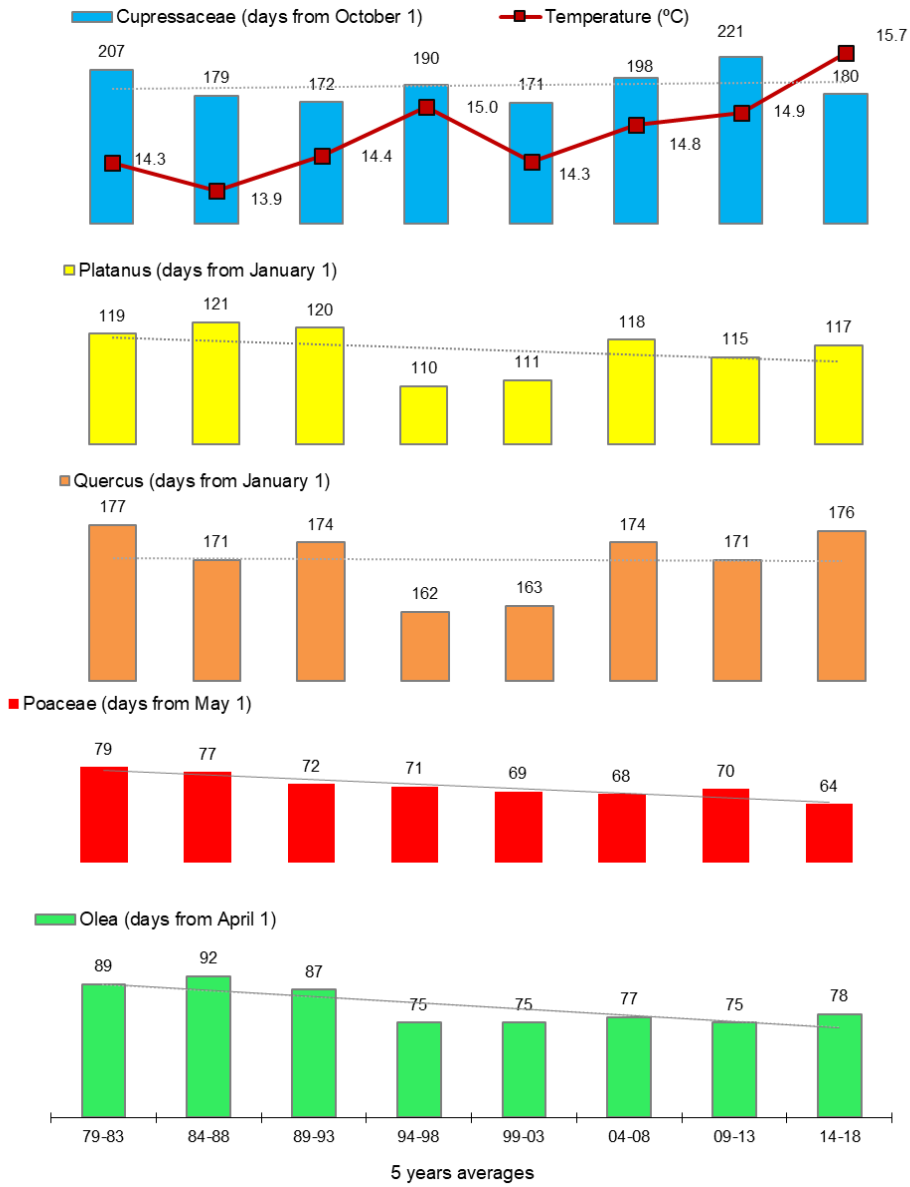

Figure 3. Pollen season, End. An early end of season was observed for Cupressaceae -18 days, *Platanus* -2 days, *Quercus* -6 days, Poaceae -7 days and *Olea*, -8 days. Comparing the average of each period, with the mean of all the periods.

Winters with More Pollinosis Symptoms

Our Madrid group carried out a study on 96 patients between 2009 and 2021, in which the patients' daily pollinosis symptoms were monitored. Although May continues to be the highest month for pollinosis globally, we observed however, that March exceeds June and February exceeds April [34]. These data point to the increasing importance of pollinosis in winter and early spring, since winters are getting warmer, and more pollen is produced.

Climatic Change and Grass Pollen

We previously reported that in Madrid, the monthly average temperatures in May and June have progressively risen, with a significant upward trend from 1979-2015. The increase in temperature has caused an increase in grass concentrations in May, but to the contrary, a decrease in grass pollen concentrations in June. The most probable reason is the increase in temperature, which in turn, causes greater simultaneous and intense flowering in May of the different species of grasses, and also an early withering in June [35]. Early withering is likely to be the cause of the horizontal trend rather than the upward trend in total grass pollen concentrations observed throughout our study (Figure 1) [29]. Furthermore, the lack of correlation between total annual grass pollen concentrations and temperature, is not surprising. We know that the pre-seasonal rainfall in Madrid and not the temperature, is mainly responsible for the total annual concentrations of grass pollen [36]. Another interesting finding, probably related to climate change, is that in a previous study carried out in Madrid, a clinically relevant out-of-season Phl p 1 was found with a positive O_3 correlation, during September-November 2009 [37]. This could have been caused by the presence of Phl p 1 in diesel exhaust particles; particles that are more common during winter anti-cyclonic days [3, 38].

Climate Change and Thunderstorm Asthma Due to Grass Pollen

Epidemic thunderstorm asthma is a global health problem that can occur without warning and can have catastrophic consequences. Due to climate change, future events are likely to become more common, more disastrous and more unpredictable [39].

On 21st November 2016, a severe thunderstorm in Melbourne, Australia, caused 9,900 patients to be hospitalized with asthma attacks. Nine deaths were also related to this episode.

Cases of epidemic thunderstorm asthma are thought to be triggered by a unique combination of high levels of grass pollen and a certain type of thunderstorm. Grass pollen grains are blown by the wind and transported long distances; some can burst and release tiny particles that are concentrated in the gusts of wind that come just before a thunderstorm. These particles are small enough to be inhaled deep into the lungs and can quickly trigger asthma symptoms, sometimes so severe that they can be fatal.

Epidemic thunderstorm asthma events are not unique to Australia. Although rare, they are a global phenomenon, with 26 known events reported around the world [39]. For more information, the reader is referred to the storm asthma chapter of this book.

Climatic Change and Fungi Asthma

Some airborne spores, such as *Alternaria*, *Cladosporium*, and *Aspergillus*, have been associated in some studies to a higher prevalence of hospital admissions for asthma [22]. Fungi in general, require high humidity and warmer temperatures. As sea levels rise, people living near coastal regions may be at a potential risk of increased exposure to water and damp living conditions, including the risk of increased indoor mould contamination and spore exposure. Consequently, it is probable that anthropogenic climate changes represent a negative influence [22]. One of

the few studies that examined the seasonality of spore production, focused on *Cladosporium* in France, and showed a downward trend in more southern locations and an upward trend elsewhere. However, such trends appeared regardless of the temperature increases that occurred continuously during the study period [40]. Corden and Millington, showed that *Alternaria* spore concentration increased during 1970-1998 in the UK, in Derby, with the increase most likely associated to increases in temperature and harvest periods [41].

Climatic Change and Dust Mites Asthma

Dust mites are very sensitive to relative humidity. In regions that become warmer and more humid with climate change, dust mite populations could reflect tropical dust mite blooms and increased egg and allergen blooms [22]. This fact could partially explain the increase in house dust mite sensitization and asthma symptoms in some countries and regions. Further epidemiological and experimental research is needed to confirm these effects and to design prevention programmes.

Conclusion

Climate change is, and will, continue to cause negative effects on respiratory tract allergic diseases. In particular, the increase in the length and severity of the pollen season.

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Chapter 10

Air Pollution and Asthma

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Abstract

Increased industrial activity and road traffic cause a rise in pollution levels, worsening air quality. This phenomenon influences people's health by increasing the prevalence of respiratory diseases, such as bronchial asthma. The main pollutants are nitrogen oxides, ozone, and particulates generated by activities that produce airborne contaminants.

Until three decades ago, allergies were a not very frequent cause of bronchial asthma. However, the prevalence of bronchial asthma has increased markedly in recent years. Pollution plays an important role in this trend. The associations between pollution and worsening lung function, new diagnoses of seasonal asthma, clinical decompensation, and the need for treatment in the emergency department have been clearly shown. Pollution causes clinical decompensation in patients with bronchial asthma, thus leading to increased medication consumption.

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Moreover, indoor pollutants, such as tobacco smoke, are an additional risk factor affecting the increase in childhood asthma, which develop a more severe type of asthma characterized by a greater functional impairment.

Pollution directly affects the immune system. Pollutants trigger the production of antioxidants and anti-inflammatories that modulate the immune response, thereby promoting the development of the allergic condition. The severity of bronchial asthma is influenced by exposure to pollutants and is impacted by both genetic and epigenetic factors. Pollution modifies allergens such as pollen, making them more allergenic; consequently, the modified allergens elicit a stronger immune response.

Keywords: grass pollen, pollution, seasonal allergic asthma, NK cells, T-CD8

Introduction

Environmental pollution and atmospheric warming are serious problems that every day affect more developed countries. The continuous population increase, its progressive concentration in large urban centres, and industrial development are causing a continuous deterioration of the air quality of cities, with negative effects on human health. These negative consequences are expressed more intensely in respiratory diseases, as bronchial asthma [1-3].

The first data obtained on the direct effect of pollution on human health were recorded in London in the middle of the last century. In December, in association with thermal inversion, black dust particles and dense fog formed “smog” caused a total of 4,000 deaths between December 4 and 9, 1952 (1,000 in only a day) [4]. However, since the 1980s, numerous studies have demonstrated the relationship between environmental pollution and bronchial asthma. Longitudinal studies were conducted in Mexico [5], Cleveland [6], Paris [7], and Barcelona [8] that confirmed a close association between particles, ozone, and nitrogen oxides and emergency care for asthmatic crises or the clinical decompensation of patients when pollutants exceeded risk thresholds for human health. In addition, when levels below these limits, there has also been a worsening of asthma in children, with greater use of emergency efforts and maintenance medication, especially in cases with more severe asthma [9, 10]. Subsequently, new advances in research on these issues have been produced, establishing the association of air pollution with impacts on lung growth in early life, the development of allergic sensitization, the development of asthma, respiratory tract inflammation, severely limited lung function, and exacerbations of asthma [11, 12].

Allergy as a cause of bronchial asthma was not very frequent until three decades ago. However, its prevalence has increased notably in recent years, especially among young adolescents [13, 14]. This increase could have occurred due to genetic or environmental factors, but it is unlikely that genetic changes could occur in such a short period of time. In addition, it has been shown that these changes manifest in populations with similar genetic or ethnic backgrounds [15]. Pollen allergens are able to trigger the release of proinflammatory and immunomodulatory mediators that accelerate the onset of IgE-mediated sensitization and allergy [2]. Pollutants disrupt the epithelial barriers of the skin and mucosal surfaces, and these disruptions have been linked to the increasing prevalence and severity of allergic and inflammatory diseases, allergic rhinitis, and asthma. Activation of epithelial cells and release of epithelial cell cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP),

followed by type 2 inflammation, play major roles in the development and exacerbation of allergic diseases [16, 17].

Similarly, outdoor air pollution derived from traffic and other human activities not only has a direct negative effect on human health but also enhances the allergenicity of some plants [3]. Recently, we analysed and compared grass pollen *(Lolium perenne)* from Madrid (high urban pollution) and Ciudad Real (low urban pollution). We demonstrated that grasses in Madrid expressed a higher degree of oxidative stress and lower photosynthetic activity than those in Ciudad Real [18].

In this chapter, we will first describe each studied pollutant, its origin, and its clinical effects associated with bronchial asthma. The second part of the chapter will focus on the differences among rural, industrial, and urban pollution since its effects are different in each area [19]. Finally, the immunological effects of pollution on asthmatic patients will be discussed.

Environmental Pollutants

Nitrogen Oxides

Nitrogen oxides are classified according to their oxidation into nitrogen dioxide (NO_2) and nitric oxide (NO). It is a reddish toxic gas that is absorbed by water droplets, precipitating in the form of acid rain. In addition to a large number of industrial processes, vehicle traffic is the most relevant source, especially in large cities, tripling its value in highly polluted areas such as Madrid (6,700.00 inhabitants) compared to Ciudad Real (75,000) [20]. Similarly, studies carried out in numerous cities have linked asthma exacerbation and a greater number of emergency service visits with high concentrations of nitrogen dioxide [6-10]. Thus, it is well established that nitrogen dioxide is a relatively specific traffic-related air pollution (TRAP) pollutant [21]. Relatedly, Cortegano [22] demonstrated the effect of a high density of vehicle traffic on the response to allergenic pollens. The results showed that in comparison to pollen from rural areas, pollen collected near motorways resulted in a significantly more intense skin response (prick test). In addition, *Cupressus arizonica* pollen associated with high traffic density expressed a new allergen from the thaumatin family (Cup a 3) because of exposure to high levels of pollution.

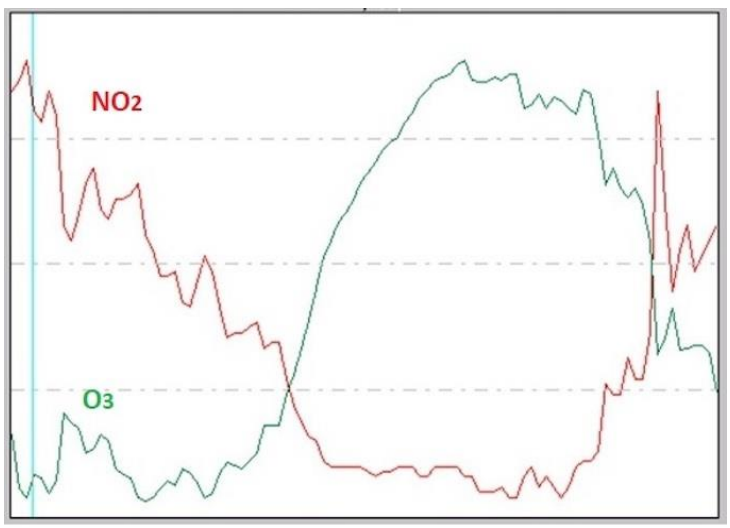
In addition to the effects of vehicle traffic, the risk of exposure to NO_2 also occurs inside homes. Although exposure to pollutants has always been related to outdoor areas, we must take into account that in some countries, people spend 90% of their time indoors [23]. NO_2 is generated from the use of gas as an energy source (kitchen, stoves, heaters, etc.). The risk is higher for children, as they spend more time in the home, especially in the autumn-winter months, and they are more vulnerable to the effect of pollutants [24].

Experimental studies have shown an increase in bronchial hyperreactivity with exposure to 0.1 ppm of NO_2, as well as an increase in immediate and delayed responses with exposure to *Dermatophagoides pteronyssinus*, if 400 ppm of the pollutant were previously inhaled. In addition, with this exposure, the cationic protein of eosinophils and concentration of the proinflammatory cytokines increased GM-CSF, IL-5, IL-6, IL-8, IL-13 and ICAM-1 as a consequence of their participation in both immediate and late immune responses [25].

Ozone

Ozone is present at different atmospheric levels and has different health effects. On the one hand, the ozone closest to the Earth's surface has oxidizing effects and is therefore harmful to health. On the other hand, stratospheric ozone in the upper layers of the atmosphere filters ultraviolet radiation, so the destruction of the so-called "ozone layer" is an environmental problem with notable repercussions, both for people and plants.

Another characteristic of ozone is that it is a secondary pollutant, and its precursors are nitrogen oxides and volatile organic compounds. The formation of ozone from its precursors is carried out in the presence of sunlight and high temperatures, so its highest levels in Europe are reached during the summer months and in Mediterranean countries. For this reason, it is known as summer "smog". Ozone can be transported over long distances by prevailing winds and can be found at high concentrations in rural areas where there are no ozone precursors. Figure 1 shows an actual situation at an automatic station in the city of Toledo, with the formation of ozone from nitrogen dioxide. The highest concentration of ozone corresponds to the maximum intensity of sunlight.



Source: *Treatise on Allergology*, 2nd Edition, Dr. I.J. Dávila.

Figure 1. Daily evolution of the concentrations of nitrogen dioxide (NO_2) and ozone (O_3) at an automatic station in Toledo. The ozone precursor character of nitrogen dioxide is observed.

Regarding the effects of ozone in asthmatic patients, its participation in the immunopathogenesis of respiratory diseases is demonstrated through an increase in Th2-type immunity. Ozone is associated with neutrophilic inflammation and underlying oxidative stress in asthma [26]. Epidemiological studies have shown a relationship between ozone and greater symptoms of asthma, emergency care due to bronchial asthma decompensation, and increased consumption of antiasthmatic medication [6, 10, 27].

Regarding the relationship of ozone with asthma, exposure to small amounts of ozone (0.12 ppm) reduces the amount of allergen necessary to cause a 15% decrease in forced expiratory volume in one second (FEV1) with respect to inhalation of uncontaminated air [25]. Thus, the increase in the amount of ozone during outdoor exercise in contaminated areas increases its

effect on the respiratory tract and has been related to a higher incidence of new asthma diagnoses in children residing in cities with high levels of ozone pollution [28, 29]. A good example involves the actions that are carried out in the cities that host the Olympic Games. With the objective of obtaining the best marks from the athletes, both Atlanta [30] and Beijing [31] significantly reduced both traffic and industrial activity. In parallel, pollutants were reduced by 33%, and bronchial asthma decompensations decreased in the same proportion. This is an obvious example of how the reduction of urban pollution has direct effects on human health.

Particles

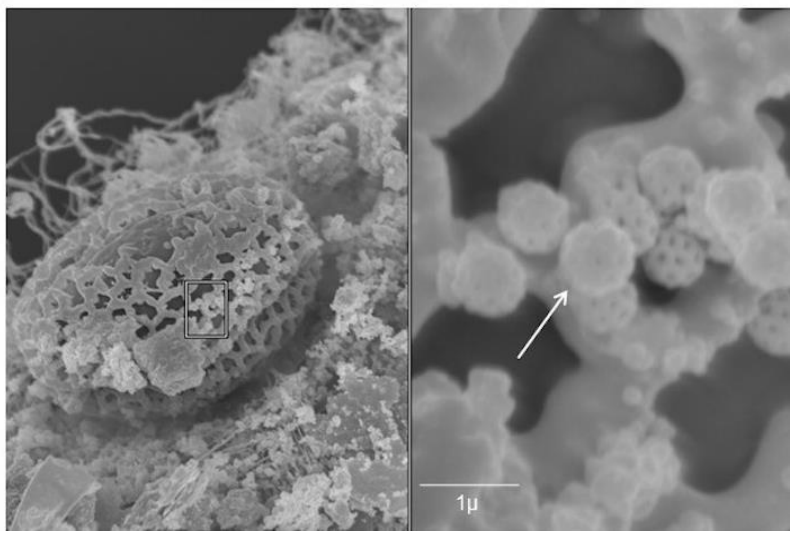
The particles that result from polluting activities are of sufficiently small sizes that they remain in suspension and are not deposited quickly on surfaces. The time that particles stay in the atmosphere depends on their size and composition, winds, rain, and atmospheric humidity.

The sizes of these particles allow them to be differentiated into coarse particles (between 2.5-10 μm) with origins in the soil, roads, industrial activities, farms, or volcanoes. Similarly, they correspond to aeroallergens such as pollen, fungi, or plant substances. Fine particles, with a size between 0.1-2.5 μm, are derived from combustion engines of gasoline or diesel vehicles, construction, or mining activities. Finally, ultrafine particles, derived from vehicle traffic and industrial activities, have a size less than 0.1 μm that allows them to pass from the alveoli to the circulatory system, producing systemic effects such as stroke, ischaemic heart disease, or haematological alterations [32].

Development in countries has led to an increase in urban populations that account for half of the world's population. It is expected that before 2050, two-thirds of the global population will live in urban areas. For example, in Japan, its industrialization caused great changes in daily activity, multiplying the number of vehicles by 500 and the amount of aromatic hydrocarbons by 1000, which are closely related to diesel exhaust particles (DEPs). Technological advances and the best gasoline engines have made diesel engines the most responsible for particle pollution, emitting up to 100 times more than gasoline engines. Thus, DEPs are considered a surrogate for TRAP. Numerous clinical studies have demonstrated the role of DEPs in worsening bronchial asthma based on emergency care for asthma exacerbations, disease reactivation, or greater consumption of rescue medication [6-10].

On the other hand, DEPs also interact with particles from pollen and allergenic plants. The sensitizing components of the plants are found in different areas of pollen grains since the particles from other parts of the plants, such as inflorescences, orbicules, leaves, stems, roots or seeds, maintain their allergenic capacity (Figure 2). Similarly, under rainy or humid conditions such as storms, grass pollens break and release up to 400-500 pollen particles for each pollen grain. During storms, intense descending, surface, and ascending air flows are produced, which drag the allergenic particles and return them again to the atmosphere and exponentially increase the risk for allergy sufferers. When this phenomenon coincides with high levels of contamination by DEPs, the DEPs can transport pollen particles to the bronchi and trigger asthma epidemics, such as those described in London and Melbourne, due to the sum of both risk factors at the bronchial level [3, 33]. Our group conducted a study of the responses of monosensitive asthmatic patients to grasses for a full year by daily recording of the sum of symptoms and medication of the patients and their relationship with pollen grains

and allergenic particles. In October and in association with storms, asthmatic patients presented moderate symptoms due to the presence of average concentrations of pollen allergens, without detecting pollen grains at the atmospheric level. Thus, the importance of allergenic particles as inducers of symptoms in asthmatic patients was demonstrated [34].



Source: *ASMA Volume 1*, Dr. Santiago Quirce.

Figure 2. Electron microscopy image of an olive pollen grain captured on an air filter. In the figure on the right, a small, spherical element with a diameter of 0.5 to 1µm can be observed between the trabeculae of the pollen (arrow). These may correspond to orbicules of *Olea europaea*.

Seasonal Asthma and Industrial Pollution

Analyses of the effects of the different types of pollutants on health began with the studies of von Mutius, who compared western Germany (Munich) with eastern Germany (Leipzig). Asthma and atopy predominated in Munich with vehicle traffic pollution and bronchitis in Lepzig with combustion of coal as an energy source. However, 5 years after the reunification of Germany, the westernization of Leipzig increased the prevalence of allergies to pollen from 2.3 to 5.1%, as an expression of the association of atopy and urban pollution [35]. Industrial pollution from coal has been followed over the years as have other polluting activities, such as the combustion of wood, and pollution from foundry plants, paper mills, and iron, steel, or cement plants. These activities have generated poor air quality, and their harmful effects have been expressed in the form of acute and chronic symptoms, as well as the development of chronic diseases, such as bronchial asthma. In addition, notably, these clinical effects occur even when the levels of pollutants are within the risk limits established by environmental agencies [6, 36].

In Spain, we have an industrial pollution model that involves examining a nearby city that does not have health risk activities. Puertollano (48,000 inhabitants) is an industrial city with a petrochemical refinery, a fertilizer factory, and two thermal power plants. On the other hand, Ciudad Real (75,000 inhabitants) is a city with administrative and service sector activities. The

cities are 37 km apart, with similar aerobiological characteristics. During two seasons, we monitored asthmatic patients allergic responses to pollens through the daily record of pulmonary function measurements (peak flow), respiratory symptoms, and anti-asthma medication. The results showed that allergic patients from Puertollano had a three-fold higher risk of clinical decompensation than those from Ciudad Real. In addition, patients in Puertollano decompensated earlier than those in Ciudad Real, so the symptoms and consumption of mediation were more intense and lasted longer. Similarly, clinical decompensation was three times higher in patients with moderate asthma than in patients with mild asthma [27, 37]. These results coincide with the results of Gent [10] and Forsberg [38], who divided patients into two levels according to their vulnerability to contaminants, with greater clinical effect in patients with more severe symptoms.

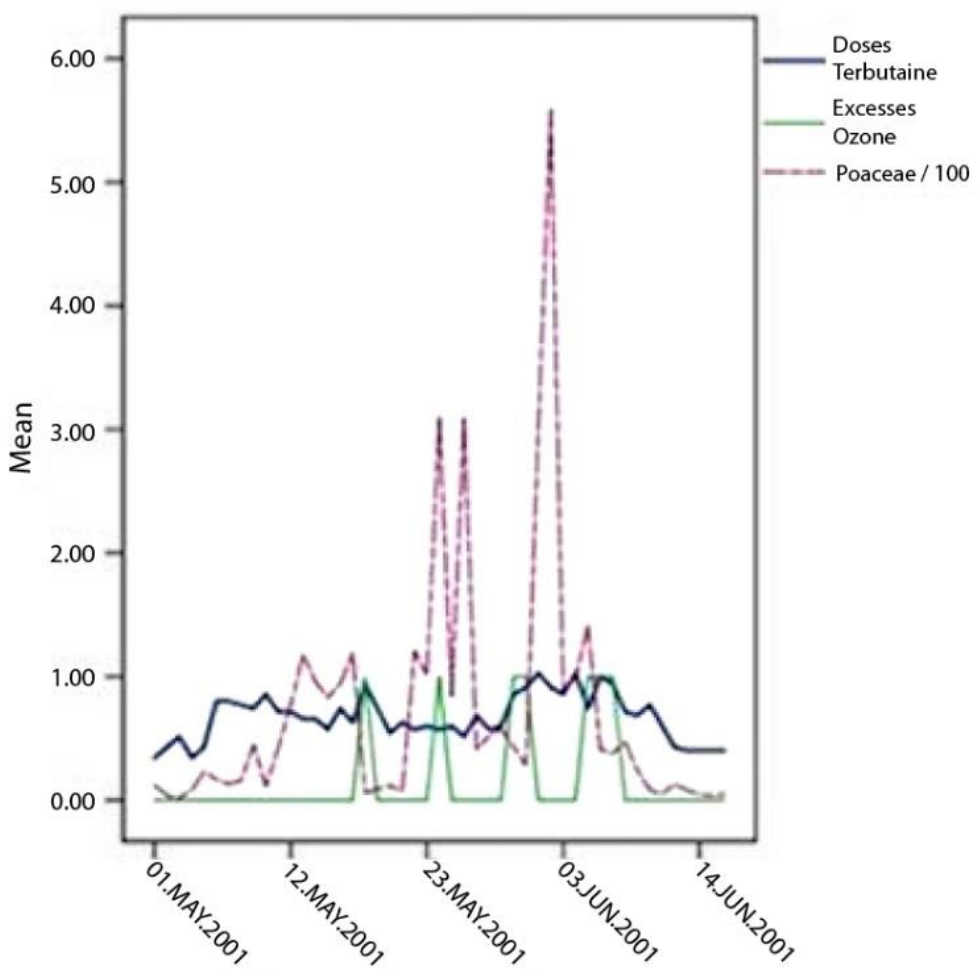


Figure 3. Consumption of rescue medication (terbutaline) by Puertollano patients and its relationship with ozone exceedances and the concentration of grass pollen (Poaceae).

The results of our study showed that ozone and particles are the pollutants most involved in the symptoms of Puertollano patients. Ozone, with a one-day lag, increased the sum of the symptoms and medication of patients by 8.5%, and particles, with a one-day lag, increased these factors by 6%. Ozone is a secondary pollutant, so its maximum effect in the production area itself is surprising, which means that ozone is "trapped" in Puertollano without reaching its maximum concentrations at a distance. Pollution has its own characteristics in each area, according to sources, meteorological characteristics, and interactions between different polluting substances [2, 39]. Another interesting aspect of these studies is the consumption of rescue medication. The maximum level of particles and ozone concentrations corresponded to higher values of consuming antiasthmatic drugs [3, 10]. Similarly, in our case, ozone exceeded its established threshold 13 times in Puertollano and only two times in Ciudad Real. The consumption of inhaled betamimetics (terbutaline) was significantly higher in the industrial city than in the city with low levels of ozone [27] (Figure 3).

Seasonal Asthma and Urban Pollution

In relation to urban pollution, data from the World Health Organization (WHO) are conclusive. Ninety percent of the global urban population is exposed to pollution levels that exceed those recommended by environmental agencies. We breathe air that will cause us not only respiratory diseases as we thought before but also cerebral infarction (stroke), ischaemic heart disease, neurological diseases, or haematological alterations. According to WHO data, each year, approximately four million people die from polluted air. This means that air pollution kills more people than AIDS (1.7 million) and malaria (660,000 lives) combined [40].

Specifically, the relationship between asthma and urban pollution is based on evidence that has accumulated over several decades. Numerous studies have demonstrated the association between pollution associated with vehicle traffic in cities and the worsening of lung function in children and adults, new diagnoses, clinical decompensation, or emergency care. In addition, urban pollution favours sensitization to new allergens [3, 9, 13, 14].

Once the effect of industrial pollution in asthmatic patients had been demonstrated, our group developed a project on the consequences of urban pollution on bronchial asthma, that study was expanded with an analysis of the botanical repercussions of the contaminants. The plants and pollens of grass *(Lolium perenne)* from Madrid were analysed to determine the expression of allergens, their physiological state, and the profile of their gene expression using the same uncontaminated city (Ciudad Real) as a control [18, 20].

In total, 106 patients were included during the two grass pollination seasons in both cities (May 1-June 15). Madrid and Ciudad Real are 160 km apart, and their meteorological characteristics are similar, with a dry, continental climate and extreme temperatures. Similarly, the concentrations of grass pollens in both cities showed very similar levels (variability +/- 5%). The criteria for patient participation included the diagnosis of moderate persistent bronchial asthma in the last two pollen seasons. The results showed that asthmatic patients in Madrid were more affected by symptoms and consumption of medication than those in Ciudad Real (records that were 30% higher) [20].

Asthma and Tobacco Smoke

Asthma is a complex disease in which tobacco has a very direct influence, although it is not among the aetiological factors of the disease. The prevalence of smoking is highly variable, with higher levels in Europe and lower levels in New Zealand, Australia, and the United States. The risk of exposure to tobacco smoke is especially prominent in children. We have evidence that shows how asthmatic children of smoking mothers have more severe asthma, greater functional impairment, and decreased FEV1 than those of mothers do not smoke. Tobacco smoke particles persist up to 2-3 days in the environment and then settle on the ground. Thus, it is not enough to avoid direct exposure to tobacco smoke since children spend much of their time on the ground and maintain closer contact with tobacco particles. In addition, preventive measures are not limited to the pollutants inhaled by children, but the risk extends to smoking mothers during pregnancy as an additional factor in the increase in asthma in childhood [41]. Recent studies have confirmed that prenatal exposure to tobacco is associated with 17q12-21-dependent asthma, with an increase in asthma exacerbation and bronchial hyperresponsiveness. Therefore, the adverse effects of tobacco in the uterus would affect lung development and would increase the susceptibility to developing allergies and type 2 inflammation [42].

Prenatal exposure also influences subsequent generations through epigenetic modifications. Experimental studies have shown that perinatal exposure to nicotine alters DNA methylation and histone 3 acetylation in germ and somatic cells of first- and second-generation descendants, which showed greater susceptibility to asthma [43].

Childhood asthma continues into adulthood, so it is difficult to determine whether exposure to tobacco smoke increases the risk of developing asthma in adults. However, it is well established that in comparison to nonsmokers, smokers with asthma have more symptoms and morbidity, and worse direct and indirect quality of life indices. In asthmatic smokers, exposure to tobacco smoke is related to bronchial hyperresponsiveness and decreased baseline lung function, which would correspond to a different inflammatory basis from nonsmoking patients with atopic asthma. As noted in another section of the chapter, the mechanism of action of tobacco smoke is similar to that of other pollutants, causing bronchial inflammation through IL-17, natural killer (NK), natural killer T cells (NKT), and γδ T cells [44].

Asthma, Environmental Pollution, and the Immune Response

Chemical substances are considered pollutants when they accumulate above natural levels or when they result in toxicity. It has long been known that the presence of chemical agents in the environment affects the immune system. The first observation was that pollution was linked to the immune-mediated diseases of the lung that cause pulmonary fibrosis, although the mechanism underlying these diseases was unknown at the time. In the 20th century, these chemical substances were identified, and the immune system components and mechanisms were characterized. Pollutants alter immune responses and can trigger immunotoxicity. It is defined as an immune-mediated reaction triggered by environmental elements that are pathological for the affected individuals. Asthma induced by elements present in the environment is an example of immunotoxicity. Environmental factors likely play a major role in the high incidence of allergies. The immune-mediated effect that pollution has on asthma

can be analyzed at 3 different levels: in the first level, the potential for pollution to increase susceptibility to an allergic condition such as asthma; in the second level, the possibility of contact with pollution exacerbating the clinical condition of patients is evaluated; and in the third level, it is determined whether patients are affected by the impacts that pollution has on environmental allergens. The information available in the literature suggests that pollution influences the immune system.

Effect of Pollution on the Development of Allergic Diseases

The results of early studies on the effect of pollution on the development of allergic diseases were inconclusive [45, 46]. Subsequently, the World Health Organization reviewed the issue [47] and, based on human [48, 49] and animal studies [50], concluded that pollution increases the risk of developing asthma and exacerbates existing cases of asthma. The body protects itself against environmental pollutants by means of detoxification processes. To facilitate these processes, many cells have xenobiotic receptors, which work as biological pollution sensors. The activation of these sensors triggers the expression of genes encoding detoxifying compounds, primarily antioxidant enzymes and antiinflammatory elements. There are essentially 2 sensor systems: the aryl hydrocarbon receptor (AHR) and the KEAP1-NFR2 systems. These systems both recognize and protect against a wide variety of environmental contaminants. Both systems work by producing transcription factors that interact in the cell nucleus, thus triggering the transcription and translation of antioxidants and antiinflammatories that mitigate the effects of environmental pollutants [51]. Emerging evidence suggests that these systems behave like key sensors, thus allowing cells to adapt to environmental conditions [52, 53]. On the one hand, the activation of xenobiotic sensors causes immunosuppression, thus leading to increased susceptibility to respiratory tract infections [54], while on the other hand, immune system modifications promote the development of allergies [49].

The pollutant-induced predisposition to allergies is caused by various events. First, oxidative stress pollutants affect the barrier function of the skin and mucous membranes, allowing potential allergens to enter locations that are more accessible to the adaptive immune system [55]. In addition, modifications produced by pollutant sensors decrease the proinflammatory Th1/Th17-type response, promoting Th2-type responses and the release of the specific associated cytokines, namely, IL-4, IL-5 and IL-13 [56, 57].

Moreover, pollution sensors in skin and mucosal cells induce the expression of artemin [58], IL-33 [59] and TSLP [60], which behave as alarmins and drive the immune response toward Th2-type responses, promoting the progression to allergic disease. *In vivo* data corroborate all these observations; it was found that diesel particles on the nasal mucosa of atopic patients stimulated the production of IL-4 and specific IgE to the new antigen. This did not occur when the nasal mucosa of patients was not stimulated by diesel exhaust particles [61]. Dimethyl fumarate, an NRF2-activating chemical, has been approved by the Food and Drug Administration (FDA) and is clinically prescribed to patients with multiple sclerosis. It works by preventing the Th1/Th17-mediated immune inflammation that causes multiple sclerosis, thus altering the Th1/Th2 balance [62].

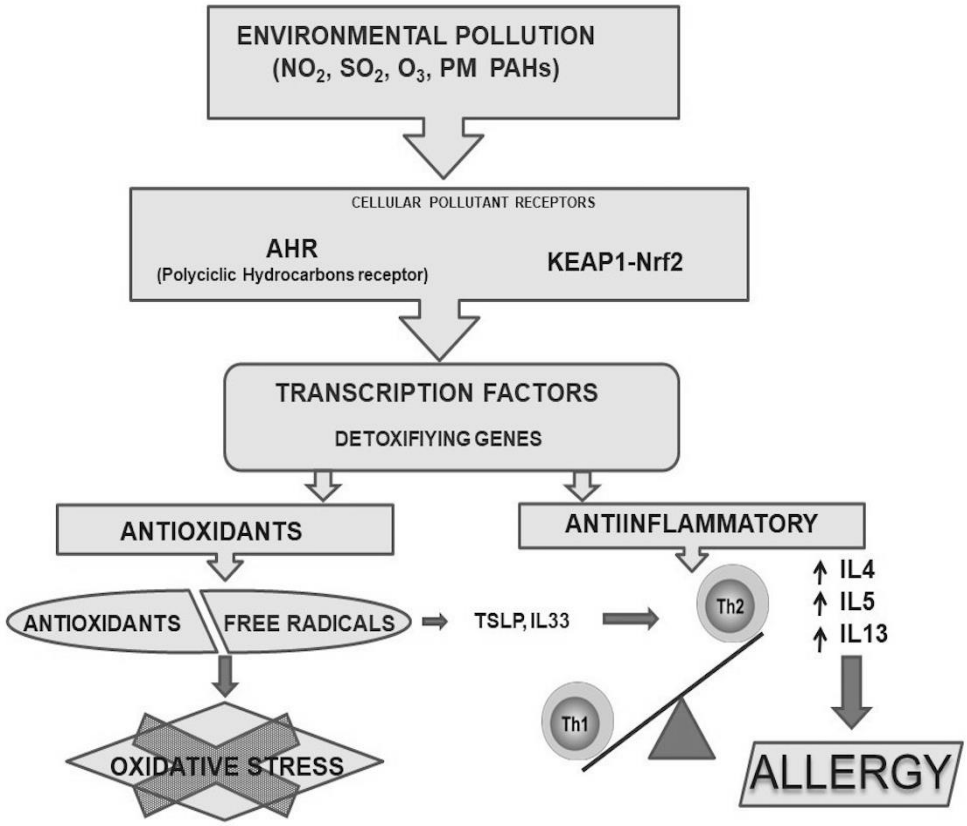


Figure 4. Molecular and cellular processes by which environmental contamination contributes to allergy development. Environmental pollution chemicals are recognized by two types of receptors (AHR and KEAP1-Nrf2) present in the cell cytoplasm that act as sensors. Binding of the chemicals to the receptors results in the generation of transcription factors that translate to the nucleus of the cell where they bind to specific DNA sequences and begin the transcription of genes that act in cellular detoxification. Transcription products are mainly antioxidant enzymes and immune system regulators to prevent inflammation. These latter modify the immune system response to antigens, decreasing Th1/Th17 responses and favoring Th2 and their cytokines (IL-4, IL-5, IL-13), which lead to susceptibility to allergy. Simultaneously, the oxidative stress mediated by the contact with pollution causes the release of TSLP and IL33 in the skin and mucosa that promotes a Th2 response to allergens.

In summary, as shown in Figure 4, pollutants are recognized by receptors that act as sensors; that recognition triggers the production of antioxidants and anti-inflammatories that modulate the immune response, thereby promoting the development of allergic condition.

Exacerbation of Asthma by Pollution

The mechanisms by which environmental pollution increases the risk of the clinical development of asthma are the same mechanisms that are responsible for exacerbating the disease when asthma patients are exposed to pollution. Genetic and epigenetic factors have been identified that help explain why patients subjected to high levels of contaminants experience a more severe clinical course. Pollution produces oxidative stress in the body, and

when this is detected, the expression of enzymes with antioxidant activity increases. The polymorphisms in regions encoding some of the antioxidant enzymes that affect enzyme activity determine the risk of significant adverse effects. Polymorphisms in the genes encoding glutathione S-transferase GSTM1 and GSTP1, modify the asthmatic response to pollutants, with increases in IgE production and histamine release [63]. Likewise, a polymorphism in the promoter region of the gene encoding TNF-α alters the proinflammatory state. This alteration leads to a higher clinical risk in asthma patients subjected to pollutants. That elevated risk is more pronounced in patients with a deficit in their antioxidant enzyme activity [64].

Other pathways through which oxidative pollutants affect asthma severity involve epigenetic modulation of the immune response. Epigenetic changes are biochemical changes that activate or inactivate the expression of genes without changing the DNA sequence. These modifications are caused by age and exposure to environmental factors (diet, exercise, medications, and chemical substances) and modify the risk of developing diseases. Biochemical modifications occur on accessory proteins, such as histones, or on DNA itself involving changes in accessibility to the coding sequences and alterations in the expression of specific genes. Polycyclic aromatic hydrocarbons and diesel exhaust particles epigenetically affect T regulatory lymphocytes (Tregs). Foxp3 is a transcription factor that drives the development of Tregs. Foxp3 gene hypermethylation alters Treg lymphocyte function and increases asthma severity [65]. Similarly, pollutant-mediated IFN-γ hypermethylation favors Th2-type responses and exacerbates asthma [66]. Combined exposure to environmental pollutants and antigens causes epigenetic modifications that promote a dual Th2 and Th17 immune response, which increases symptoms in patients with allergic asthma. Several environmental factors, such as pollutants, have been shown to have an epigenetic influence on the genesis of allergic diseases and asthma.

Effect of Pollution on Allergens that Trigger Asthma

Pollen grains and pollution interact with each other, and pollution is one of the main stressors affecting plants. The effects of climate change and environmental pollution result in elevated expression levels of allergenic and inflammatory molecules in pollen grains, which serve as a means of adapting to the environment. Stress causes plants to modify the expression of certain enzymes and proteins to counteract the effects of pollution, thus modifying their immunogenic properties [67]. A wide variety of pollen contains NADPH oxidase. The activity of this enzyme leads to the formation of radicals that produce oxidative stress in the respiratory tract of asthmatic patients. Pollen-mediated oxidative stress was also proven to be clinically relevant in immediate hypersensitivity reactions.

A recent study has shown that pollen collected in cities with a high level of pollution has higher NADPH oxidase activity and induces more oxidative stress in patients, thus contributing to the development of pollen-mediated allergic inflammation [18]. The effect of pollen on health should be quantified not only based on its ability to bind IgE but also based on its potential to polarize a Th2-type immune response. As shown in Figure 5, the components of pollen in high-pollution and low-pollution areas were similar and were recognized in the same way by IgE. Considering that the structure of pollen is very complex, components of pollen other than the known allergens may induce allergies. Pollution can alter pollen components or enzymes such as NADPH oxidase, and these alterations can result in a Th2 polarized immune

response. This introduces a new concept, allergenicity, which is the evaluation of the allergenic potential of pollen affected by pollution with respect to that of its natural counterpart.

Environmental pollution modifies the allergenicity of pollen in various ways, leading to a greater clinical impact of asthma [68]. Pollen from areas with high contamination levels contains a higher count of Enterobacteriaceae, which prompts the release of greater amounts of enterotoxin. Enterotoxin has been linked to an increased immune response, which leads to increased airway inflammation [69]. The effect of pollen from highly polluted areas on patients was described in a recent study comparing pollen from two cities, one with high pollution levels and one with low pollution levels.

An *in vitro* study was performed that involved using pollen collected in those two cities to stimulate different lymphocyte subtypes in patients with grass pollen-induced asthma. Pollen from the city with a high pollution level resulted in greater stimulation of all the different types of lymphocytes studied, with particularly robust effects in CD8 T lymphocytes and NK cells. This effect was independent of the origin of the patients studied and was entirely caused by pollution at the site where the pollen was collected [20]. The activity of CD8 T lymphocytes and NK cells is involved in determining the severity of pollen-induced asthma and even the associated risk of mortality. Surprisingly, patients in the low-pollution area responded more actively to pollen from the polluted area than patients living in the high-pollution area. A correlation was found between the level of pollution and the clinical severity of asthma, as reflected in symptoms and medication requirements. Reactivity, as indicated by skin tests and basophil activation tests, was relatively higher in the location with the highest level of contamination [70]. Pollen from areas with higher levels of pollution induces a more severe clinical manifestation of asthma because pollution modifies pollen, resulting in higher allergenicity and, consequently, a stronger immune response.

Figure 5. Electrophoresis of protein components of grass pollen. Line 1 standard pollen, line 2 pollen from an area with low pollution and line 3 pollen from an area with high pollution. No differences are observed in their components.

Conclusion

Global warming, associated with air pollution, is a phenomenon that results in constantly increasing severe consequences on human health. In the case of bronchial asthma, pollutants act both directly on the bronchial of humans and indirectly by altering the physiology of plants. The effects that pollutants produce, both in people and in plants, are carried out by oxidative stress.

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Chapter 11

Allergic Asthma and Viruses

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Abstract

Asthma, Allergens and Viral Infections: An Intricate Relationship

Airborne bacteria, virus, fungal spores, pollen and other bioparticles are essential for the reproduction and propagation of organisms through diverse ecosystems, and may cause or worsen diseases in humans, animals and plants. Their interaction is implicated in serious pathologies such as asthma, stroke, ischemic heart disease and cancer.

Viruses and other selfish genetic elements are dominant entities in the biosphere, with respect to both physical abundance and genetic diversity. In eukaryotes, RNA viruses account for the majority of the virome diversity although ssDNA and dsDNA viruses are common as well. Viruses may cause respiratory allergic pathology in both the upper and lower airways in susceptible people, and have been implicated in other allergic disorders such as chronic obstructive pulmonary disease, pneumonitis and alveolitis [1, 2].

In this chapter, we will discuss the latest developments in research and knowledge on virus-induced asthma exacerbations, COVID-19 and asthma relationship during the pandemic and consider recent advances in treatment options.

Keywords: allergy, asthma, virus, allergens, RNA viruses, ssDNA viruses

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Introduction

Are Viruses a Hazard or Protection for Allergic Sensitization? Does Allergy Diminish the Antiviral Response Promoting Exacerbation of Asthma?

Allergy and viral respiratory infections have long been recognized as two of the most important risk factors for exacerbations of asthma. Many research studies have raised questions as whether allergy diminishes the antiviral response promoting exacerbation of asthma. Alternatively, do viral respiratory infections potentiate allergic inflammation in the airway? [3]. More and more evidence related both roles with viral pathogens, especially human rhinovirus and respiratory syncytial virus [4]. Once asthma is present, viral infection is a common precipitant of asthma exacerbation. Bacterial infection has also been associated with exacerbation and recurrent asthma. Atypical bacterial infections, such as those caused by *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, and fungi are found in most cases of severe asthma [5], and also play a potential role in exacerbating this disease, with viral infection as cofactor.

In addition, certain individuals may have a genetic predisposition toward viral-induced wheezing and the development of asthma [4]. A severe infection by syncytial respiratory virus, the leading cause of lower respiratory tract infection in pre-school children, is linked to development of asthma later in life [3, 4]. Understanding the mechanisms that cause and exacerbate allergic asthmatic disease is difficult and of great clinical interest. Clinical studies revealed some interactive inflammatory mechanisms between virus and allergens [6]:

1. Deficiency in virus-induced interferon responses.
2. Defective epithelial barrier function.
3. Increased release of epithelium-derived cytokines (thymic stromal lymphopoietin (TSLP), interleukins (IL-25, IL-33).
4. Dysregulation of lymphocytes (e.g., innate lymphoid cell (ILCs), regulatory T cell (Tregs).
5. Altered activation of purinergic receptors.

The importance of developing these possibilities is to provide targets for new therapies to prevent asthma exacerbations [7]. The combined influence and interaction of early life viral wheezing and aeroallergen sensitization is important because it is common that allergic sensitization precedes the onset of viral wheeze [3-7].

Human studies demonstrate that inactivated virus can trigger eosinophil activation in vitro through antigen presentation and memory CD4+ lymphocytes. In animal models infected with live parainfluenza virus (PIV), allergen sensitized and challenged with ovalbumin, developed airway eosinophilic inflammation and hyperreactivity when re-exposed to UV-inactivated, while non-sensitized animals did not. The airway hyperreactivity was inhibited by pre-treatment with dexamethasone. The authors suggest that the response of allergic inflammation to virus antigen is a significant factor causing asthma exacerbation and this mechanism explains how corticosteroids prevent virus-induced asthma attack and are useful in exacerbation induced by respiratory syncytial virus [8, 9, 10].

As a recent practical example, corticosteroids have become the standard of care in critically ill COVID-19 patients undergoing mechanical ventilation and admitted to Intensive Care Units, after demonstrating a survival benefit [11]. However, the underlying mechanism explaining this phenomenon has not been elucidated yet. The inflammatory profile orchestrated by T helper cells (Th) in severely ill COVID-19 patients supports the predominance of a Th2 polarization, which primarily activates a humoral response, via interleukins 4 and 6 [12-14]. This response has been linked to a dysregulated cytokine release or "cytokine storm," and has been associated with poor prognosis [13]. The Th2 pathway is involved in allergen-specific and IgE-related events, since IL-4 induces production of these antibodies by B cells (Figure 1) [12, 14].

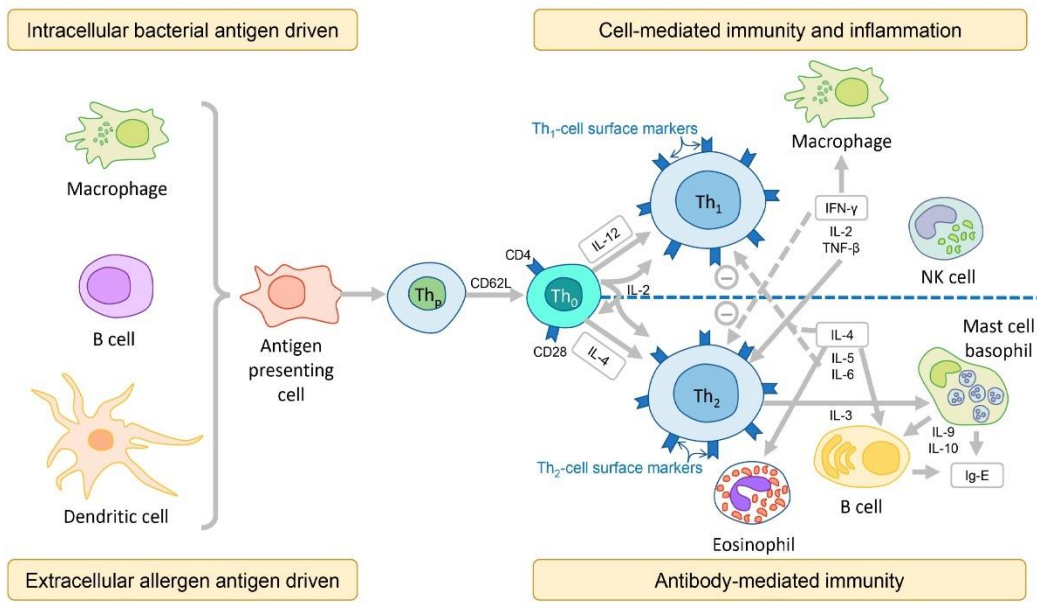

Figure 1. T helper (Th) cell polarization after exposure to an antigen presenting cell. The immune response tends to polarize towards the Th1 pathway when driven by an intracellular bacterial antigen, leading to predominance of cell-mediated immunity and inflammation. Contrarily, the immune response tends to polarize towards the Th2 pathway when driven by an extracellular allergen antigen, leading to predominance of antibody-mediated immunity.

Clinical Course and Management Principles of Viral-Induced Acute Asthma Exacerbations

Signs and symptoms of asthma include intermittent shortness of breath, cough (especially during night-time), wheezing (an exhalatory whistle-like sound) and chest tightness. Asthmatic patients develop respiratory symptoms after exposure to a trigger, and commonly improve with avoidance of it and/or adequate therapy. Acute asthma exacerbations or asthma attacks are characterized by a worsening of basal asthma symptoms and a decline in lung function due to airway inflammation, increase in mucus production and bronchospasm. These episodes may be the presenting manifestation of asthma in a patient without a previous diagnosis or arise in

patients with a previous diagnosis of asthma in response to a trigger. A wide range of potential triggers for asthma attacks have been proposed, including viral infections, allergen exposure, environmental irritants (such as cigarette smoke or air pollution), suboptimal adherence to prescribed medications and combinations of these [15,16].

Viral respiratory tract infections are the most frequent cause of acute asthma exacerbations in both children and adults [17], and are also a major reason for worsening pulmonary symptoms in patients of any age with preexisting asthma [18-20]. Epidemiological studies have concluded that viral respiratory tract infections are responsible of up to 85% of asthma attacks in children and trigger about one half of exacerbations in adult patients [21,22]. Human rhinovirus (HRV), the most common virus causing infections in humans and the predominant cause of common cold, is also the main infectious agent isolated in school-aged children and adult patients with acute asthma exacerbations [23]. Three species of HRV (HRV-A, HRV-B and HRV-C) have been recognized to include more than 150 antigenically different virus subtypes, which explains life-long susceptibility to the virus [24]. Infections by HRV-C are associated with more severe disease in children requiring admission for acute asthma exacerbations [25]. In patients younger than 1-year, respiratory syncytial virus (RSV) is the leading cause of bronchiolitis, which plays a contributory role in the development of asthma [24]. Other viruses such as enterovirus, bocavirus, parainfluenza, influenza, adenovirus, metapneumovirus and coronavirus can trigger acute exacerbations in both children and adults with asthma [26].

In the last century, the mortality rate from asthma attacks has substantially decreased. The main reasons for this are the improvements in chronic therapy and the advances in intensive care medicine. However, the risk for asthma attacks is not evenly distributed. Requirement of an ICU admission is the stronger predictor for a subsequent life-threatening or fatal asthma exacerbation. Thus, these patients should be closely followed-up by an asthma specialist in the outpatient setting. It remains unclear whether certain viral respiratory infections can lead to asthma or if wheezing with viral infections in general are predictors for the development of asthma [27].

The best strategy for the management of acute asthma exacerbations is a prompt recognition and intervention, which may prevent attacks from becoming more severe and potentially life-threatening [28]. The main therapies required to control an acute asthma exacerbation in an adult patient include the repeated administration of short-acting beta agonists (SABA), early administration of a systemic glucocorticoid, and supplemental oxygen aiming a peripheral arterial oxygen saturation above 92% [29]. Indications for endotracheal intubation and initiation of mechanical ventilation are clinical, and include: inability to maintain an adequate oxygenation, bradypnea, a decreased level of consciousness, worsening hypercarbia with respiratory acidemia, an increased work of breathing, inability to cooperate with administration of inhaled therapy and hemodynamic instability [30]. In refractory cases to these treatments, magnesium sulphate may be considered, since it produces smooth muscle relaxation, acting as a bronchodilator [31]. Last tier therapeutic options should be considered individually, as no definitive evidence support a general recommendation. These therapies include the use of anesthetic agents (ketamine or isoflurane), helium-oxygen mixtures, parenteral administration of SABA and veno-venous extracorporeal membrane oxygenation (vv-ECMO) [32].

Systemic corticosteroids reduce symptoms in acute asthma exacerbations and the risk of relapse in both children and adult patients. Eosinophilic inflammation may play an important

role in this observation, since a maintenance therapy with systemic corticosteroids leads to eosinophil apoptosis which, in turn, reduces airway inflammation and mucus production [33]. When the acute situation is under control, treatment with inhaled glucocorticoids prevents recurrence of asthma attacks and helps prevent the potential decline in lung function associated with any future severe asthma exacerbation. A great number of available biologic therapies and vaccines can successfully reduce the frequency of exacerbations among patients with severe asthma [34]. Therefore, patients with frequent asthma exacerbations will likely benefit from referral to an asthma specialist.

Asthma and COVID-19: What Have We Learned?

Coronavirus disease 2019 (COVID-19), a respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became a pandemic worldwide in 2020. Risk factors for severe COVID-19 include older age, ethnicity, sex, comorbidities, and living conditions. Although asthmatics and those with allergies are susceptible to more severe outcomes to viral infections, interestingly, asthma has not been reported to be a major comorbidity of COVID-19. However, there are some conflicting reports on the impact of asthma on COVID-19. The underlying immunological and molecular mechanisms may explain at least in part these observations. Furthermore, environmental factors like air pollution that have detrimental effects on asthma and respiratory illnesses also have an impact on COVID-19.

Trying to answer whether inhaled corticosteroids have a protective effect against coronavirus infection we made a cross-sectional observational descriptive study of COVID-19 infection in difficult-to-control asthmatics receiving inhaled corticosteroids and nursing home residents from the Valladolid Health Area, Spain [35]. The aim was to compare respiratory symptoms in nursing home residents and asthmatic patients: 139 patients (122 adults and 17 children), 84% of them extrinsic asthmatics due to different etiologies, who were assumed to be the patients most of risk of infection. All asthma patients were treated with inhaled corticosteroids (IC), 82 at low doses of IC+LABA, 37 at medium doses and 20 at high doses. Thirteen patients were infected with coronavirus, but none became uncontrolled. None of the asthmatic patients sensitized to allergens contracted the Coronavirus infection. None of asthmatics that received immunomodulatory treatment (7 mepolizumab, 4 omalizumab and 1 benralizumab) and 40 with specific immunotherapy with allergens were infected or became uncontrolled during the COVID-19 pandemic (Figure 2). Of the 134 nursing home residents, 80 (60%) were infected by coronavirus. Of these, 43% received inhaled corticosteroids (for COPD, respiratory failure, asthma, etc.) and, of these, 80% evolved significantly better than patients not treated with inhaled corticosteroids (Figure 3).

Our results show that asthmatic patients did not have asthma relapses, despite high pollen levels during the study period, especially *London plane* (400 grains/m^3) and *rye grass* (21 grains/m^3). This may be due to home confinement measures when the state of alarm was declared in Spain on March 14th 2020, although some patients had allergies to indoor allergens. In addition, patients were advised not to cease inhaled corticosteroids as it was thought this could destabilize them.

In both asthmatic patients and nursing home residents, prior allergic sensitization was associated with a favorable evolution. A possible explanation is that COVID-19 appears to polarize a Th2 immune response, as occurs during respiratory syncytial virus infection and coronavirus gastroenteritis, where the systemic and local immune response switched from Th1 to a Th2-based immune response. This is the same route used by parasites, which may generate competition for allergic patients or may suppose a viral defense in areas where parasitosis is endemic.

Studies have found that patients with common allergic diseases did not develop severe courses [36, 37]. Allergic disease, asthma and COPD are not risk factors for SARS-CoV-2 infection but, in our patients, older age, greater comorbidity and more prominent laboratory abnormalities were associated with severity.

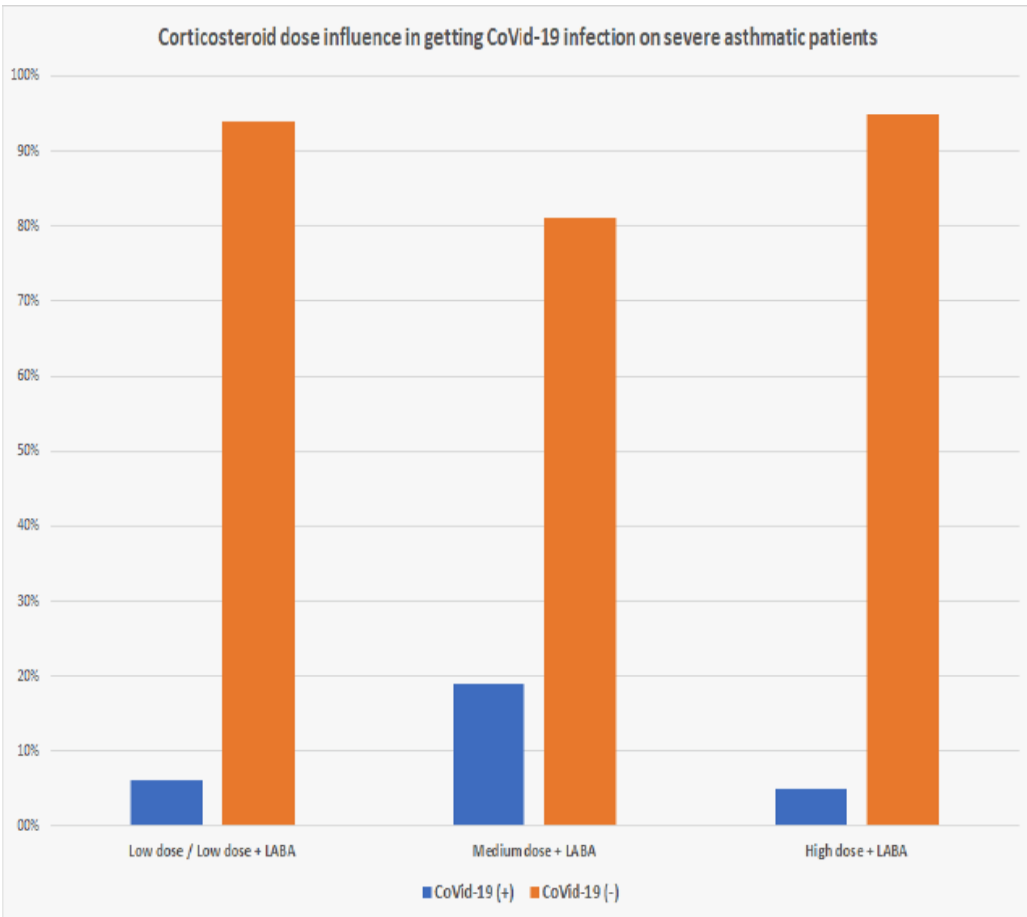

Figure 2. All asthma patients were treated with inhaled corticosteroids, 82 at low doses of IC+LABA, 37 at medium doses and 20 at high doses. Thirteen patients were infected with coronavirus, but none became uncontrolled [35].

The clinical syndrome manifests as an inflammatory syndrome due to cytokine release or hypersensitivity pneumonitis [38], and if so, corticosteroids would be the best treatment. Inhaled corticosteroids administered at the onset of viral infection might block the inflammatory response and hypersensitivity.

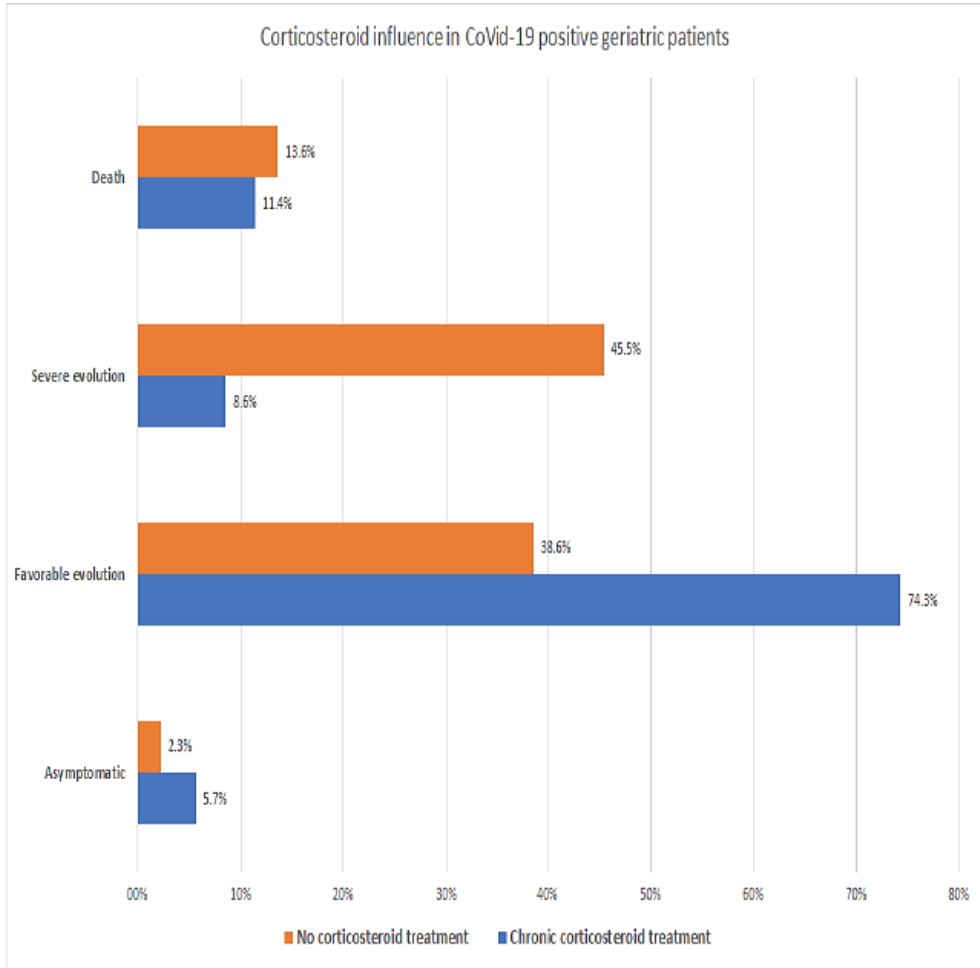

Figure 3. Of the 134 nursing home residents, 43% received inhaled corticosteroids and, of these, 80% evolved significantly better than patients not treated with inhaled corticosteroids [35].

Like other studies, we suggest that in patients with severe COVID-19, early, short-term, low-dose methylprednisolone was beneficial and did not delay SARS-CoV-2 RNA clearance and influence IgG antibody production [39]. The WHO indicated that parenteral corticosteroid therapy (hydrocortisone 100 mg IV) showed no benefits in the SARS and MERS epidemics, but it was parenterally applied at advanced disease stages. We suggest that inhaled corticosteroids might exert a targeted effect on the lungs without a risk of increased viral infection, just as they improve outcomes in children with respiratory syncytial virus bronchiolitis.

Despite the use of corticosteroids and other drugs, COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in high mortality worldwide. SARS-CoV-2 exhibits a great number of mechanisms to evade the immune response, including suppression of the host's cortisol stress response [40]. Several potential mechanisms for adrenal insufficiency (AI) have been proposed [41]. One of them is based on the expression of amino acid sequences by SARS-CoV-2 that are remarkably similar to those of the host adrenocorticotropic hormone (ACTH) [40-43]. Production of antibodies against viral antigens may also lead to destruction of the host ACTH, avoiding the increase of cortisol and resulting in a relative AI. However, there is little data on the evidence of the existence of anti-ACTH antibodies and on the levels of cortisol in COVID-19 patients [40-42].

We performed a proof-of-concept study to assess whether anti-ACTH antibodies were detectable in critically ill COVID-19 patients [43]. We measured plasma levels of cortisol and ACTH as exploratory variables. All the COVID-19 patients undergoing mechanical ventilation (MV) admitted to the Intensive Care Unit (ICU) of our University Hospital within a 10-week period (January to March 2021) were eligible to participate. Inclusion criteria were: 1) SARS-CoV-2 infection confirmed by real-time polymerase chain reaction (RT-PCR) in a nasopharyngeal swab or bronchoalveolar lavage specimen; 2) Development of at least two signs or symptoms of AI: hyponatremia, hyperkaliemia, lymphopenia, eosinophilia, hemodynamic instability and/or hyperthermia; and 3) The signs or symptoms of AI were not straightforwardly explained by alternative confounders (i.e., infection/sepsis, pulmonary embolism, diuretics, etc.) The only exclusion criterion was unwillingness to give informed consent. Measurements were also performed in a control group of patients admitted to the hospital ward for acute respiratory failure for reasons other than COVID-19, without suspected AI, and Addison's disease patients from the outpatient clinic, all of them within the recruitment period.

The inflammatory profile in COVID-19 patients supports a predominance of the Th2 immune response, the pathway involved in allergen-specific and IgE-related events [12-14]. So, we measured specific anti-ACTH IgE-class antibodies (ImmunoCAP™ IgE assays, Thermo Fisher Scientific/Phadia, Uppsala, Sweden), and plasma levels of cortisol and ACTH, at the moment when inclusion criteria were fulfilled (Table 1). We found specific anti-ACTH IgE-class antibodies in 60% of COVID-19 patients with suspected AI, half of them together with low levels of plasma cortisol and ACTH. Note 70% of the patients were receiving corticosteroids at the time of assessment. We did not find detectable titers of these antibodies in controls.

Table 1. Clinical characteristics of the patients, anti-ACTH IgE, cortisol and ACTH levels. Normal reference limits are described in parentheses. In bold, levels outside the reference ranges. Note laboratory data in COVID-19 patients represent the greatest deviation during the ICU stay (lowest value of lymphocyte count and sodium, and highest value of eosinophil count and potassium)

Patient	Age	Sex	APACHE-II	COVID-19	Atopy	Lymphocyte count, $\times 10^3/\mu L$ (0.8 – 5)	Eosinophil count, $\times 10^3/\mu L$ (0.1 – 0.65)	Sodium, mmol/L (136 – 146)	Potassium, mmol/L (3.5 – 5.1)	Hemodynamic instability	Hyperthermia	Anti-ACTH IgE, KU/L (<0.10)	Cortisol, µg/dL (6.7 – 22.6)	ACTH, pg/mL (5 – 46)	Corticosteroids at the time of assessment?	Days from admission to assessment
1	56	M	13	Yes	Yes	**0.7**	**2.3**	**135**	4.4	No	**Yes**	**12.9**	**1.1**	**<5**	Yes	33
2	67	M	21	Yes	Yes	**0.5**	0.1	**133**	**4.9**	**Yes**	**Yes**	**0.19**	**6.1**	**<5**	Yes	61
3	79	F	21	Yes	No	1.3	**1.4**	**131**	**5.2**	**Yes**	No	<0.10	15.5	16	Yes	24
4	76	M	20	Yes	No	**0.2**	0.4	**130**	5	**Yes**	No	**0.82**	8.9	**<5**	No	20
5	67	F	18	Yes	Yes	**0.4**	**1.7**	**127**	4.9	No	No	<0.10	**1.8**	**<5**	Yes	38
6	68	M	12	Yes	No	**0.4**	**0.9**	**132**	**6**	No	**Yes**	**9.01**	15.4	5.6	No	34
7	70	M	16	Yes	No	**0.3**	0.6	**133**	4.8	No	**Yes**	<0.10	12.1	<5	Yes	63
8	78	F	24	Yes	No	**0.5**	0.1	137	**5.8**	No	No	**21**	**1.8**	**<5**	Yes	18
9	24	M	20	Yes	No	**0**	**1**	**129**	4.8	No	No	**0.62**	16.81	17.7	No	23
10	70	M	15	Yes	No	**0.6**	**1**	**132**	4.7	No	No	<0.10	**2.32**	**<5**	Yes	22
C1	64	M	5	No	No	1.6	0.1	142	4	No	No	<0.10	15.2	16	No	6
C2	70	F	7	No	Yes	1.9	**0.7**	146	3.8	No	No	<0.10	16.1	29.7	No	5
C3	82	M	8	No	No	1.5	0.4	140	4.1	No	No	<0.10	18	19	No	7
C4	59	F	7	No	Yes	1.9	0.6	139	3.9	No	No	<0.10	12.8	22.4	No	6
C5	67	M	7	No	No	1.9	0.1	142	4.2	No	No	<0.10	13.1	17.6	No	7
C6	90	M	10	No	Yes	2.3	0.6	141	3.7	No	No	<0.10	12.5	19.2	No	5
C7	75	F	10	No	No	3.6	0.1	139	3.9	No	No	<0.10	15.3	21	No	4
C8	75	M	8	No	No	1.4	0.2	145	4.1	No	No	<0.10	16.2	18.9	No	8
C9	80	M	10	No	No	1.9	0.1	140	4.2	No	No	<0.10	14.3	12.3	No	7
C10	91	M	12	No	No	1.5	0.3	141	4.4	No	No	<0.10	15.4	40.1	No	6
A1	56	F	6	No	No	1.4	**0.7**	**126**	**5.6**	No	No	<0.10	**1.2**	**50**	Yes	-
A2	78	F	10	No	No	1	**0.8**	**130**	5	No	No	<0.10	**1.9**	**150**	Yes	-
A3	62	F	4	No	No	1	**0.7**	138	**5.4**	No	No	<0.10	**7.3**	**150**	Yes	-

C = controls, A = Addison's disease patients. Anti-ACTH IgE levels: <0.10 KU/L, negative; 0.10-1.9 KU/L, mildly elevated; 2-14.9 KU/L, moderately elevated; >15 KU/L, highly elevated.

The reason why we decided to measure specific IgE-class antibodies is that the Th2 pathway is involved in allergen-specific and IgE-related events, since IL-4 induces production of these antibodies by B cells [12-14]. We found a 60% prevalence of anti-ACTH IgE-class antibodies in patients with suspected AI. Levels of plasma ACTH were low in 4 out of 6 patients, with a plausible contribution of anti-ACTH IgE-class antibodies. A partial explanation by negative feedback from exogenous administration of corticosteroids is feasible in 3 out of 4 patients, but not in the remaining. We observed 2 out of 4 patients without anti-ACTH IgE-class antibodies exhibited low levels of plasma cortisol and ACTH, suggesting either exogenous administration of corticosteroids, or implication of other potential mechanisms of AI in COVID-19 patients, like the negative feedback toward the hypothalamic-pituitary-adrenal axis provided by the immune dysregulation caused by SARS-CoV-2, or the entrance of the virus in the glands through the angiotensin-converting enzyme 2, causing hypophysitis and adrenalitis [41].

To our knowledge, this was the first time that anti-ACTH antibodies have been described in severely ill COVID-19 patients. We suggest SARS-CoV-2 may take advantage of its homology with human ACTH to evade the host immune system by blocking the primary immune response and blunting the natural response of the hypothalamic-pituitary-adrenal axis to stress, leading to a relative AI state. We believe anti-ACTH antibodies play a plausible role in the pathophysiology of severe COVID-19, which may have potential clinical implications and merits further research [43].

Future Therapeutic Advances in the Treatment of Asthma Exacerbated by Viruses

Conventional therapeutics for rhinitis and asthma including inhaled corticosteroid, allergen immunotherapy (AIT) and anti-IgE monoclonal antibody, might also reduce the risk of asthmatics suffering infection from viruses through alleviating inflammation enhancing antiviral defense [44, 45].

Treatment that inhibits inflammation (corticosteroids, omalizumab) effectively decrease rhinovirus-induced wheezing and asthma exacerbations. The anti-RSV monoclonal antibody, palivizumab, decreases the risk of severe RSV illness and subsequent wheeze. The problem is that the evolutionary dynamics of a virus can differ within hosts and across time and populations [46].

To achieve an effective treatment of asthma exacerbated by viruses, it is necessary to know the molecular mechanisms of viral infection in the different host and try to control them. Virus hijack host cellular receptors and functions for replication, thereby posing a complication in identifying therapeutic targets [47].

Several viruses (including HIV and Herpesvirus) have evolved ingenious strategies to evade host-immune system and persist life-long. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is an ancient antiviral system recently discovered in bacteria that has shown tremendous potential that can provide a successful antiviral mechanisms and treatment modalities facilitating the clearance of virus-infected cells and/or prohibiting virus infection or replication [48]. This technology has changed the landscape of molecular biology and may be applied to repair genetic disorders and severe infections in future therapies [49].

In COVID-19, angiotensin-converting enzyme 2 (ACE2) is the receptor for the attachment and entry of SARS-CoV-2 into the host cells that is upregulated by Th1-mediated responses. In asthmatics, ACE2 gene expression is generally reduced and recent studies have shown a negative correlation between the levels of Th2 cytokines including IL-4, IL-5, and IL-13 in airway epithelial cells and other type 2 biomarkers with ACE2 expression. This may explain in part the potential protective role of asthma on COVID-19 and raised the possibility of treatment with allergen immunotherapy.

Pneumovirus infection induces airway epithelial cell necroptosis. Inhibition of this necroptosis may be a viable strategy to limit the severity of viral bronchiolitis and break its nexus with asthma [50].

Conclusion

Viruses play a pathophysiological role in the development of allergic diseases, including asthma. Understanding the mechanisms that cause and exacerbate allergic asthmatic disease is difficult and of great clinical interest.

Acute asthma exacerbations or asthma attacks are characterized by a worsening of basal asthma symptoms and a decline in lung function due to airway inflammation, increase in mucus production and bronchospasm. Viral respiratory tract infections, particularly those caused by human rhinovirus and respiratory syncytial virus, are the most frequent cause of these exacerbations. In the setting of an exacerbation, prompt recognition and intervention may prevent attacks from becoming more severe and potentially life-threatening.

Although asthmatics and those with allergies are susceptible to more severe outcomes to viral infections, interestingly, asthma has not been reported to be a major comorbidity of COVID-19. Outpatients with COVID-19 treated with inhaled corticosteroids had significantly better outcomes than patients who did not receive them. In both asthmatic patients and nursing home residents, prior allergic sensitization was associated with a favorable evolution, possible due to a polarized Th2 immune response. SARS-CoV-2 exhibits similarity to host ACTH, which constitutes an immune evasion mechanism: production of antibodies against viral antigens may behave as anti-ACTH antibodies, which avoids the increase of cortisol resulting in a relative adrenal insufficiency in critically ill COVID-19 patients.

Recent advances in the treatment of asthma exacerbated by viruses include therapies such as omalizumab (anti-IgE monoclonal antibody), palivizumab (anti-RSV monoclonal antibody) and use of CRISPR technology. The discovery of new pathogenic pathways may reduce the burden of asthma in the future.

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Chapter 12

United Airway Disease

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Abstract

The unified airway theory and the concept of united airway disease (UAD) are relatively recent. However, there is a body of growing evidence to support it. This book chapter will delve into the anatomical, epidemiological, pathophysiological, and clinical data that have been instrumental in expanding this theory. In addition, the increasing focus on precision and personalised medicine has allowed for the description of new endophenotypes involving both upper and lower airways, which will further support the concept of UAD and allow for optimised diagnostic and management pathways (e.g., targeted treatments like biologics or allergen-specific immunotherapy).

This chapter will explore those endophenotypes involving allergic rhinitis, as other chapters in this book will address most of the other endophenotypes described here.

Keywords: asthma, allergy, rhinitis, united airway disease, local rhinitis

Introduction

The larynx divides the respiratory tract into upper and lower airways [1-3]. Although artificial, this subdivision is undeniable, even with two different medical specialities managing the upper

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and lower respiratory tract [2, 4]. However, growing evidence has supported a unified airway theory, which is understanding the airways as a single functional entity, both in health and illness [2, 5]. See figure 1 for a schematic summary.

United airways disease (UAD) is a surprisingly recent concept [6, 7]. However, it soon had a significant impact on the understanding and management of rhinitis and asthma with the advent of the Allergic Rhinitis and its Impact on Asthma (ARIA) collaboration, which still 20 years later is fundamental in bringing together under one roof definitions, research aims, and summary recommendations to guide comprehensive medical management of UAD [8].

UAD has been primarily studied in the interaction between rhinitis and asthma, and recent approaches suggest that applying the concepts of Precision Medicine and identifying the different endophenotypes of this condition could entail better patient outcomes [1]. Moreover, there is increasing interest in applying the concept of UAD to other disease areas, such as chronic obstructive pulmonary disease (COPD) and sarcoidosis [9, 10].

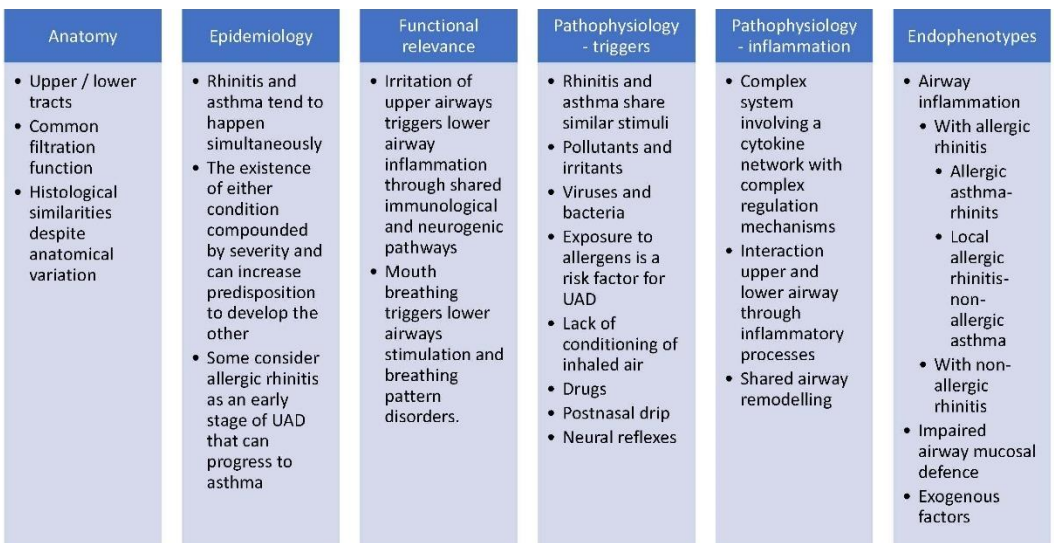

Figure 1. Summary of key points. Evidence in favour of a United Airway Disease (UAD) comes from different angles, and our current knowledge has led to the description of clinical endophenotypes with consequences in management.

Anatomy

The larynx divides the respiratory tree into upper (nose, pharynx, and larynx) and lower (trachea, bronchi, bronchioles, alveolar duct, and alveoli) tracts [2, 3].

In the upper respiratory tract, the inhaled air is heated and humidified [2, 3]. Indeed, the nasal sinuses are significantly involved in thermal regulation [2].

Moreover, the turbulent flow of the air through the upper tract helps with particle deposition and filtration, which protects the lower tract [2]. Interestingly, the lower respiratory tract has a similar filtration function. Whilst the upper tract stops the larger particles (5-10 μm) and dissolves irritant soluble gases, the mucociliary escalator of the lower tract filters the smaller inhaled particles [3].

There is evident anatomical differentiation in all these areas. For example, the larynx plays a significant role in phonation and acts as a protective valve against foreign bodies. Whereas trachea and bronchi have a cartilaginous wall to avoid collapse to withstand air pressure changes in these air conducting areas, and they divide progressively into gas-exchanging areas with no cartilage, the bronchioli [2, 3]. Despite these anatomical variations, there are histological similarities throughout the airways, including basement membrane, lamina propria, ciliary epithelium, goblet cells, and glands [3].

According to Licari et al.:

> The main difference between the nose and the lungs is that upper airway obstruction is mainly caused by vasodilatation and oedema, whereas the lower airway patency is influenced by smooth muscle function. [3]

Epidemiology

Asthma and rhinitis are strongly interlinked, and they are a growing public health problem due to their prevalence, the economic burden, and impact on patient's quality of life [3, 5, 8, 11]. It seems an established fact that allergic rhinitis in children and adults is a risk factor for asthma independent of allergy [11-14]. Furthermore, regardless of allergic aetiology, rhinitis increases susceptibility to asthma with an odds ratio of approximately 3 [11, 15-17].

However, some authors propose that, instead of merely a risk factor, allergic rhinitis is an early stage of UAD that can progress to asthma [2, 11, 18]. Unsurprisingly, some argue that successful management of this chronic respiratory syndrome needs an integrated view of the airways [11, 19].

Notably, most people with asthma suffer from rhinitis, and -conversely- the prevalence of asthma in subjects without rhinitis is usually less than 2% [11, 17, 20, 21]. Moreover, although not all patients with rhinitis have asthma, those with a more persistent clinical picture or on the more severe end of the clinical spectrum are more likely to have asthma, and around 19-38% of patients with allergic rhinitis have co-existing asthma [11, 22, 23]. In addition, there is evidence of lower airway hyperresponsiveness (methacholine or histamine bronchial challenge) in around 30% of patients who suffer from rhinitis alone [5, 24, 25]. Conversely, 78% of asthmatics report symptoms of allergic rhinitis, with severe or persistent rhinitis more likely to occur with co-existent asthma [26-28].

Thus, the available epidemiological data support this view of a UAD. Indeed, rhinitis and asthma tend to happen simultaneously, and the functional relevance of this upper airway association can be summarised as follows:

1) Allergen and irritant challenges to the upper airway, including nasal challenges, elicit lower airway inflammation via shared immunological and neurogenic pathways, which is in line with the unified airway hypothesis [29].
2) Mouth breathing, facilitated by nasal blockage, results in impaired humidification and filtration of inspired air and leads to lower airway stimulation [30].
3) Nasal obstruction results in mouth breathing that can be linked to increased breathlessness and breathing pattern disorders in patients with asthma [31].

Moreover, the existence of either condition may be compounded by the severity of the disease and, in turn, increase an individual's predisposition to the development of the other [32].

Pathophysiology Triggers

Another aspect supporting the epidemiological evidence for a UAD is that asthma and rhinitis share similar stimuli, including viruses/bacteria, irritants/pollution, allergens, drugs, lack of conditioning of the inhaled air, cold air, physical exercise, postnasal drip, and neural reflexes [2, 5].

Similar viruses and bacteria can cause upper and lower tract infections and exacerbate chronic bronchitis and asthma [2].

Atmospheric pollutants, occupational pollutants, and cigarette smoke can also induce similar injuries both in the upper and lower respiratory tract [2]. These injuries might range from direct damage to the epithelium and chronic inflammation to even metaplasia and cancer [2]. For example, tobacco smoke is a significant risk factor for chronic bronchitis, larynx cancer, bronchial cancer, and even rhinitis [2, 33, 34].

Exposure to allergens is a known risk factor for UAD [3, 22]. Both rhinitis and asthma show a high prevalence of sensitisation to airborne aeroallergens, subdivided into outdoor allergens (i.e., pollen or mould), indoor allergens (i.e., animal dander or house dust mites), and occupational agents [3, 21].

Regarding pharmacological triggers, NSAID-exacerbated respiratory disease causes symptoms in both the upper and lower airways [35].

The complex interaction between upper and lower airways is highlighted by how triggers might have a more substantial impact on the lower airway if the function of the upper airway is impaired. For example, the nose acts as a filter of higher molecular weight particles. However, if the air is inspired through the mouth as the nose is blocked, it will not be optimally filtered, warmed, humidified, or purified [2, 5, 19, 36]. This lack of conditioning of the inhaled air through the nose could damage the bronchial epithelium and allow allergens with a higher molecular weight (e.g., pollen) to reach the lower tract [2, 19, 36]. In addition, cold and dry air may directly induce bronchoconstriction [5].

Moreover, more moderate nasal mucosal congestion can induce patients to bypass nasal breathing in favour of oral breathing, thus missing the innate and adaptative immune defences of the nasal mucosa, which protect the lower airway from allergens and pathogens [5].

Postnasal drip could potentially be a relevant trigger [5]. For example, accumulated secretions in the lower pharyngeal area can stimulate irritant receptors, thus generating morning cough in patients with rhinitis [5, 36].

The existence of a nasobronchial reflex is controversial, but this reflex would allegedly produce smooth-muscle contraction through the vagus nerve after a stimulus on the sensory nerve endings in the nose [5, 36]. Regardless, there is data on patients with asthma experiencing airway hyperresponsiveness after being exposed to nasal allergens or cold, dry air [5, 37-39]. Conversely, the relevance of the bronchonasal reflex -different to the nasobronchial reflex- is unclear, but an increase in nasal airway resistance has been observed after inhaling nebulised distilled water [5, 40].

Pathophysiology Inflammation

Airway inflammation is one of the key pathogenic factors in respiratory tract diseases [1]. However, inflammation is not a fixed phenomenon; instead, inflammation is dynamic and is affected by a range of modulating factors from the natural history of the disease to environmental exposures, treatment, or infections [1]. Inflammation of the respiratory tract is a complex system involving a cytokine network with complex regulation mechanisms [4].

The interaction between upper and lower airways regarding inflammatory processes is another reason to support the united airways hypothesis [1, 3, 4].

Even if the exact mechanisms are yet unknown, it seems that propagation of the inflammation happens through postnasal drip and systemic circulation [3, 5]. A possible explanation is that local inflammation in lower or upper airways might trigger a systemic response, including increased bone marrow production of white blood cells tropic to the respiratory tract [3]. Consequently, progenitor cells would be released and recruited to tissues [3]. Most cases of rhinitis, particularly allergic and rhinitis with nasal polyps, are mediated by immune cells of the T helper lymphocyte type-2 (type 2) side of the immune system, including mast cells and eosinophils, and the same cell types are responsible for the lower airway inflammation in nearly all cases of asthma.

Indeed, there is good evidence to sustain that respiratory tract inflammation can lead to systemic inflammation [5, 36]. For example, systemic eosinophilic inflammation can be observed both in upper and lower tract respiratory diseases, and it is indeed observed both in eosinophilic asthma and chronic rhinosinusitis with nasal polyps (CRSwNP) [1, 5]. In addition, there are even data to support the UAD hypothesis when we look at neutrophilic inflammation: there is a correlation between nasal and sputum IL-8 concentrations in COPD patients [1, 41-43].

Remodelling is another aspect of inflammation that shows data to support the concept of UAD; for example, thickening of the epithelial basement membrane, typical of lower airway remodelling, can be seen in atopic patients without asthma and patients with allergic rhinitis [3].

Endophenotypes

A recent review article by Yii et al., efficiently summarised the endophenotypes of UAD [1]. These authors classify all the UAD subtypes -endophenotypes- into 3 major categories, which feature the main ‘treatable traits’: airway inflammation, impaired airway mucosal defence, and exogenous cofactors [1]. Each of these 3 main subgroups encompasses different conditions. Namely:

- Airway inflammation:
  - allergic asthma-rhinitis
  - local allergic rhinitis-non-allergic asthma (local mucosal IgE production)
  - non-allergic rhinitis with eosinophilia syndrome-non-allergic eosinophilic asthma
  - asthma-chronic rhinosinusitis
  - chronic obstructive pulmonary disease chronic rhinosinusitis

- Impaired airway mucosal defence:
  - cystic fibrosis: bronchiectasis-chronic rhinosinusitis due to airway surface liquid viscosity
  - primary ciliary dyskinesia: bronchiectasis-chronic rhinosinusitis due to ciliary dysfunction
  - primary antibody deficiency
- Exogenous cofactors:
  - occupational asthma-rhinitis
  - aspirin-exacerbated respiratory disease
  - asthma-chronic rhinosinusitis: hypersensitivity to *Staphylococcus aureus* enterotoxins

Many of these 'endophenotypes' are already mentioned in this book or even have a chapter of their own, such as occupational asthma-rhinitis or aspirin-exacerbated respiratory disease. However, this chapter will focus on the two endophenotypes that feature allergic rhinitis.

Allergic Asthma-Rhinitis

This endophenotype is usually defined by the presence of atopy, which is traditionally demonstrated by the existence of mast cell's membrane allergen-specific IgE (using skin prick testing) or free serum allergen-specific IgE (e.g., ImmunoCAP, Thermo Fischer) against an airborne allergen [1, 44]. Preemptively testing for food allergy in the evaluation of rhinitis is not recommended, as food allergy is virtually never the cause of rhinitis alone [45].

Throughout this chapter, we have explored the close relationship between allergic rhinitis and asthma and the many possible allergic triggers for these conditions, and next, we will briefly focus on management. The first steps of treating allergic rhinitis are mainly topical steroids (which can be combined with topical antihistamines) and systemic antihistamines [8, 44, 45]. Other add-on alternatives with varying evidence include montelukast, sodium cromoglicate, or topical anticholinergics [44]. These medications are used in the general approach to allergic rhinitis globally; however, prescription patterns and specific stepwise strategies might vary locally depending on many factors [46].

Fortunately, international consensus is moving forward to multimorbidity guidelines to ensure consistent management of UAD (not only allergic rhinitis as an isolated entity) and bearing in mind the digital transformation of healthcare [46].

Importantly, good control of rhinitis is important to achieving asthma control, another supporting factor for the UAD hypothesis [47].

However, not all patients reach control with the standard treatments, so they need to progress to other treatment steps. When a specific causative allergen can be found, patients have the option of a disease-modifying therapy that can target that allergen, which is allergen-specific immunotherapy.

As defined by Alvarez-Cuesta et al., [48] in a classical document by the European Academy of Allergy and Clinical Immunology (EAACI) Immunotherapy Task Force, where the practice standards for immunotherapy were described, allergen-specific immunotherapy is:

> The practice of administering gradually increasing quantities of an allergen product to an individual with IgE-mediated allergic disease in order to ameliorate the symptoms associated with subsequent exposure to the causative allergen. Allergen-specific immunotherapy induces clinical and immunologic tolerance, has long-term efficacy and may prevent the progression of allergic disease. Allergen-specific immunotherapy also improves the quality of life of allergic patients. This definition is based on category I evidence.

The classification of immunotherapy products, the indications for treatment, and the practical management of allergen-specific immunotherapy greatly exceed the objectives of this chapter. However, consensus documents and guidelines are widely available for further consultation [46, 48-54].

Notably, allergen-specific immunotherapy with airborne allergens is mainly indicated or licensed for the treatment of allergic rhinitis; however, the controversial use of allergen-specific immunotherapy for the treatment of asthma is explored in a different chapter of this book. Despite controversies and not entering into further discussion, the fact that this therapy seems to have beneficial effects both on upper and lower airways is another factor supporting the UAD hypothesis.

Non-specific immunotherapy with biologics is also available for patients with this endophenotype, specifically in severe asthma patients [55]. However, the use of biologics in asthma is explored specifically in a different chapter of this book.

Local Allergic Rhinitis-Asthma (Local Respiratory Allergy)

Recent evidence in children and adults suggests that allergic rhinitis can be divided into 3 different phenotypes: allergic rhinitis, local allergic rhinitis, and dual allergic rhinitis -the coexistence of both- [56, 57].

Local allergic rhinitis is an underdiagnosed entity that features classic symptoms of allergic rhinitis affecting the quality of life of patients with allergen-specific reactivity upon nasal allergen provocation test but, strikingly, no signs of systemic IgE-sensitisation, i.e., negative skin testing and serum allergen-specific IgE [58, 59].

Even if its pathophysiology is still under research, this type of rhinitis does not seem to be an initial phase of allergic rhinitis but is a clinical entity in its own right [58-60].

These patients can show specific IgE in nasal secretions, findings in line with type 2 inflammation, and a remarkable rate of asthma progression [58-61].

Patients with local allergic rhinitis and asthma show what has been defined as a new asthma phenotype: ‘local allergic asthma’ [62, 63]. Just as with local allergic rhinitis, this type of asthma lacks confirmation of systemic atopy but, interestingly, patients show positive allergen-specific bronchial challenges [62, 63].

These findings have prompted the coining of a new term for this eosinophilic phenotype of chronic airway disease, and this term is very much in line with this UAD chapter: ‘local respiratory allergy’ [60].

There is extensive research on *in vitro* diagnostic tests for local respiratory allergy, such as basophil activation testing or specific IgE quantification in nasal lavage [60, 64]. However, nasal and bronchial allergen challenges remain the 'gold standard' technique. Unfortunately, these are complex techniques that require expert personnel and adequate installations [60, 64].

Even if combined standard therapy (e.g., intranasal steroids or antihistamines) would be expected to improve eosinophilic inflammation and control symptoms, the effect of this approach has not been specifically assessed in patients with local allergic rhinitis [65]. However, the beneficial impact of allergen-specific immunotherapy in controlling symptoms, decreasing medication needs, and improving quality of life has been demonstrated in several randomised clinical trials and one observational study [66-69].

Conclusion

This chapter delved into the unified airway theory and the concept of UAD. The growing evidence for UAD comes from different angles, namely, anatomical, epidemiological, pathophysiological, and clinical. In addition, the improved understanding of UAD has allowed clinicians to expand on disease endophenotypes that involve both upper and lower airways. A deeper understanding of these endophenotypes is essential to ensure ideal diagnostic and management pathways, including targeted treatments (such as biologics or allergen-specific immunotherapy), in line with precision and personalised medicine.

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Chapter 13

Allergic Bronchopulmonary Aspergillosis

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Abstract

Fungal-related lung diseases can be infective, toxic or allergic in nature. Allergic bronchopulmonary aspergillosis (ABPA) is caused by hypersensitivity to allergens of the common saprophytic filamentous fungi *Aspergillus*, mainly *A. fumigatus*. ABPA is characterized by an early allergic response and late-phase lung injury in response to repeated exposure to *Aspergillus* antigens, because of persistent fungal colonization of the airways.

ABPA is mostly found in patients with asthma and cystic fibrosis. Patients present with respiratory symptoms including wheeze, hemoptysis, and productive cough plus other systemic symptoms, such as fever and weight loss and can suffer recurrent exacerbations. ABPA can be a cause of large airway collapse and lead to bronchiectasis. The central histological feature of ABPA is allergic eosinophilic mucin-harboring hyphae in the bronchi, for which the formation of extracellular DNA trap cell death of eosinophils induced by viable fungi is essential. When the spores contact the immune system, the *A.*

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fumigatus antigens cause a polyclonal antibody response leading to elevated levels of total IgE, *A. fumigatus*-IgE, and *A. fumigatus*-IgG antibodies. Then, eosinophilic mucus plugs containing fungal hyphae are formed in the respiratory tracts.

ABPA is characterized by eosinophilic recruitment to the airways and peripheral eosinophilia. Type-2 (T2) cytokines promote eosinophil participation. The diagnosis of ABPA requires a combination of clinical, serological, and radiological findings. ABPM should be considered when peripheral blood eosinophil counts or serum IgE levels increased in patients with asthma who are sensitized to fungal antigens, or when pulmonary opacities, central bronchiectasis, or mucus plugs were accompanied by peripheral blood eosinophilia.

All criteria proposed by different authors utilize the results of several investigations to aid diagnosis. These criteria represent biomarkers of the mainly involved T2 immune mechanism and of the fungi colonization of the airways. In ABPA, systemic corticosteroids are the first line of treatment. Antifungal agents are regularly added. If the patient becomes treatment dependent, alternative antifungals, pulsed methylprednisolone, nebulized amphotericin, or biologic agents against T2 asthma targets could be considered.

Keywords: allergic bronchopulmonary aspergillosis, antifungal therapy, aspergillus, asthma, bronchiectasis, cystic fibrosis, eosinophilia, eosinophilic extracellular traps (EETs), glucocorticoids, high attenuation mucus, immunoglobulin G (IgG), immunoglobulin E (IgE), interleukin 5 (IL-5), interleukin 4 (IL-4), interleukin 13 (IL-13), precipitins, type-2 cytokines, type-2 targeted biologics

Introduction

Inhalation of airborne components, including airborne conidia, hyphae and fungal fragments may cause a spectrum of disease in some persons, ranging from sensitization to severe invasive infection [1]. Fungal-related lung diseases represent a heterogeneous group of conditions (Table 1). They can be infective, toxic or allergic in nature; however, there is a degree of overlap.

Environmental fungi such as *Alternaria* and *Cladosporium* can act as aeroallergens, triggering symptoms directly related to airborne concentrations of fungal substance, including acute severe asthma exacerbations. Thermophilic filamentous fungi like species from the Aspergillus and Penicillium genera have the additional property of being able to germinate in the airways, colonizing the lungs and causing a persistent allergenic stimulus [1].

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic pulmonary condition caused by hypersensitivity to allergens of the common saprophytic filamentous fungi *Aspergillus*. ABPA is characterized by an early allergic response and late-phase lung injury in response to repeated exposure to *Aspergillus* antigens, because of persistent fungal colonization of the airways (Figure 1).

A. fumigatus is the most common pathogen in this genus; it is abundant in both indoor and outdoor environments [1, 2]. The conidia of *A. fumigatus* are able to reach the distal alveoli due to their small size (2-5 μm). Following germination and subsequent growth, *A. fumigatus* produces a multitude of elements that may either mediate or aggravate asthma symptoms [3]. Moreover, its ability to colonize the respiratory tract, if not readily cleared by immune system, means it may drive a sustained release of allergens and other products over a prolonged period. The cardinal histological feature of ABPA is allergic eosinophilic mucin-harboring hyphae in

the bronchi, for which the formation of extracellular DNA trap cell death of eosinophils induced by viable fungi is essential.

ABPA was first described in 1952 [4]. It is mostly found in patients with asthma and cystic fibrosis (CF), and much less frequently in chronic obstructive pulmonary disease, post-tuberculous fibrocavitary disease or other rarer conditions, such as chronic granulomatous disease and hyper-immunoglobulin E syndrome. Individuals with ABPA present with respiratory symptoms including asthma, wheeze, hemoptysis, and productive cough plus other systemic symptoms, such as fever and weight loss and can suffer recurrent exacerbations [5-7]. ABPA can typically be a cause of large airway collapse and lead to bronchiectasis [5-7].

Table 1. Pulmonary aspergillosis clinical syndromes

Invasive aspergillosis		Allergic/hypersensitivity reactions	Saprophytic colonization	Mycotoxicosis
Generalized	Limited			
Aspergillosis pneumonia Angioinvasive aspergillosis Lung abscess and multiple cavities Aspergillosis bronchitis Infarction Pleural effusion and empyema	Chronic necrotizing pulmonary aspergillosis	Allergic asthma Allergic bronchopulmonary aspergillosis Extrinsic allergic alveolitis Bronchocentric Granulomatosis	Aspergilloma (Mycetoma or fungus ball)	Chemical pneumonitis

Epidemiology

The epidemiological drivers of respiratory fungal disease are multifaceted and dependent on a range of factors, many of which may have an anthropogenic basis. Global warming and the global trade in plants may have rapidly accelerated the propensity for new and ecologically invasive species, as well as the rapid global emergence of triazole resistance, recently characterized in A. fumigatus.

The estimated prevalence of ABPA in asthma range between 0.72% and 3.5%, and therefore is expected to affect 4.8 million patients worldwide [8]. The prevalence in severe asthma is likely to be much higher; a prospective study of patients with severe asthma attending a tertiary hospital in Northern India showed a prevalence of 70% [9]. The prevalence of ABPA in CF range, in recent studies, between 8.9% and 10.5% [10, 11].

Pathophysiology

Aspergillus species are ubiquitous molds in the environment, especially present in the organic matter. *A. fumigatus* is the most common fungus involved in ABPA but other species including *A. flavus*, *A. niger*, and *A. oryzae* may also be involved. Other fungal species including *Schizophyllum commune* or *Candida albicans* may rarely cause similar pathology to ABPA [12, 13], and this disease is named allergic bronchopulmonary mycosis (ABPM).

Viable fungi are essential for developing ABPA. Fungi colonization depend on the form and size of fungal conidia. Conidia of *Alternaria* are 25-60×3-3.5 μm, and conidia of

Cladosporium are generally 15-25×7-10 μm. However, conidia of *Aspergillus* and *Penicillium* are spherical and smaller in size, approximately 3-6 μm, and therefore can easily move into the lower airways. Another important factor is fungus thermophilicity, which can allow the fungi to germinate at human body temperatures. The optimum germination temperature of *A. fumigatus* is 37°C-42°C, which is approximately the same as that of the human body.

Unlike invasive aspergillosis -largely associated with underlying neutropenia and/or macrophage dysfunction-, APBA pathophysiology stems from immune deviation toward florid Th2 responses and a component of eosinophilic inflammation, suggesting different immune-pathogenic mechanisms. Arising from *A. fumigatus* activation of pattern recognition receptors (PRRs) and proteolytic activity, epithelial and dendritic cells drive innate lymphoid cells type 2 (ILC2) and Th2 differentiation.

Host factors can determine ABPA development. Genetic predisposition influences the host innate and adaptive immune response to *Aspergillus* [14-21].

There are several phases for the inhaled fungi to induce ABPA pathology (Figure 1). In the first phase, inhaled conidia are moved into the lower airways because of their small size. In the second phase, the conidia germinate and form hyphae, which activates the immune system of the host. Hyphae do not penetrate the lung tissues but settle in the mucus plugs of the bronchi. When the spores contact the immune system, the *A. fumigatus* antigens cause a polyclonal antibody response leading to elevated levels of total IgE, *A. fumigatus*-IgE, and *A. fumigatus*-IgG antibodies. Then, eosinophilic mucus plugs containing fungal hyphae are formed in the respiratory tracts. They play a major role in the pathophysiology of ABPA (Figure 2).

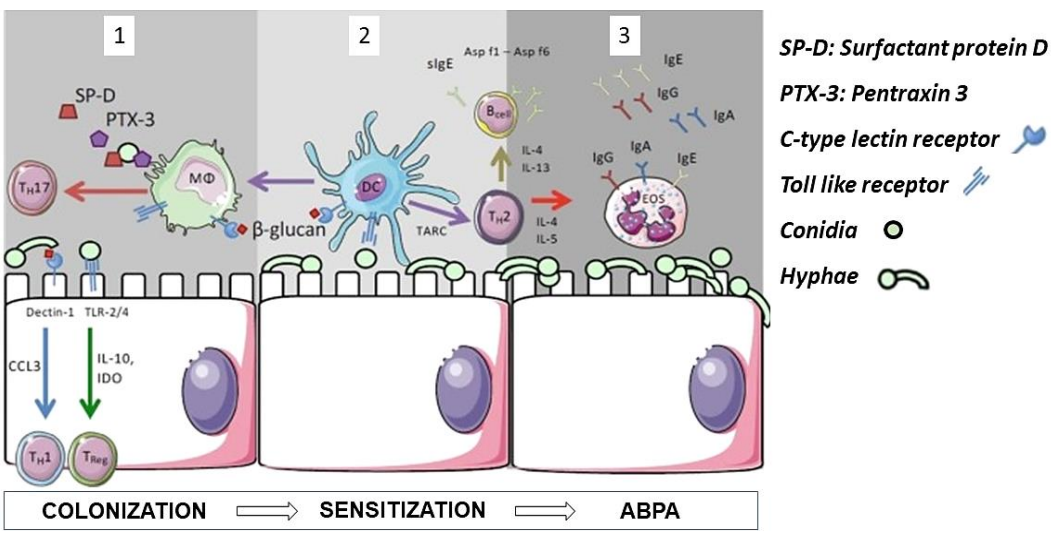

Figure 1. Phases of immune response to Aspergillus in ABPA.

Innate cells can participate through PRRs located on the cell wall that identify pathogen-associated molecular patterns (PAMPs) including B glucan, chitin, and galactomannan [6, 7]. Toll-like receptors (TLRs) are located within the plasma membranes of cells and on intracellular endosomes and detect PAMPs on the fungal cell wall. Binding to these receptors, triggers secretion of pro-inflammatory cytokines, and TLR activation on dendritic cells causes propagation of an adaptive immune response by presenting allergens to naïve CD4 T cells (Th0). A failure in phagocytosis or an alteration in the presentation of antigens can facilitate

the type 2 immune response [19]. Genetic polymorphisms in TLR and mannose binding lectin can augment the susceptibility to ABPA.

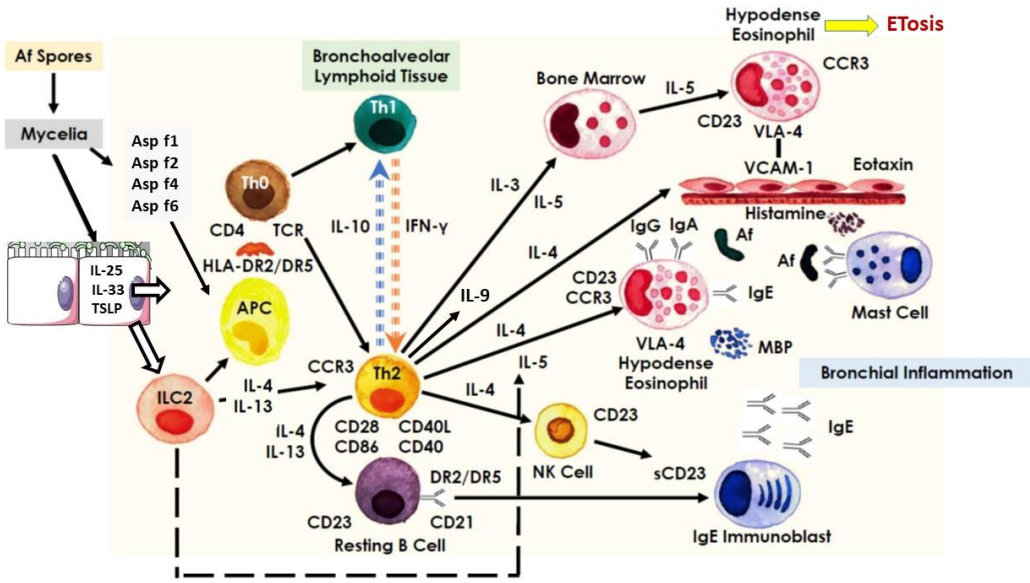

Figure 2. Immuno-pathogenesis of ABPA.

A. fumigatus has also several mechanisms that drive the adaptive immune response towards a Th2 response rather than a Th1 response. Viable fungi, particularly *A. fumigatus*, can stimulate an intense type 2 immune response [21] through a reduction of chemokine CXCL 10 by inhibition of IFN-β signaling through the JAK-STAT pathway and activation of protease-dependent receptor 2 and tyrosine-protein phosphate non-receptor type [22]. Exposure to fungal or protease antigens induce the extracellular release of IL-33 and production of IL-25 and thymic stromal lymphopoietin (TSLP) by the airway epithelium [20]. IL-33, IL-25 and TSLP, cytokines known as alarmins, activate CD4+ T cells (Th2) and ILC2 to produce a large quantity of type 2 cytokines, such as IL-5, IL-13, IL-9, and amphiregulin. Basophil-derived IL-4 may facilitate ILC2 production of cytokines. ILC2-derived IL-13 enhances antigen uptake and migration of dendritic cells and promotes proliferation and differentiation of Th2-type CD4+T cells. Dendritic cells (antigen presenting cells) capture first and after present allergens to naïve CD4 T cells in presence of HLA-DR2/DR5, and the differentiation of Th2-type CD4+T cells is obtained. The risk of ABPA is increased in patients who express HLA-DR2 and/or DR5 but not HLA-DQ2. HLA-DRB1*1501 and 1503 are associated with higher risk of ABPA, and HLADQB1*0201 are associated with lower risk [23].

Furthermore, ILC2s and Th2-type CD4+ T cells may interact directly to sustain production of type 2 (T2) cytokines. The intense T2 response to active *Aspergillus* causes progressive disease and promote IgE and eosinophilic responses in ABPA. ILC2 and Th2 cytokines include IL-4, IL-5, IL-9, and IL-13. IL-4 stimulates activated B cells and promotes differentiation of B cells into IgE producing plasma cells. IL-5 is a mediator for eosinophil production and activation. IL-13 induces airway hyper-responsiveness, goblet cell metaplasia, and mucous hypersecretion.

ABPA is characterized by eosinophilic recruitment to the airways and peripheral eosinophilia. T2 cytokines promote eosinophil participation. Extracellular DNA trap cell death (ETosis) of eosinophils has been suggested as a mechanism for the formation of eosinophilic mucus plugs in T2-induced airways diseases. ETosis was formerly reported in neutrophils. Neutrophil extracellular traps (NETs) have no fungicidal action. Eosinophils experience a process of extracellular trap cell death in response to *Aspergillus*. Eosinophils suffer cytolysis and release filamentous chromatin fibers, forming eosinophilic extracellular traps (EETs) [16-18]. EETs can immobilize *Aspergillus*; however, EETs are not so effective in killing *Aspergillus*, may increase mucus viscosity and sputum plug formation, and so may contribute to ABPA pathogenesis [16-18]. Repair mechanisms in response to damage by *Aspergillus* proteases, mast cell degranulation, eosinophils, and EETs result in proliferation of epithelial cells, endothelial smooth muscle cells and fibroblasts, resulting in remodeling of the airways, and development of bronchiectasis [18].

Once in the airways, *A. fumigatus* tries to persist and the immune system of the host tries to expel it. There are substances that the fungus generates to maintain itself on airways that are initially stimuli for the immune system based on the T2 response to act to expel it. If there are defects in the host's immune system -mainly in phagocytosis or mucus drainage [14, 24, 25], the T2 response will become the most important part of the problem in ABPA. The host response to *Aspergillus* in ABPA is driven towards an uncontrolled T2 response, and this is thought to cause progressive disease.

The first innate barrier to infection is the airway epithelium. The epithelial layer contains goblet cells and ciliated cells. Goblet cells produce mucus, which captures foreign bodies, and ciliated cells move the mucus up the airways towards the mouth. This mucociliary escalator is impaired in individuals with CF or asthma. *Aspergillus* can also reduce the efficacy of the mucociliary escalator because it produces metabolites such as gliotoxin that weaken ciliary beating [14].

The first immunologic line of defense against *Aspergillus* in the airways is the macrophage, which is capable of ingesting and killing spore [19, 23, 24]. *A. fumigatus* conidia bind surfactants A and D; various extracellular matrix proteins, such as laminin, fibronectin, and fibrinogen; and mannose-binding lectin efficiently, as well as C3 [19]. Surfactant augments phagocytosis of conidia. The composition of mucous may also be a factor predisposing to ABPA. Polymorphisms in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which regulates the flow of sodium and chloride ions across cell membranes, augment the viscosity of mucous and increase the risk of ABPA [15]. Several studies showed an increased probability of seeing CFTR mutations in patients with ABPA [15]. In the smaller airways, type-II pneumocytes secrete surfactant proteins A and D. They are opsonins that bind *Aspergillus* conidia and target them for phagocytosis by neutrophils and macrophages [19]. Changes in the function of surfactant proteins may contribute to worsened disease [20].

Diagnosis

The diagnosis of ABPA requires a combination of clinical, serological, and radiological findings. ABPM should be considered when peripheral blood eosinophil counts or serum IgE levels increased in patients with asthma who are sensitized to fungal antigens, or when pulmonary opacities, central bronchiectasis, or mucus plugs were accompanied by peripheral blood eosinophilia (Tables 2 and 3).

Table 2. Comparison of diagnostic criteria for ABPA [7, 26, 28-30]

Rosenberg-Patterson (26)	Consensus Conference for diagnosis of ABPA in CF (7)	ISHAM consensus (28)	Asano et al. (29)	Saxena et al. (30) Modified ISHAM-AWG
Major criteria (1–6 suggestive, +7 definite) 1. Asthma 2. Peripheral blood eosinophilia 3. Positive immediate skin test to Aspergillus 4. Positive precipitin test to Aspergillus 5. Increased total serum IgE > 1000 IU/mL 6. History of transient or fixed lung infiltrates 7. Proximal bronchiectasis with normal tapering of distal bronchi *Minor criteria* 1. Brown plugs/flecks in sputum 2. Positive late (6–12 h/Arthus) skin test to Aspergillus	*Classic criteria* 1. Acute or subacute clinical deterioration not attributable to another etiology (cough, wheeze, exercise intolerance, exercise induced asthma, change in pulmonary function, or increased sputum production) 2. Serum total IgE >1000 IU/mL (2400 ng/mL) unless receiving systemic corticosteroids 3. Positive immediate skin test to *Aspergillus* or serum IgE antibody to *A. fumigatus* 4. Precipitating antibodies or serum IgG antibody to *A. fumigatus* 5. New or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy *Minimal diagnostic criteria* 1. Acute or subacute clinical not attributable to another etiology 2. Serum total IgE >500 IU/mL. If ABPA is suspected and the total IgE level is 200–500 IU/mL then repeat in 1–3 months. If on steroids, repeat once steroid treatment stopped. 3. Positive immediate skin test to *Aspergillus* or serum IgE antibody to A. fumigatus 4. One of the following: - *A. fumigatus* precipitins - IgG antibody to *A. fumigatus* - New or recent anomalies on chest radiography or chest CT not cleared with antibiotics and standard physiotherapy	Predisposing conditions asthma or cystic fibrosis should be present *Obligatory criteria* 1. Positive immediate skin test to *Aspergillus* antigen) or specific IgE levels against *A. fumigatus* 2. Elevated total IgE levels (>1000 IU/mL) *Supportive criteria (2 or more)* 1. Presence of precipitating or IgG antibodies against *A. fumigatus* in serum 2. Radiographic pulmonary opacities consistent with ABPA 3. Total eosinophil count >500 cells/μL in steroid naive patients	*Require 6 or more for diagnosis* 1. Current or previous history of asthma or asthmatic symptoms 2. Peripheral blood eosinophilia (≧500 cells/ mm3) 3. Elevated total serum IgE (≧417 IU/mL) 4. Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi 5. Presence of precipitins or specific IgG for filamentous fungi 6. Filamentous fungal growth in sputum cultures or bronchial lavage fluid 7. Presence of fungal hyphae in bronchial mucus plugs 8. Central bronchiectasis on CT 9. Presence of mucus plugs in central bronchi based on CT/bronchoscopy or mucus plug expectoration history 10. High attenuation mucus in bronchi on CT	*All of the following:* 1. Asthma 2. *A. fumigatus*-specific IgE >0.35 KUA/L 3. Serum total IgE levels >500 IU/mL *Two of the following*: 1. *A. fumigatus*-specific IgG >27 mg/L 2. Bronchiectasis on CT chest 3. Eosinophil count >500 cells/mL

ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis; CT, computed tomography; ISHAM, International Society of Human and Animal Mycology.

Table 3. Newer criteria from modified ISHAM-AWG criteria for ABPA [30, 31]

Modified ISHAM-AWG criteria	Presence of both: (1) serum Af-specific IgE >0.35 kUA/L, and (2) serum total IgE >500 IU/mL
	AND two of the following: (1) serum Af-specific IgG >27 mgA/L; (2) bronchiectasis on CT chest; (3) TEC >500 cells/μL
Newer Criteria	
Criteria 1	Both: (1) serum Af-specific IgE >0.5 kUA/L, and (2) serum total IgE >500 IU/mL AND any of the following: (1) serum Af-specific IgG >27 mgA/L; (2) bronchiectasis on CT chest
Criteria 2	Both: (1) serum Af-specific IgE >0.5 kUA/L, and (2) serum total IgE >500 IU/mL AND any of the following: (1) bronchiectasis on CT chest; (2) TEC >500 cells/μL
Criteria 3	All the following: (1) serum Af-specific IgE >0.35 kUA/L; (2) serum total IgE >500 IU/mL; (3) bronchiectasis on CT chest
Criteria 4	All the following: (1) type 1 *Aspergillus* skin test positive; (2) serum total IgE >500 IU/mL; (3) bronchiectasis on CT chest
Criteria 5	Both: (1) serum Af-specific IgE >0.35 kUA/L, and (2) serum total IgE >500 IU/mL AND any of the following: (1) serum Af-specific IgG >27 mgA/L; (2) bronchiectasis on CT chest
Criteria 6	Both: (1) serum Af-specific IgE >0.35 kUA/L, and (2) serum total IgE >500 IU/mL AND any of the following: (1) bronchiectasis on CT chest; (2) TEC >500 cells/μL
Criteria 7	Both: (1) serum Af-specific IgE >0.35 kUA/L, and (2) serum total IgE >500 IU/mL AND any of the following: (1) serum Af-specific IgG >27 mgA/L; (2) TEC >500 cells/μL

Af, *Aspergillus fumigatus*; AWG, ABPA working group; CT, computed tomography; ISHAM, International Society of Human and Animal Mycology; TEC, total eosinophil count.

Rosenberg and Patterson [26] proposed the classic diagnostic criteria of ABPA in 1977. In 1988, Greenberger and Patterson [27] added a new disease concept, ABPA-seropositive, which refers to cases that lack central bronchiectasis, but fulfil other criteria including serological tests (Table 4).

Table 4. ABPA according to radiographic findings [27, 36, 37, 39]

Classification	ABPA-S	ABPA-CB	ABPA-HAM	ABPA-CPF	SAFS
Findings	Patients that meet minimum requirements of ABPA but do not have central or peripheral bronchiectasis	ABPA with central bronchiectasis	ABPA with high attenuation mucus	ABPA with chronic pleuropulmonary fibrosis, which includes other radiological features including pulmonary fibrosis, parenchymal scarring, fibro cavitary lesions, aspergilloma, and pleural thickening without the presence of mucoid impaction or HAM	Patients who have severe asthma and sensitivity to fungi but do not meet the criteria for ABPA

ABPA: allergic bronchopulmonary aspergillosis; ABPA-S: ABPA-seropositive; ABPA-CB: ABPA with central bronchiectasis (CB), ABPA-HAM: ABPA with high attenuation mucus (HAM) and ABPA-CPF: ABPA with chronic pleuropulmonary fibrosis (CPF); SAFS: severe asthma associated with fungal sensitivity.

In 2013, the International Society of Human and Animal Mycology (ISHAM) proposed new diagnostic criteria [28]. ISHAM defines asthma and cystic fibrosis as predisposing conditions and proposes two obligatory criteria: 1.) immediate cutaneous hypersensitivity to *Aspergillus* antigen or elevated IgE levels against *A. fumigatus*, and 2.) elevated total IgE levels > 1,000 IU/mL. In addition, at least two of the following 3 criteria should be fulfilled: 1.) the presence of precipitating or IgG antibodies against *A. fumigatus* in serum, 2.) radiographic features in the lungs consistent with ABPA, and 3] total eosinophil count > 500 cells/μL.

Asano et al. have recently proposed new criteria in 2020, requiring 6 or more for diagnosis [29]:

1. Current or previous history of asthma or asthmatic symptoms.
2. Peripheral blood eosinophilia (≧500 cells/ mm^3).
3. Elevated total serum IgE (≧417 IU/mL).
4. Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi.
5. Presence of precipitins or specific IgG for filamentous fungi.
6. Filamentous fungal growth in sputum cultures or bronchial lavage fluid.
7. Presence of fungal hyphae in bronchial mucus plugs.
8. Central bronchiectasis on CT.
9. Presence of mucus plugs in central bronchi based on CT/bronchoscopy or Mucus plug expectoration history.
10. High attenuation mucus in bronchi on CT.

Saxena et al. have modified ISHAM criteria in 2021, based on latent class analysis [30], a relatively modern computational approach for refining the diagnostic criteria for ABPA. There

are different criteria used for patients with CF, proposed by the CF Foundation [7], and for patients with ABPM [29, 30].

Recently, Agarwal et al. have evaluated seven simpler models for diagnosing ABPA [31] from the modified ISHAM criteria. The modified ISHAM criteria [30] established the presence of both: 1) serum *A. fumigatus* specific IgE >0.35 kUA/L, and 2) serum total IgE >500 IU/mL; and two of the following: 1) serum *A. fumigatus* specific IgG >27 mgA/L; 2) bronchiectasis on CT chest; 3) TEC >500 cells/µL.

The simpler new criteria proposed were [31]:

Criteria 1:

- Both: 1) serum *A. fumigatus* specific IgE >0.5 kUA/L, and 2) serum total IgE >500 IU/mL.
- And any of the following: 1) serum *A. fumigatus* specific IgG >27 mgA/L; 2) bronchiectasis on CT chest.

Criteria 2:

- Both: 1) serum *A. fumigatus* specific IgE >0.5 kUA/L, and 2) serum total IgE >500 IU/mL.
- And any of the following: 1) bronchiectasis on CT chest; 2) peripheral blood total eosinophil count >500 cells/µL.

Criteria 3:

- All the following: 1) serum *A. fumigatus* specific IgE >0.35 kUA/L; 2) serum total IgE >500 IU/mL; 3) bronchiectasis on CT chest.

Criteria 4:

- All the following: 1) type 1 *Aspergillus* skin test positive; 2) serum total IgE >500 IU/mL; 3) bronchiectasis on CT chest.

Criteria 5:

- Both: 1) serum Af-specific IgE >0.35 kUA/L, and 2) serum total IgE >500 IU/mL.
- And any of the following: 1) serum *A. fumigatus* specific IgG >27 mgA/L; 2) bronchiectasis on CT chest.

Criteria 6:

- Both: 1) serum *A. fumigatus* specific IgE >0.35 kUA/L, and 2) serum total IgE >500 IU/mL.
- And any of the following: 1) bronchiectasis on CT chest; 2) peripheral blood total eosinophil count >500 cells/µL.

Criteria 7:

- Both: 1) serum *A. fumigatus* specific IgE >0.35 kUA/L, and 2) serum total IgE >500 IU/mL.
- And any of the following: 1) serum *A. fumigatus* specific IgG >27 mgA/L; 2) peripheral blood total eosinophil count >500 cells/μL.

The analysis of these criteria [31] showed that *A. fumigatus* specific IgE-based criteria performed better than skin test-based criteria. Of the seven criteria, the combination of IgE (total and *A. fumigatus* specific IgE) and either an elevated A. fumigatus specific IgG or bronchiectasis (criteria 5) could be a feasible alternative to the modified ISHAM criteria. The specific IgE-based minimal essential criteria (criteria 3) might be another alternative, especially in resource-constrained settings, as it includes only three components. The skin test-based minimal essential criteria (criteria 4) may be used to confirm ABPA (specificity >99%) in settings without access to immunoassays. However, criteria 4 cannot be used to rule out ABPA, given its poor sensitivity (73%). In this study [31], the results provide clinicians with evidence regarding the certainty of diagnosing ABPA when one or more of the components of the existing criteria are missing.

All criteria proposed utilize the results of several investigations to aid diagnosis. These criteria represent biomarkers of the mainly involved T2 immune mechanism and of the fungi colonization of the airways (Table 2 and 3).

Serology: Total IgE and Specific IgE to *Aspergillus fumigatus*

Serology plays a central role in ABPA diagnosis. Rosenberg and Patterson's diagnostic criteria [26], used since 1977, include an elevated total IgE of at least 1,000 ng/mL. In recent literature, threshold values for total IgE of 500 kU/L or 1,000 kU/L are frequently used [28]. In addition, specific IgE to A. fumigatus must be present, which can be assessed by a positive skin test and a serum specific IgE > 0.35 kU/L, although much higher IgE concentrations are usually present. Increased levels of total IgE in serum are useful for diagnosis but also for monitoring the disease activity. Reductions in levels of total IgE of 25–50% correlate with improved symptoms, while radiological appearances with increasing IgE levels (e.g. >50% in IgE level) suggest an imminent exacerbation [28]. Conversely, different cut-off values have been employed depending on the diagnostic criteria (Table 2). The ISHAM emphasize the usefulness of higher cut-off values to distinguish ABPA from severe asthma with fungal sensitization [28].

Recent advances in technology have enabled cloning of several proteins of A. fumigatus [32, 33]. Asp f 1, the ribonuclease mitogillin, a member of the ribotoxin family, is a major allergen of A. fumigatus, and an indicator of genuine sensitization to A. fumigatus. It is associated to all allergic fungal airway diseases. Asp f 1 is a secreted protein. It exerts cytotoxic and proinflammatory effects. Asp f 2, a cell wall-associated extracellular protein, is also a major allergen of A. fumigatus, an agent of all allergic fungal airway diseases. Asp f 2 binds fibrinogen, plasminogen, and extracellular matrix proteins, contributing to fungal defense and host colonization. Asp f 3, located in peroxisomes, belongs to the highly conserved family of peroxiredoxins, involved in redox homeostasis and response to oxidative stress. Asp f 3 is a

minor allergen; it is an agent of allergic fungal airway diseases. Asp f 3 displays cross-reactivity with homologues from other fungal species and genera. Antibodies against Asp f 4 and Asp f 6 are elevated only in ABPA.

A recent work from India has now shown that the combination of IgE antibodies against Asp f 1 (at a cut-off of 4.4 kU/L) and Asp f 2 (at a cut-off of 1.3 kU/L) have a sensitivity of 100% and a specificity of 81% in differentiating between Aspergillus-sensitized asthma patients and patients with ABPA in asthma [32]. For ABPA screening in asthma patients, in the future, based on the current data from India, the estimable combined sensitivity of Asp f 1 and Asp f 2 may allow these two parameters to be used in combination with total IgE. The determination of specific IgE against Asp f 4 and f 6 can then be performed for confirmation due to the very good specificity of these two parameters. It would be desirable to verify the value of the two parameters Asp f 1 and f 2 in European patients since genetic differences of the patients as well as variations of the Aspergillus strains may play a role.

Serology: Serum Precipitins and Specific IgG to *Aspergillus fumigatus*

Serum precipitins -specific IgG- against *A. fumigatus* are present in 69-90% of patients with ABPA, but also in 10% of asthmatics with or without severe asthma with fungal sensitization. IgG antibodies, including precipitins, against *A. fumigatus* can be demonstrated using double gel diffusion techniques, enzyme linked immuno-assay (ELISA), fluorescent enzyme immunoassay (FEIA) or other methods. ImmunoCap method has good reproducibility. *A. fumigatus* IgG may not be specific for ABPA as high levels are seen in other forms of aspergillosis. Various cut-offs for *A. fumigatus* IgG determined by FEIA have been reported in different studies. In the United Kingdom, a cut-off of 40 mg/L was determined based on unpublished observations. An age-dependent decline in the levels of *A. fumigatus* IgG can be observed. Thus, cut-offs for *A. fumigatus* IgG levels can be determined separately by age as 60 mg/L for patients aged <55 years, and 45 mg/L for those aged ≥55 years. For Asp f 1 IgG, 6.6 mg/L was set as the cut-off regardless of age [34]. However, Saxena et al. have modified ISHAM criteria in 2021 [30], and the cut-offs for *A. fumigatus* IgG determined by FEIA is IgG >27 mgA/L.

Sputum Cultures

Classically, mycological criteria have typically been from fungal cultures from either the airway or on tissue biopsy; however, these would not necessarily be diagnostic on their own as the airway has always been considered non-sterile from a microbiological perspective and fungi are ubiquitous components of the aerial microbiota. Further progress has been made with respect to more systematic use of both β-1,3 glucan and galactomannan as markers of respiratory fungal disease where β-1,3 glucan is used in serum primarily as a screening assay and galactomannan has utility both in serum and airway samples for specific diagnosis of aspergillosis. Sputum cultures may be important in determining azole resistance prior to treatment [27]. Culturing fungi from sputum is a supportive test in the diagnosis of ABPA but is not 100% specific for ABPA as *A. fumigatus* is ubiquitous and commonly isolated from lung

expectorant in other diseases. Nevertheless, between 40% and 60% of patients have positive cultures depending on the number of samples taken [27].

Lung Function

Lung function determine the severity of underlying lung disease and allow monitor response to treatment. Fixed airflow obstruction and reduced lung volumes can be found in progressive disease.

Radiology

Radiological findings are non-specific or subtle in the early stages of the disease, and the diagnosis is often difficult. Radiological findings can contribute to assess the stage of ABPA (Table 5 and 6). There is preferential involvement of the upper lobes. The chest radiographic appearances of ABPA are myriad and can be broadly classified as transient or permanent. The active stage is characterized radiographically by transient and recurrent infiltrates that may clear with or without glucocorticoid therapy, although steroid therapy does accelerate the clearing of opacities.

Table 5. ABPA Staging according to Patterson et al. [37].
Radiographic findings and corresponding IgE levels

Stage	Description	Radiographic Findings	Total IgE levels
Stage I: acute	The patient is diagnosed with ABPA. Some features such as Aspergillus-specific IgE, radiological abnormalities, peripheral blood eosinophilia, and Aspergillus-specific serum precipitins may be presented.	There may be homogenous infiltrates, mucus plugging, lobar consolidation or collapse, “tree-in-bud” appearance, bronchiectasis. Predominantly in upper lobes.	Overall elevated
Stage II: remission	Asymptomatic patient with underlying controlled asthma but no new radiological infiltrates and no rise in total IgE for a minimum of six months.	Complete or significant resolution of pulmonary infiltrates and clearance mucoid impaction.	Normal or elevated IgE level but less than stage I level
Stage III: exacerbation	New pulmonary infiltrates appear on x-ray with peripheral blood eosinophilia and double the remission level IgE levels.	The same findings as seen in acute stage.	Elevated IgE levels usually double the level of stage II
Stage IV: steroid-dependent asthma	Patients become dependent on corticosteroid treatment and are unable to completely taper off from it.	Significant resolution of pulmonary infiltrates and mucoid impaction. There can be atelectasis or hyperinflation from asthma. Fixed pulmonary opacities may be present. If exacerbation occurs then the findings will resemble stage I.	Normal or elevated IgE level
Stage V: end-stage fibrotic disease	Chest x-ray and CT scans will show irreversible fibrosis and chronic cavitation. Despite this, serological parameters are usually negative.	There is lung-scarring, hyperinflation, chronic infiltrates, evidence of bronchiectasis, pulmonary fibrosis or cavities or fibrocavitary findings, pulmonary hypertension.	Normal or elevated IgE level

ABPA: allergic bronchopulmonary aspergillosis; IgE: immunoglobulin E; CT: computed tomography.

Central bronchiectasis is one of the hallmarks of ABPA [25, 28, 31, 33], although the diagnosis can be made without bronchiectasis. Central bronchiectasis is defined as bronchiectasis confined to the medial two-thirds or medial half of the lung. Bronchiectasis can however extend to the periphery as well. The bronchiectasis in ABPA usually involves the upper lobes although, rarely, there may be involvement of the lower zones without involvement of the upper lobes. While some patients may not have bronchiectasis, its presence, especially multi-lobar central or proximal bronchiectasis on high-resolution CT (HRCT) scan warrants further evaluation for ABPA. HRCT is far more sensitive than chest radiography for the detection of bronchiectasis. Mucus impaction is another common finding on computed tomography imaging and high attenuation mucus is a pathognomonic trait of ABPA [33, 35, 36]. The bronchial mucus plugging in ABPA is generally hypodense but may also have high CT attenuation values. High-attenuation mucus is visually denser than the paraspinal skeletal muscle. The constituents of high-attenuation mucus are not entirely clear. Atelectasis is usually subsegmental or segmental, occasionally lobar, and rarely can involve an entire lung. It is important to note that a normal radiographic appearance does not completely exclude the diagnosis of ABPA.

Table 6. Clinical staging as proposed by the ISHAM working group [28]

Stage	Definition	Features
0	Asymptomatic	Global Initiative for Asthma (GINA) definition of controlled asthma. Meets diagnostic criteria for ABPA. No previous diagnosis of ABPA.
1	Acute	Uncontrolled asthma/constitutional symptoms. Meets diagnostic criteria for ABPA. No previous diagnosis of ABPA.
1a	With mucoid impaction	Meets all criteria with mucoid impaction on chest X-ray, CT, or bronchoscopy.
1b	Without mucoid impaction	Meets all criteria without mucoid impaction on chest X-ray, CT, or bronchoscopy.
2	Response	Clinical improvement (resolution of constitutional symptoms and improvement in asthma control). Major radiological improvement. IgE decline by ≧25% of baseline at 8 weeks.
3	Exacerbation	Clinical or radiological deterioration with increase in IgE ≧50%.
4	Remission	Sustained clinic/radiological improvement with IgE levels remaining below baseline or increase <50% for ≧6 months on or off therapy other than systemic steroids.
5a	Treatment dependent ABPA	Relapse on ≧2 consecutive occasions within 6 months of stopped treatment or has worsening clinical, radiological, or immunological parameters on tapering oral steroids/azoles.
5b	Glucocorticoid dependent asthma	Patient requires oral or parenteral glucocorticoids for asthma control while activity of ABPA is controlled as reflected by IgE levels of chest radiograph.
6	Advanced ABPA	Type-II respiratory failure and/or cor pulmonale with radiological evidence of fibrotic findings consistent with ABPA on CT chest after excluding reversible causes of acute respiratory failure.

ABPA, allergic bronchopulmonary aspergillosis; CT, computed tomography; ISHAM, International Society of Human and Animal Mycology.

Evolution and Staging

There is a spectrum of allergic fungal airway disease ranging from simple sensitization to fungal asthma, to severe asthma with fungal sensitization to ABPA. Diagnosis of APBA lies

along a continuum, with a gradation of symptoms, and serologic and radiographic features. The staging defined by Patterson et al. [37] include five categories: acute, remission, exacerbation, steroid dependent asthma, and fibrotic lung disease (Table 5). The ISHAM group proposed a new staging system (Table 6), with seven stages ranging from 0 (asymptomatic) to 6 (advanced ABPA) [27, 38, 39]. Radiological staging (Table 4) has also been proposed by the ISHAM group [39, 40]. Prognosis of ABPA is controversial due to lack of studies. However, early diagnosis and treatment of ABPA seems to be crucial in preventing the development of serious and potentially irreversible lung damage, such as bronchiectasis or fibrosis [25-27, 41, 42]. Remission can be observed in 33% patients, steroid dependent asthma in 20%, and 2% developed end-stage fibrotic lung disease after a following over 4 years [43].

Treatment

The goals of treatment in ABPA are to control symptoms of asthma or cystic fibrosis, to prevent or treat pulmonary exacerbations of ABPA, to maintain and normalize lung function, and to prevent radiological progression to end-stage fibrotic or cavitary disease. We need to minimize or downgrade the pro-inflammatory response and reduce airway fungal colonization to achieve our objectives. At the same time, treatment options should also have minimal or no adverse reactions.

The treatment of ABPA has traditionally included steroids and antifungal therapy. Novel treatments including monoclonal antibodies, immunotherapy, and novel antifungal agents may be of benefit in the treatment of ABPA but have not been studied in large-scale randomized control trials.

Glucocorticoids

At present, systemic glucocorticoids remain the most effective drugs for treating ABPA [44, 45]. In CF associated ABPA, it is recommended 0.5–2.0 mg/kg/day prednisone equivalent (maximum 60 mg/day) for 1-2 weeks, then 0.5–2.0 mg/kg/day prednisone equivalent every other day for 1-2 weeks, then tapering on the basis of clinical and immunologic improvement. An attempt should be made to begin to taper off corticosteroids in 2-3 months [7].

In patients with asthma with ABPA, the commonly used treatment strategy is an initial dose of prednisone 0.5 mg/kg daily for 14 days, followed by 0.5 mg/kg every other day, and then further tapered by 5–10 mg every 2 weeks and finally discontinued at three months.

An unblinded randomized clinical compared the previously mentioned treatment strategy to prednisone 0.75 mg/kg/day for six weeks followed by a more gradual taper [44]. No significant difference was noticed except that the rate of adverse effects was higher in the 0.75 mg/kg/day prednisone group [44].

Response to prednisone treatment was demonstrated by the following reduction in serum IgE levels and the clearing of radiographic infiltrates. Serum IgE levels should have a decrease of 25% or more [27] to achieve the response. A total serum IgE level decrease of 35% is considered a good therapeutic response. Blood eosinophil counts also return to normal levels [44].

High doses of inhaled corticosteroids should not replace systemic corticosteroids [27, 46].

Intravenous pulse steroid therapy in ABPA has been used in patients who have adverse effects with daily corticosteroids or do not respond to standard doses of oral steroid therapy. In several reports, pulse methylprednisolone was successfully used in oral steroid-dependent patients with CF and ABPA (10-20 mg/kg/day for 3 consecutive days every month) [47, 48]. In an 11-year-old child with CF who was unresponsive to oral steroids, the use of intravenous pulse methylprednisolone made an improvement in clinical stabilization and better control of ABPA (20 mg/kg for 3 days followed by 10 mg/kg for 3 days) [49]. In most of the studies, intravenous pulse steroid therapy was well tolerated and patients were able to stop the pulse therapy after 6–12 months with disease control [47, 48].

The use of systemic glucocorticoids is limited by significant side effects including obesity, osteopenia, development of type-2 diabetes, insomnia and many other effects. In addition, long-term glucocorticoid consumption can cause downregulation of glucocorticoid receptors inducing a steroid-resistant state. Given the significant side effect profile of steroid treatment, antifungal strategies are considered in steroid dependency.

Antifungal Therapies

Reducing the fungal colonization in the airways will diminish the antigenic stimulus and therefore reduce inflammation, improving symptoms, and possibly slowing progression. Patients may therefore be advised to avoid high-risk environments, areas with decomposing matter and moldy indoor environments [3, 4, 41]. The strategies to reduce fungal burden should include treatment with antifungal agents. Oral triazole antifungal drugs are first-line therapy in the management of *Aspergillus*-related infection and allergy in chronic respiratory disease [50-54]. First-generation of triazole antifungals includes itraconazole and fluconazole and second-generation triazole antifungals include voriconazole, posaconazole, and isavuconazole. Oral corticosteroids are more effective than itraconazole alone [100 versus 88%] in the treatment of acute-stage ABPA [50]. Antifungal drugs are commonly employed in steroid-resistant cases or for reducing steroid dose and duration. Thus, antifungal drugs can act as steroid-sparing agents.

The most widely used azoles in the management of ABPA are itraconazole and voriconazole [50-54]. The initial dose of itraconazole should be 5 mg/kg/day, which may be given once or twice daily (maximum 200 mg/dose) during 16 weeks (3-6 months) because of the emerging risk of azole-resistant *Aspergillus* species. Itraconazole should be added to therapy if there is a slow or poor response to corticosteroids, for relapse of ABPA, in corticosteroid toxicity, and corticosteroid-dependent ABPA [50-54].

Posaconazole and voriconazole induced clinical response in 78% and 70% of patients, respectively, in a study of 25 patients with previous itraconazole treatment failure [53]. In 26% of patients treated with voriconazole, treatment cessation was required due to adverse events, while no significant adverse effects were observed with posaconazole. In the CF population, posaconazole may be more effective than other triazole drugs in the treatment of ABPA [54].

Novel inhaled azole compounds are being investigated in clinical trials with the aim to reduce the systemic side effect profile often related to azole therapy [55, 56].

Other antifungals like the echinocandin group [e.g., caspofungin, micafungin, anidulafungin, rezafungin, ibrexafungerp], the polyenes [e.g., amphotericin] or other novel drugs [olorofim or fosmanogepix] could be used in ABPA, because they have been employed

or investigated in other *Aspergillus*-induced diseases, where azole resistance is a problem [57-63]. Amphotericin B in nebulized form has been administered in ABPA, but nebulized in sodium deoxycholate formulation can produce bronchospasm [59]. The lipid formulations [e.g., ambisome] may be better tolerated for its use, especially in pediatric patients with CF [60, 61].

Monoclonal Antibodies

There is raising evidence for the use of monoclonal antibodies in treating ABPA, because many patients do not respond to standard care. Biologic agents inhibit some of the fundamental pathways for the development of ABPA. To date, there has been a lack of randomized controlled trials to support the use of biologic agents, but there have been several case reports and case series [64].

Omalizumab is a humanized monoclonal IgG antibody against IgE. Omalizumab fixes free serum IgE and down regulates cell-surface high-affinity receptors for IgE (FcεR1) on basophils, mast cells and other T2-related cells. It is an attractive approach, but the levels of free circulating IgE often far exceed the binding capacity of omalizumab at its highest licensed dose. Furthermore, the strategy of treating ABPA with an anti-IgE monoclonal antibody does not address the unopposed effects of IL-5 and IL-13 produced by Th2 and ILC-2 cells. The omalizumab dose is dependent on the initial IgE level (0.016 mg/kg/IU) with an upper IgE limit of 1500 IU/mL and a maximum dose of 1200 mg monthly. Thirteen patients with chronic ABPA were randomized to a four-month treatment with omalizumab (750 mg monthly) or a placebo followed by a three-month washout period in a crossover design; and there was a significant reduction in FeNo and exacerbations in the treatment arm. A recent literature review included 161 patients treated with biologics agents [64]; 60% of the studies investigated omalizumab use, and the remaining studies were distributed among the rest of the biologics. With regard to omalizumab, 40% of patients had a significant reduction in IgE post treatment (>35%), 66% had a reduction in their steroid dose, and 95% had a reduction in exacerbation frequency.

Mepolizumab is a humanized monoclonal antibody to IL-5, which is a key mediator in eosinophil differentiation, activation, migration, and survival. Nine studies including 32 patients treated with mepolizumab were reviewed [64]; in these studies, 90% of patients were able to discontinue steroids, and the remaining 10% had a dose reduction to a dose between 2.5 mg and 5 mg of prednisone. A reduction in IgE of 66.5% from baseline was seen in the four patients for whom pre- and post-treatment IgE was reported. There were no adverse effects identified.

Reslizumab is another anti-IL-5 monoclonal antibody and benralizumab is a monoclonal antibody against the α unit of the IL-5 receptor. Both have been shown to reduce blood eosinophil levels in asthma patients with potential efficacy in ABPA [65]. Two case reports of patients treated with benralizumab were included in previous report [64]. Both cases showed a clinical improvement; however, the follow-up time was short, and not all variables were described.

Dupilumab is an IL4-Rα antibody that has been used in atopic dermatitis, severe asthma, and chronic rhinosinusitis; and it inhibits Th2 cytokine signaling via IL-4 and IL-13. In cases reported with dupilumab treatment [64], all patients had a reduction in total IgE levels, and 20 out of 21 patients reported an improvement in exacerbation frequency. This review [64]

highlighted that although most patients responded with a decrease in total IgE levels, there were cases where the IgE did not reach the 35% decrease; however, there was significant clinical improvement, suggesting that patient-centered outcomes are crucial for monitoring response. A phase-III randomized control trial of dupilumab in asthma patients with ABPA is currently underway [NCT04442269].

Tezepelumab is a monoclonal antibody against TSLP, which acts as an upstream mediator of the inflammatory response to common asthma precipitants including viruses, allergens, and other airborne irritants [66]. Given its actions on Th2-mediated immune responses and airway remodeling, tezepelumab could be a future therapeutic agent in ABPA.

Other potential therapeutics targeting IgE mediated Th2 inflammation includes designed ankyrin proteins (DARPins) which prevent IgE mediated activation of effector cells [67]. DARPins act to disrupt the formation of IgE/receptor complexes and break down formed complexes. Further prospective clinical trials are required to analyze the long-term effectiveness of omalizumab, other anti-IgE monoclonal antibodies [e.g., ligelizumab and quilizumab] and other biologics in ABPA.

Mucolytics

Treatments that reduce mucus viscosity could be beneficial inpatients with ABPA. Hypertonic saline in conjunction with nebulized salbutamol can be used to reduce sputum viscosity and promote mucus clearance. There are a number of other licensed mucolytic available including nebulized Dornase-alpha and N-acetylcysteine; however, no ABPA-specific clinical trials have been performed. Dornase-alpha is potentially of benefit in ABPA, given the significant contribution of filamentous chromatin-rich EETs to sputum viscosity.

Management of APBA

In acute ABPA, systemic corticosteroids are the first line of treatment. Antifungal agents are added if we observe a slow or poor response to corticosteroids, for relapse of ABPA, in corticosteroid toxicity, and corticosteroid-dependent ABPA. Additionally, it is also very important to identify and exclude any potential environmental exposure source of *A. fumigatu*s because it can trigger new exacerbations.

Antifungal therapy is commonly begun with itraconazole. Newer azoles are reserved for patients who fail therapy or experience adverse reactions with itraconazole. Some patients may need chronic corticosteroid treatment. We should monitor the therapy for acute ABPA with clinical evaluation, serum total IgE levels, spirometry, and chest radiography.

The levels of *Aspergillus*-specific IgE and IgG during the treatment are not correlated with the reduction in the serum total IgE or clinical or radiologic improvement.

Serum total IgE concentrations should be assessed every 6-8 weeks, especially in the first year. The main purpose of therapy is to decrease serum total IgE levels by 35–50% at 8 weeks, which is consistently accompanied with clinical and radiographic improvement. The lowest value of total IgE achieved after treatment is considered the new baseline. An increasing level over 100% of the new baseline of total IgE along with worsening respiratory symptoms and the

consistent radiologic findings suggest an exacerbation of ABPA. Many relapses [20-35%] are asymptomatic and are found radiographically and serologically. The treatment of the first exacerbation is similar to the treatment of acute disease.

If the patient becomes treatment dependent, alternative antifungals, pulsed methyl-prednisolone, nebulized amphotericin, or biologic agents could be considered.

Pulse steroids may be considered in steroid-dependent patients, which worse clinical, serological and/or radiological findings on tapering steroids/azoles. If they are taking itraconazole, newer azoles may be considered. Biologic agents have shown promise in such cases. Targeted immune treatments against aspects of the aberrant T2 response have been shown to be effective and novel antifungal agents may be better tolerated and should be considered in patients with hard-to-treat disease or recurrent exacerbations. However, despite the potential role of novel biologic and antifungal therapies there is a critical lack of large-scale randomized control trials in ABPA. The current evidence for novel biologic and antifungal therapies is limited to case series and subgroups of larger trials, limiting the therapeutic options for patients. An understanding of disease heterogeneity in ABPA and endotypes will also be critical to ensure therapeutic stratification and success of future clinical trials.

Remission may be considered if the patient has remained asymptomatic with stable IgE levels (persisting at/below baseline or increase by <50%) for at least 6 months without the requirement of corticosteroid or antifungal therapy. In the remission period, monitoring may be performed every 3 months for a year and every 6 months thereafter with a clinical examination and serum total IgE levels. Chest radiography may be obtained if clinically indicated. Spirometry is performed in routine follow-ups and in response to changes in symptoms. Antifungal therapy is not used to prevent exacerbations given the potential toxicity and lack of proven benefit. Another considerable point of management is that chronic respiratory tract infections are almost inevitable in ABPA patients with CF. These patients are especially vulnerable to *Pseudomonas aeruginosa* or nontuberculous mycobacteria because of the combined effects of structural deformities in the airways and compromised immunity caused by systemic and local administration of corticosteroids. In that case, monoclonal antibodies such as omalizumab may be a good choice since it can prevent the use or reduce the doses of systemic corticosteroids. In summary, clinical improvement is generally achieved with proper diagnosis, follow-up, and treatment.

Conclusion

Our knowledge of the pathogenesis of ABPA is still progressing. This knowledge can drive us to new personalized therapy. The goal of our treatment should be to offer maximum benefit to each patient with the least occurrence of adverse reactions and toxicity. Some patients may require several different therapeutic protocols before symptoms are maintained under reliable control. Improvement in symptoms may offer an opportunity to stop certain drugs associated with adverse events. With proper treatment, ABPA is a controllable, albeit chronic, illness. Response to treatment in ABPA needs to be evaluated in multiple spheres over time, including clinical, immunologic, physiologic, and structural evaluations and measures. While data on long-term prognosis is quite limited, treatment is effective in maintaining lung function and

overall health, but late diagnosis and/or untreated ABPA leads to progressive and potentially fatal pulmonary fibrosis.

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Chapter 14

Aspirin-Exacerbated Respiratory Disease

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Abstract

Aspirin/acetyl salicylic acid (ASA)-exacerbated respiratory disease (AERD) is characterized by the combination of chronic rhinosinusitis, nasal polyps, bronchial asthma, and hypersensitivity reactions involving upper and/or lower airways after the exposure to ASA and other nonsteroidal antiinflammatory drugs (NSAIDs). It is a sub-endotype of T2 asthma in which dysregulation of arachidonic acid metabolism with an overproduction of cysteinyl-leukotrienes occurs after NSAIDs intake, although the underlying mechanisms are not fully understood.

The confirmatory diagnosis is crucial for an adequate management, being ASA challenge the gold standard. The management of these patients is complex and should be multidisciplinary. It encompasses avoidance of ASA and other NSAIDs as well as treatment of asthma and rhinosinusitis including pharmacological and non-pharmacological measures according to the currently guidelines. ASA desensitization followed by daily ASA therapy has shown to be useful when standard medical treatments are not effective.

Keywords: aspirin, asthma, challenge, cysteinyl-leukotrienes, eosinophil, rhinosinutis

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Introduction

Aspirin-exacerbated respiratory disease (AERD) is a clinical syndrome characterized by the triad asthma, chronic rhinosinusitis (CRS) with recurrent nasal polyps (NPs) (CRSwNPs), and aspirin/acetyl salicylic acid (ASA) and other nonsteroidal antiinflammatory drugs (NSAIDs)-induced hypersensitivity reactions manifested as nasal and/or bronchial symptoms [1-3]. It is considered a specific phenotype of NSAID hypersensitivity and a difficult-to-treat asthma phenotype [1]. The underlying mechanisms remain incompletely explained. It has been proposed an overproduction of cysteinyl-leukotrienes (cys-LT) with dysregulation of arachidonic acid metabolism and increased type 2 chronic eosinophilic inflammation in the upper and lower airways [4-7].

The prevalence of AERD is unknown and figures reported depend on the population studied and the diagnostic criteria used. Therefore, in the general population, prevalence has been reported to range from 0.3% to 12.4% [8, 9]; in adults asthmatics from 5.5% to 21%, with a mean prevalence of 7.1%; in severe asthmatics of 15% [8, 9], 24% in asthmatic patients admitted to the intensive care unit [10]; and up to 30-40% among asthmatics patients with CRS [9].

Classically, it is diagnosed when the triad of CRwNPs, ASA/NSAIDs hypersensitivity, and asthma is identified. However, the confirmatory diagnosis is achieved by ASA challenge and supported by potential biomarkers [1]. In addition, radiological imaging as well as other diagnostic methods, such as rhinoscopy and nasal endoscopy can aid in AERD diagnosis [1, 7].

The management of these patients is complex and should be multidisciplinary. It comprises the treatment of the underlying asthma and CRS, and the avoidance of ASA and others NSAIDs in order to prevent exacerbations. Management of asthma and NPs must include a guideline-based medical and surgical approach. ASA desensitization followed by daily ASA therapy may be considered when standard medical treatments are not effective.

Clinical Features

The typical patient is a female in her third or fourth decade of life and non-atopic. Most patients report upper airway symptoms that evolve into CRS with/without NPs, being usually refractory to conventional treatment [1, 3, 11]. Partial loss of smell or even anosmia occurs more frequently in AERD patients than in CRS due to other causes, being loss of smell considered a clinical marker to identify AERD patients [1].

One to five years later, patients show symptoms of asthma. Most of AERD patients experience a severe course of asthma. In fact, the prevalence of severe asthma among AERD (15%) is twice the general asthma population. The risk of severe asthma and asthma attacks in AERD increases by 60%, emergency room visits by 80%, and asthma hospitalization by 40% [12].

Subsequently, rhinitis and asthmatic symptoms are followed by ASA and other NSAIDs hypersensitivity reactions, although they can occur at any time in the evolution of the disease, and even NSAID hypersensitivity may be the precipitator of the first asthma exacerbation [1, 3, 11]. Clinical reaction to ASA or other NSAIDs is manifested within 30-180 min with upper

and/or lower airway symptoms. In patients with unstable asthma, the symptoms may appear faster, and rapidly progress to severe bronchospasm or even lead to death [13]. The onset and the severity of the reactions are dose-dependent. Dose provoking a reaction has reported to vary between 10 and 300 mg of ASA, although most patients experience symptoms after the intake of a dose of 60 mg of ASA [14]. It is of note that despite avoidance of NSAIDs, patients continue to suffer from chronic airway symptoms [3].

Nasal and bronchial symptoms after consuming alcoholic beverages have been reported among AERD patients [15].

In children, AERD appears rarely, being diagnosed in up to 5% of asthmatic children [16]. Unlike adults, asthma usually develops before CRS and severity varies from mild-moderate to severe-persistent asthma [16].

Pathogenesis

AERD is a sub-endotype of T2 non-allergic asthma [4]. This entity is characterized by a more severe clinical presentation compared to other T2 asthma endotypes [4]. Phenotypically AERD is defined by a very high mucosal and peripheral eosinophilia [17]. In this regard, eosinophil accumulation in the airway wall is driven by the high systemic availability of IL-5 [17]. In T2 non-allergic asthma, microbial antigens or pollution stimulate the airway epithelium to produce high levels of IL-25 and IL-33, which in turn activate group 2 innate lymphoid cells (ILC2) [6]. These cells reside in the airway mucosa during homeostasis, but they are also increasingly recruited from the blood stream during T2 inflammatory conditions [6]. This recruitment is possible because ILC2 constitutively express CCR3 [6]. Once ILC2 have been activated, epithelial-derived thymic stromal lymphopoietin (TSLP) contributes to their survival and resistance to the antiinflammatory effect of inhaled corticosteroids (ICS) [18].

During AERD, ILC2 activation can occur through an alternative mechanism. Bacterial antigens can pass through an impaired airway epithelium and reach the lamina propria where they encounter complement components [17]. The bacterial wall triggers complement activation which results in the release of anaphylotoxins (C3a and C5a) [17]. Of note, resident mast cells express anaphylotoxin receptors and react to these mediators by secreting prostaglandin (PG) D2 (PGD2) [6]. ILC2 constitutively express PGD2 receptor (usually termed PD2 or CRTh2), thus being activated by this lipid mediator [18]. Either way, ILC2 stimulation results in the release of high amounts of IL-5 and IL-13. IL-5 activates the airway endothelium to recruit circulating eosinophils [19].

As opposed to mast cells and ILC2, eosinophils do not reside in the airways during homeostasis. Eosinophils accumulate in the lamina propria and can also pass through the epithelium to be found in the airway lumen [20]. Moreover, IL-5 directly activates mucosal eosinophils through IL-5 receptor, thus mediating the release of preformed proteases, galectin-10, and eosinophil DNA extracellular traps (EET) [21]. Eosinophils also produce cytokines and chemokines with key regulatory functions in extra-pulmonary organs (adipose tissue, mammary gland, etc.) [20], but these functions are not relevant in the specific case of AERD [5, 21].

Pre-formed proteases contained in eosinophil granules exert a prominent role in driving tissue remodeling, especially sub-epithelial fibrosis, thickening of the basement membrane of the epithelium, and impairment of the epithelial barrier function [5]. On the other hand, EET contribution to airway remodeling occurs via the induction of goblet cell metaplasia (transformation of ciliated cells into goblet cells) and direct stimulation of mucus secretion [22]. EET also collaborates with the recruitment and activation of monocytes to the airway lumen [19]. To exert these roles, EET interact with pulmonary neuroendocrine cells through CCDC25 receptor [23]. The binding of EET to CCDC25 requires the concurrence of eosinophil peroxidase to be stable enough to induce intracellular signaling [22]. In this regard, EET are naturally decorated with nuclear, cytoplasmatic and granular proteins including eosinophil peroxidase [23]. CCDC25 signaling induces the release of neuropeptides and neurotransmitters (e.g., GABA) by pulmonary neuroendocrine cells, which in turn mediate airway remodeling and T2 inflammation [24, 25].

Galectin-10 is the most abundant protein in eosinophil cytoplasm and is among the few subsisting molecules with the capacity to crystalize *in vivo* at physiologic pH conditions, thus forming Charcot-Leyden crystals (CLC) [26]. CLC have been long regarded as mere bystanders or inert markers of T2 inflammation [24]. Nevertheless, the evolutionary preservation of the crystallization property probably indicates a more relevant immunomodulatory role for them [25]. In this regard, the mechanical damage induced by CLC on resident myeloid dendritic cells is required for inflammasome activation, which is in turn needed for efficient dendritic cell maturation and priming of adaptive T cell responses [27]. In any case, adaptive immunity does not play a prominent role on AERD, therefore the deleterious impact of CLC in this condition is more probably related to the increase of mucus viscosity [27].

Importantly, EET also contribute significantly to the enhanced viscosity of airway secretions [22]. The airway epithelium expresses functional receptors for IL-5 and IL-13 (type II receptor) [28]. Of note, ILC2 release vast amounts of these two cytokines [26]. IL-5 and IL-13 signaling induces the loss of tight junctions and the apoptosis of ciliated cells, whereas IL-13-stimulation of goblet cells also mediates mucus secretion [29]. Thus, AERD patients have both more abundant and denser respiratory secretions [27]. The infiltration of airway epithelium by mast cells is also present in AERD individuals [24].

During homeostasis, mast cells reside within the lamina propria of the airway mucosa and express a relatively antiinflammatory pattern of granular proteases (especially tryptase and chymase) [5]. Conversely, in AERD individuals, mast cells migrate to the epithelium where they release IL-5 [19]. This cytokine generates an epithelial gradient driving the recruitment and accumulation of intra-epithelial eosinophils [25]. These cells are also activated by IL-5 with the subsequent release of cys-LT [22]. These lipid mediators induce inflammatory changes in intraepithelial mast cells including a switch in their granular protease profile (increased level carboxypeptidase), and the release of IL-13 [27].

Intraepithelial mast cells also communicate with ciliated cells to induce the synthesis of IL-33, thus boosting T2 inflammation [30]. Of note, there is a strong correlation between intraepithelial IL-5 and indirect bronchial hyper-reactivity (as measured by mannitol challenge) in AERD patients [31]. Figure 1 shows a summary of the inflammatory mechanisms observed in eosinophilic non-allergic asthma.

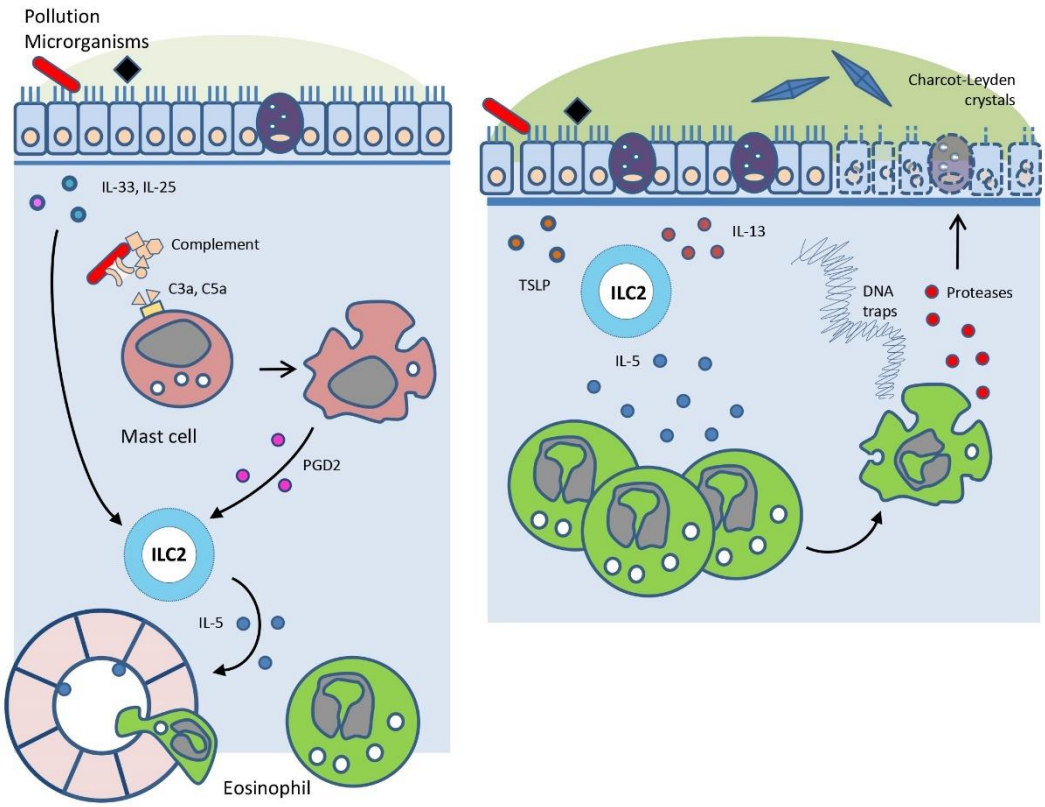

Figure 1. Eosinophilic inflammation in non-allergic asthma.

The pathological mechanisms described so far apply for the whole inflammatory disease (asthma and CRS) affecting the airways in AERD. Nevertheless, the inflammatory alterations driving CRS also involve the extravasation of macrophages and fibrinogen to the lamina propria of the naso-sinusal mucosa [19]. Under T2 conditions, macrophages produce factor XIIIA, which mediates the cross-linking of fibrin meshes from fibrinogen [32]. Fibrin meshes also increase the viscosity of mucosal secretions and provide the "scaffolds" or architecture permitting the polypoid degeneration of the ethmoidal mucosa in CRS with NPs [33].

Smell impairment (often complete smell loss or anosmia) is also a clinical hallmark of AERD [21]. These individuals display a decreased density of basal cells and immature neurons in the olfactory epithelium, together with the infiltration of these layers by mast cells and eosinophils [34]. The olfactory cleft in AERD patients is occupied by a dense mucus layer impairing volatile aromatic compounds to reach the distal edges of mature olfactory neurons [28]. The mucus is also rich in several inflammatory cytokines, especially IL-5 which shows a strong correlation with the impairment of smell function in AERD individuals [26].

Figure 2 shows a summary of the inflammatory mechanisms observed in eosinophilic CRSwNP.

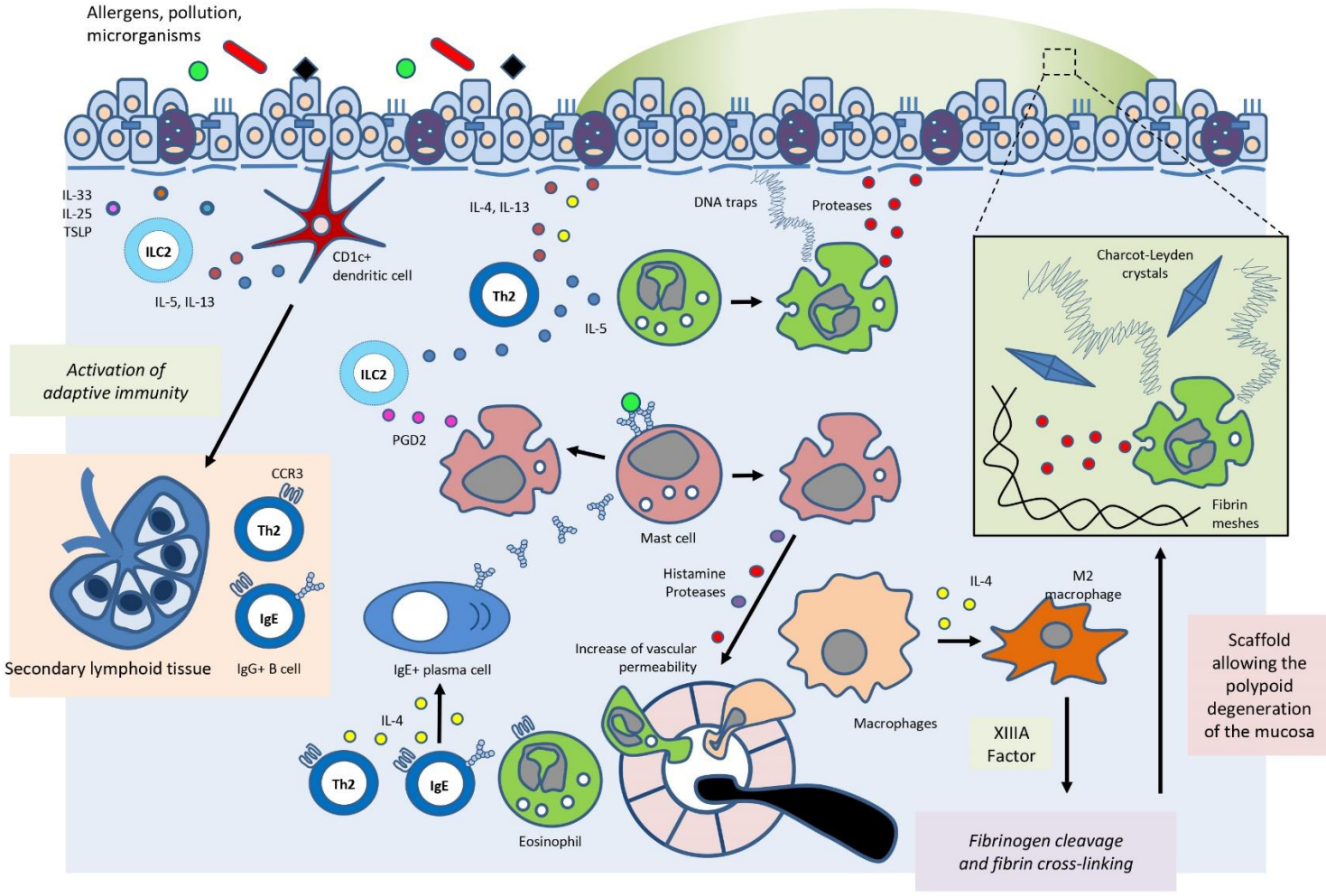

Figure 2. Eosinophilic inflammation in CRSwNPs.

The occurrence of an intense eosinophilic inflammation of the airways is a prerequisite for the development of the clinical phenomenon called NSAID hypersensitivity [5]. AERD patients experience respiratory symptoms (either naso-sinusal, bronchial, or both) after the intake of strong COX-1 blockers of any pharmacological group (mainly ASA), arylacetic acids, arylpropionic acids or pirazolones) [6]. Importantly, NSAID hypersensitivity in AERD is not mediated by the recognition of specific chemical structures (shared by all drugs) by the adaptive immune system, but by the metabolic effect of strong COX-1 blockers on eosinophils and other cells involved in T2 inflammation [35].

Eosinophils use their membrane phospholipids to obtain arachidonic acid (AA), which is metabolized through the COX and LOX pathways [30]. The former enzyme synthetizes mainly PG, whereas the later gives rise to cys-LT. The intake of a COX-1 inhibitor blocks the COX pathway and directs all the AA to the synthesis of cys-LT [27]. Eosinophils from healthy individuals or from patients with non-T2 inflammation or with non-severe T2 inflammation are able to adapt to these increased metabolic demands and quickly react by catabolizing the excess cys-LT with no associated clinical symptom [36]. Conversely, eosinophils from subjects with severe T2 inflammation display an extremely high baseline activation ("exhausted" status), thus having lost the capacity to adapt to additional metabolic demands [26].

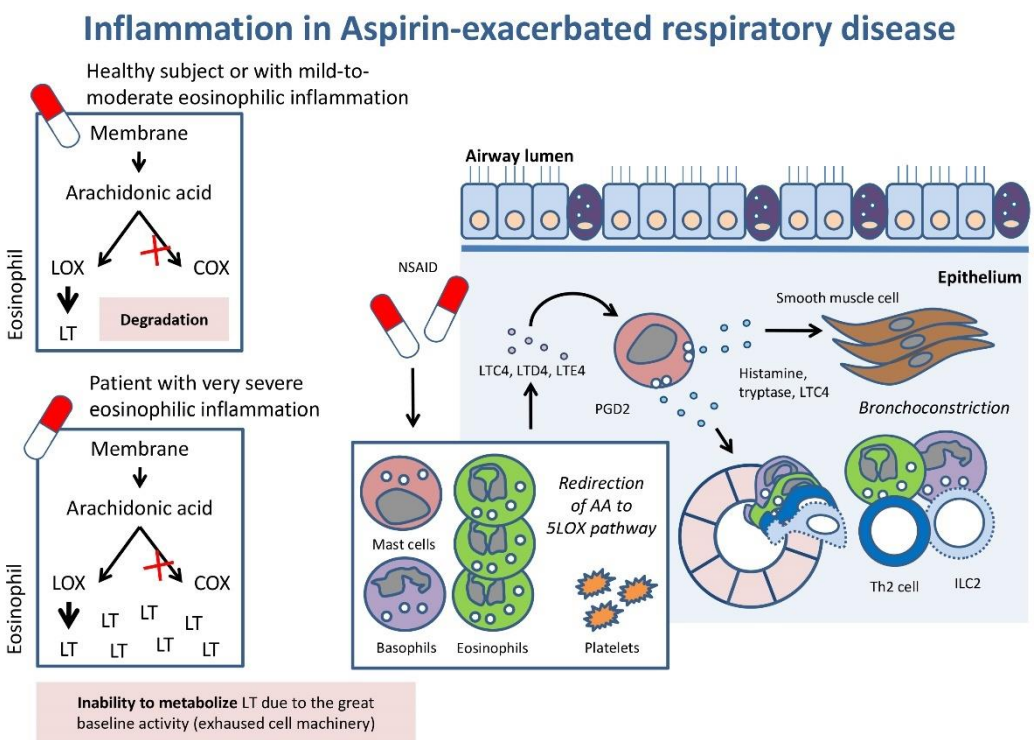

Figure 3. Inflammatory mechanisms in AERD.

Therefore, excess cys-LT cannot be catabolized and accumulates in the respiratory mucosa inducing bronchoconstriction in the lower airways and obstruction/congestion in the upper airways [37]. Other cells (basophils or platelets) can also participate in the exhausted phenotype explaining AERD mechanism [28]. Of note, AERD onset often follows a respiratory viral

infection, which constitutes the prototypical scenario for increased metabolic demands [38]. In summary, AERD, defined as the clinical reactivity to strong COX-1 blockers, is a phenomenon occurring in the context of preexisting severe eosinophilic inflammation of the airways.

Figure 3 shows a summary of the inflammatory mechanisms involved in NSAID hypersensitivity in patients with pre-existing eosinophilic asthma and/or CRSwNP.

Biomarkers

Quantification of Peripheral and Local Eosinophils

Besides the intense airway eosinophilia, AERD patients also display elevated blood eosinophils [18]. Nevertheless, their cutoff to identify T2 asthma is not clearly defined. 300-400 cells/μL is usually proposed to define eosinophilic asthma, or to prescribe biologicals targeting the IL-5 or IL-4/IL-13 pathways [39]. In patients with maintained oral corticosteroid intake 150 cells/μL is usually accepted as cutoff for the T2 endotype [40]. Of note, blood eosinophils are greatly sensitive to oral corticosteroids and high-dose of inhaled corticosteroids [39]. Because asthma phenotyping is commonly performed in severe patients under maintenance therapy with these medications, the interpretation of blood eosinophilia poses many challenges in the clinical practice [6]. One solution to overcome this limitation is the investigation of the historical level of blood eosinophils [17]. Nevertheless, it is not clear how long back is acceptable to consider the value of peripheral eosinophils to take clinical decisions in the present time [40]. In this regard, blood eosinophils experience significant variation over time, which is not necessarily connected to asthma control and severity [39].

Because CRS and asthma are airway conditions, the investigation of mucosal eosinophils has been long regarded as the gold standard for the identification of T2 phenotypes [41]. In this sense, tissue specimens are often obtained during endoscopic sinus surgery and they can be easily subjected to the analysis of infiltrating granulocytes. On the other hand, the collection of a transbronchial biopsy or of a bronchoalveolar lavage sample is less often performed [39]. Another alternative to assess airway eosinophilia in asthma is the analysis of induced sputum [42]. As this technique is easier to implement in the clinic, it is considered the gold standard by many research studies [40]. The suggested cutoff point to identify T2 asthma by sputum eosinophilia is 2-3% [43]. Of note, there is a significant correlation between blood and sputum eosinophilia, in spite of the correlation being far from perfect. Interestingly, considering 3% of sputum eosinophils as the gold standard for T2 asthma, a 300-cells/μL cutoff is associated with a 40-50% rate of both false negative and false positive results [40, 41, 43].

Other T2 Biomarkers

The biomarkers used in the clinic to identify T2 asthmatics are often also elevated in AERD individuals, although they do not exactly mark eosinophil infiltration of the airways [44].

In this regard, elevated total IgE in serum (≥100 IU/mL has been proposed as cutoff point) confirms the activation of the adaptive immune system in T2 asthma [45]. This biomarker has

little diagnostic or prognostic value, although it is required to decide the dose of omalizumab in severe allergic asthma [44].

Similarly, the value of fractional exhaled nitric oxide (NO, FeNO) shows a positive correlation with airway eosinophilia [45]. Because of this relationship, FeNO has been long regarded as a marker of eosinophilic airway inflammation [46]. Nevertheless, the production of NO by the airway mucosa is not directly related to eosinophil recruitment or activation [44]. Airway epithelial cells respond to IL-13, a key cytokine in T2 immune responses, by up-regulating NO synthase expression [46]. This enzyme mediates the conversion of L-arginine into NO and L-citrulline [47]. NO is a volatile metabolite with bronchodilatory effect which represents a mechanism to compensate inflammation in the airways [45]. It is important to note that NO measured in exhaled air originates from both the naso-sinusal and bronchial mucosae, with the quantification of the relative contribution of each compartment being currently impossible [48]. FeNO is useful to predict the response and monitor the adherence to ICS, as well as to predict the loss of control, the exacerbations and the accelerated decline of lung function in T2 asthmatics, including those with AERD [46]. On the other hand, the cutoff points for each of these FeNO utilities are not clearly established [47]. This biomarker shows also a great inter-individual variability and is greatly dependent on exposome components (e.g., tobacco smoke, alcohol, etc.) [44]. Fractional nasal NO (FnNO) has been also investigated as a potential biomarker of T2 inflammation in the upper airways [45]. Of note, the sinusal mucosa represents the main source of NO in the airways [44]. Nevertheless, AERD patients often display a recalcitrant form of CRSwNP. In these cases, the extent of nasal obstruction prevents the sinusal NO to reach the nostril and to be measured in the expiratory flow [47]. Thus, many AERD patients with severe CRSwNP show a paradoxically low value for FnNO [46].

Similarly to FeNO, serum periostin also marks the effect of IL-13 over the airway epithelium [48]. During homeostasis, periostin is stored in the cytoplasm of ciliated cells and fibroblasts, but after IL-13 stimulation this mediator is released locally [49]. Periostin contributes to several features of the airway remodeling connected to T2 inflammation including the disruption of the epithelial barrier, the subepithelial fibrosis, and the mucus hyper-secretion [50]. Periostin shows a good correlation with other T2 biomarkers and is useful to predict the response to ICS [48]. Nevertheless, its clinical utility is still under investigation and reliable cutoff points remain to be established [51].

In summary, despite the potential elevation of other T2 biomarkers, peripheral and local eosinophilia are regarded as the most specific biomarkers of the pathophysiological alterations leading to AERD.

Atopy and Allergy Tests

Because AERD is characterized by an intense eosinophilic inflammation of the airways, it is not uncommon that these patients become sensitized to aeroallergens [18]. In this regard, the high baseline activation status of the airway mucosa in AERD individuals makes them prone to mount IgE immune responses against many exponent components (e.g., aeroallergens or microbial antigens like *Staphylococcus aureus* enterotoxins) reaching the nasal or bronchial lumens [52]. Nevertheless, unlike allergic rhinitis and asthma, eosinophilic inflammation precedes atopy in AERD patients [17]. In most cases, atopy is not clinically relevant (patients are atopic but not allergic) or contributes only to a minor extent to the clinical expression of the

disease (e.g., seasonal conjunctivitis due to pollen allergy in the context of perennial nasal obstruction and anosmia) [53]. However, in some cases, exposure to aeroallergens can exacerbate nasal and/or bronchial symptoms in a significant manner (the patient is atopic and allergic), and the patient might improve with adequate avoidance measures [54]. In this regard, skin prick test and the quantification of serum allergen-specific IgE can help identify IgE-sensitizations in AERD subjects, whereas the nasal and/or bronchial allergen challenges (NAC and BAC, respectively) can help discriminate asymptomatic and symptomatic sensitizations [53, 54]. Because many AERD patients will not tolerate a BAC, the NAC is a good alternative to investigate the clinical relevance of IgE-sensitizations in these individuals [52]. This utility of the NAC derives directly from the united airway concept [54]. On the other hand, the NAC is difficult to interpret in individuals with severe CRSwNP [53].

Specific Biomarkers for AERD

The oral provocation with ASA and the nasal and bronchial provocations with lysine aspirin (L-ASA) are the most accurate biomarkers to diagnose AERD [55]. Indeed, these tests are able to identify precisely patients with NSAID hypersensitivity among the heterogeneous population of individuals with eosinophilic CRSwNP and/or asthma [55]. Their utilities and differently features will be extensively reviewed in the "Diagnosis" section of this chapter.

Figure 4 shows a summary of the biomarkers available in the clinic to phenotype patients with asthma and CRSwNPs.

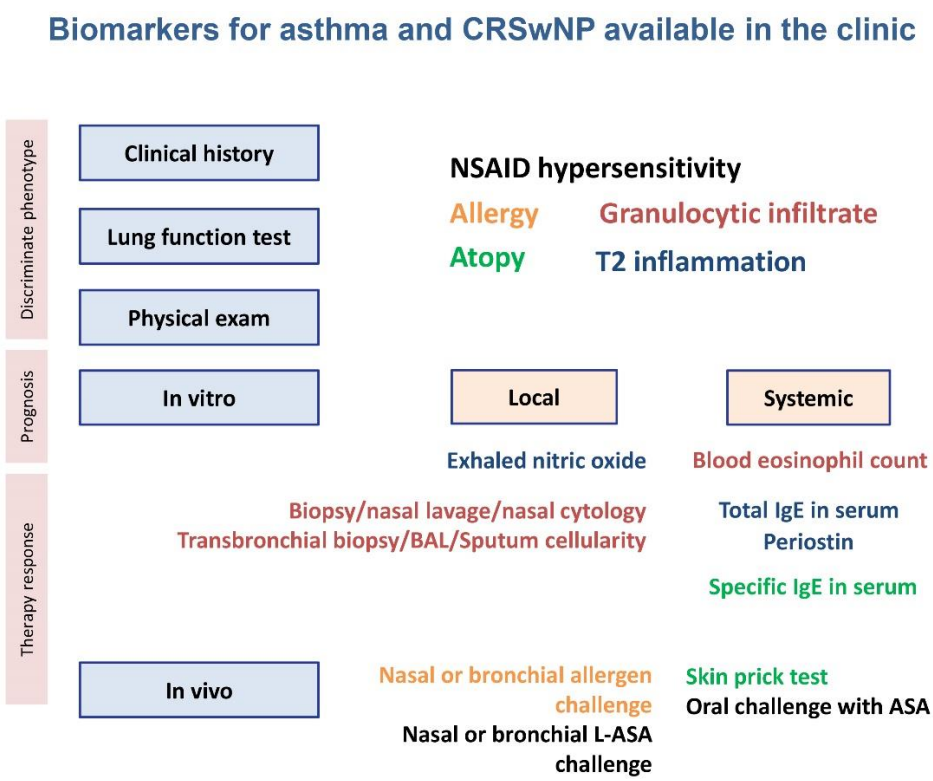

Figure 4. Biomarkers available in the clinic to phenotype patients with asthma and CRSwNPs.

Diagnostic Approach

The triad of CRwNPs, ASA/NSAIDs hypersensitivity, and asthma allows identifying patients by clinical history. However, the confirmatory diagnosis is achieved by ASA challenge [1, 56]. In addition, radiological imaging as well as other diagnostic methods, such as rhinoscopy and nasal endoscopy can aid in AERD diagnosis [1, 7].

Clinical History

A diagnosis of AERD is fundamentally based on the clinical history, being suspected in patients with adult-onset asthma and CRSwNPs whose symptoms exacerbate after ingestion of ASA and/or other NSAIDs [1]. However, clinical history is often not reliable. Many patients have not experienced hypersensitivity reactions to ASA/NSAIDs and lack of history of respiratory reactions in a patient with asthma and CRSwNPs does not exclude the presence of hypersensitivity [57]. Therefore, ASA challenge is needed to avoid both under- and overdiagnosis being the gold standard for diagnosing AERD [1].

ASA Challenge

Challenge tests involve the administration of doses of ASA in fixed-time intervals and the evaluation of the response that may include objective methods such as respiratory function tests (FEV1), peak nasal inspiratory flow (PNIF), rhinomanometry or acoustic rhinometry, and subjective methods, as visual analogue scale and symptoms scale [1, 56]. There are 3 types of the ASA challenge test depending on the route of ASA administration: oral, bronchial, and nasal (Table 1).

Table 1. Indications, contraindications, advantages, and limitations of oral, bronchial and nasal challenge with ASA in AERD diagnosis approach

	Oral challenge	**Bronchial challenge**	**Nasal challenge**
Indications	-Confirmation/exclusion of hypersensitivity to NSAIDs in patients with ambiguous history -Verification of negative results of bronchial or intranasal tests -Assessment of provocation dose of aspirin before oral desensitization -Research purposes	-Confirmation of AERD suspicion especially when lower airways are involved in the reported reactions induced by NSAIDs -Research purposes	-Confirmation of AERD suspicion with involvement of the upper airways in the reported reactions induced by NSAIDs, especially when oral and bronchial challenges are contraindicated -Research purposes

Table 1. (Continued)

	Oral challenge	**Bronchial challenge**	**Nasal challenge**
Contraindications	-Uncontrolled asthma, cardiac, immunologic, oncologic, or other important systemic - FEV1 <70% of the predicted value, -History of chronic renal failure or gastrointestinal bleeding -Respiratory tract infection or exacerbation of asthma (wait 4 weeks) -Pregnancy -Patients who cannot discontinue temporarily the intake of non-selective β blockers -Patients who cannot perform reproducible spirometry maneuvers		-Pathology of the nasal cavity which interferes with nasal challenge -Upper respiratory tract infection (wait 4 weeks) -Surgery of the nose or paranasal sinuses (wait 6-8 weeks) -Uncontrolled severe asthma -Pregnancy
Advantages	-Gold standard	-Higher sensitivity (77-90%) than nasal challenge -Faster than oral challenge (4-5 hours) -Safer than oral (symptoms are milder and appear earlier)	-Safer than oral and bronchial challenge -Faster than oral and bronchial challenge (2 hours)
Limitations	-Risky -Time consuming (2-3 days) -Resources consume	-Need for controlled asthma	-Nasal hyper-reactivity -Need for a relatively preserved nasal anatomy -Lower sensitivity than oral and bronchial challenge (80-87%)

The *oral challenge* test is considered to be the gold standard for diagnosing hypersensitivity to NSAIDs, as it mimics natural exposure to the drug [1, 56]. Increasing doses of ASA are given, usually in a single-blind manner, for confirming or ruling out a diagnosis of hypersensitivity to the culprit drug and cross-reactivity between NSAIDs. Challenge administering other NSAIDs than ASA is also used for providing a safe alternative therapy in cases of confirmed as hypersensitivity of AERD [1, 56]. It is a costly and time-consuming procedure, and not risk-free as it can provoke severe systemic reactions. Therefore, patients should be monitored continuously and the test should be performed in a specialized clinical setting with resuscitative equipment under the supervision of experienced physicians [1, 56].

Bronchial challenge consists of continuous inhalation at incremental volumes of L-ASA. It is as sensitive as oral but safer and quicker to perform. Similarly to oral challenge, it is a risky technique and patients with lower FEV1 (<70% of the predicted value) or unstable asthma status are not recommended to undergo this procedure [1].

Nasal challenge involves the control exposure to L-ASA or ketorolac in order to reproduce a response in the nasal mucosa. It is recommended for patients with predominant nasal symptoms, is safer and quicker, but the sensitivity is lower than oral and bronchial challenge [1, 56]. Nasal challenge can be used initially to diagnose the most sensitive subjects and may be a diagnostic alternative for patients in whom oral or inhaled challenge is contraindicated [1].

Management

The management of AERD should be individualized and include avoidance of ASA and other NSAIDs as well as treatment of asthma and CRS, which includes pharmacological and non-pharmacological measures following the currently established guidelines [58, 59].

Avoidance of NSAIDs

Reactivity in AERD patients depends on the potency of COX-1 inhibition of the NSAIDs. Therefore, AERD patients should avoid not only the agent responsible for the symptoms but also cross-reacting NSAIDs. Highly selective COX-2 inhibitors (etoricoxib and celecoxib), weak COX-1 inhibitors (paracetamol), and preferential COX-2 inhibitors (meloxicam or nimesulide) are generally well tolerated and can be provided as safe alternatives after proving tolerance by oral challenge [60]. Although avoidance of COX-1 inhibitors prevents exacerbations, airway inflammation will progress and most patients with AERD will experience worsening asthma and sinus disease [3].

Pharmacological Treatment

Pharmacological management of asthma and CRS in AERD patients should follow general guidelines focusing on underlying mucosal eosinophilic inflammation of the respiratory tract.

Corticosteroid Treatment

It constitutes the first line of pharmacological treatment. Inhaled corticosteroid in combination or not with long acting β2-agonists is sufficient as initial treatment as control asthmatic inflammation for most AERD patients [61]. Intranasal corticosteroid has shown to reduce the eosinophilic mucosal nasal inflammations, being highly effective decreasing the size of NPs [62, 63] and reducing polyp regrowth after nasal polypectomy [64]. Nasal drops have shown to be more effective than sprays because of their distribution within the sinus cavities [65]. Systemic corticosteroids can be additionally required to control bronchial inflammation and severe CRS symptoms and to improve quality of life in certain patients [61]. Side effects must be considered in long-term high dose treatment [66, 67]. Adding antihistamines or oral/nasal decongestants may provide symptom relief [58, 59].

Leukotriene-Modifying Drugs (LTMDs)

AERD is strongly associated with overproduction of cys-LT (4-7). Therefore, cys-LT1 receptor antagonists (montelukast, zafirlukast and pranlukast) and 5-LO inhibitors (zileuton) should be considered. Several studies have shown that these LTMDs improve asthma symptoms, respiratory function, quality of life, and help reduce the use of rescue bronchodilators [58, 59]. However, montelukast has shown not to be more effective in the treatment of AERD compared to NSAIDs-tolerant patients [68] and the long-term benefit in asthma and NPs is not completely clear.

Antibiotics

Antibiotic treatment should be used in cases with underlying infections [69]. There are controversies about whether long-term macrolide treatment has antiinflammatory effects as it blocks the production of proinflammatory cytokines and the migration and adhesion of neutrophils [70]. Doxycycline has shown to improve nasal symptoms as it decreases size of NPs and mucosal and systemic markers of inflammation, such as myeloperoxidase, ECP, and matrix metalloproteinase in nasal secretions [71].

Aspirin Therapy after Desensitization

ASA given after desensitization can be beneficial for AERD patients when standard medical treatments are not effective, such as uncontrolled asthma, recalcitrant NPs, recurrent purulent sinusitis, need for repeated courses of systemic corticosteroids and/or repeat sinus surgeries [1, 72].

ASA desensitization is a procedure by gradually increasing doses of ASA given orally or intranasally at 90-120 minutes intervals over a period of 1 to 3 days [1, 73]. It is a risky procedure that should be carried out in a well-equipped hospital under the supervision of experienced physicians [1].

In the majority of AERD patients, ASA therapy after desensitization has shown to be effective and to improve nasal symptoms, with a decrease in the doses of topical and oral corticosteroids and a reduction in NPs size, the recurrence of NPs, and in the need for sinus surgery [74, 75]. However, the overall effect on lower airways seems to be less important than in CRS, although it has been reported an improvement in asthma symptoms, pulmonary function and a decrease in the number of emergency room visits, hospitalizations and in the maintenance doses of oral and inhaled corticosteroids [76-78].

The mechanism explaining the improvement on ASA therapy is not completely understood. It has been proposed that daily ASA treatment reduces interleukin-4-caused expression of cys-LT and induces downregulation of the cys-LT1 receptor by inhibiting the transcription factor, signal transducer, and activator of transcription 6 (STAT-6) [79].

The benefit of ASA has been associated with high doses such as 325 mg twice daily up to 650 mg twice daily. Although a recent study has shown the long-term safety of ASA therapy in patients who underwent continuous daily treatment for more than 10 years [80], ASA therapy is associated with adverse effects (mostly gastrointestinal) in up to 34% of subjects receiving this treatment. In order to reduce the prevalence of adverse effects associated with ASA treatment, appropriate preventive measures such as the use of proton pump inhibitors and H2blockers during the treatment has been proposed [1].

Prolonged repeated intranasal application of L-ASA has also been shown to be effective in reducing the recurrence rates for NPs [81] as well as to improve nasal peak flow, olfaction, and nasal nitric oxide levels in AERD patients. Moreover, in a subgroup of patients it showed to improve asthma outcomes including a decrease in emergency visits, hospitalization, and oral steroid use [82].

It is of note that there are no data indicating that ASA is beneficial in the treatment of patients with asthma and NPs but without NSAIDs intolerance.

Biological Agents

To date, no biological has been specifically approved for AERD treatment. Nevertheless, due to the intense eosinophilic inflammation associated with the disease, most AERD patients fulfill criteria one or more of the 5 monoclonal antibodies (mAb) approved for severe T2 asthma [83].

Omalizumab (anti-IgE mAb) is indicated in severe allergic asthma due to perennial allergens (84). Nevertheless, in the clinical practice, omalizumab is often prescribed on the basis of atopic sensitization (83). Therefore, many AERD patients have received this drug regardless of the presence of allergy [83]. Surprisingly, omalizumab induced a significant benefit in many AERD individuals (84). Further studies demonstrated the effectiveness of omalizumab also in CRSwNP, or even in non-atopic asthmatics [84]. The mechanisms of action of omalizumab in non-allergic airway disease are poorly understood, but they could be related to the increase of the activation threshold of inflammatory cells [83].

Mepolizumab and *reslizumab* (anti-IL-5 mAbs), and *benralizumab* (anti-IL-5Rα mAb) target the IL-5 pathway and are indicated in severe eosinophilic asthma (85). Both clinical trials and real-life studies have demonstrated an excellent performance of these 3 mAbs in asthma associated with AERD [86]. Indeed, NSAID hypersensitivity, together with a high blood eosinophil count, are regarded as the most trustful response biomarkers for mAbs targeting the IL-5 pathway [55]. This fact is not surprising since NSAID hypersensitivity is just a phenomenon occurring in the most severe cases of eosinophilic airway inflammation [83]. Therefore, anti-IL-5 mAbs should be considered the option of choice in AERD individuals not responding to standard inhaled or intranasal therapy [85]. Importantly, mepolizumab is the only mAb of this group which is also indicated in eosinophilic CRSwNP, yet the other 2 drugs might have a beneficial effect in the naso-sinusal inflammation as well [86].

Dupilumab is an anti-IL-4/IL-13 mAb (it blocks IL-4Rα chain) which is approved for severe T2 asthma and for CRSwNP [83]. Dupilumab can be administered to patients with high blood eosinophil count or FeNO [87]. Real-life studies report a good performance of this drug for both conditions, including AERD patients [88]. Nevertheless, the mechanism of action seems less specific for AERD as compared to mAbs targeting the IL-5 pathway [86]. Moreover, dupilumab raises more safety concerns than other mAbs approved for severe asthma, since peripheral hyper-eosinophilias and parenchymal eosinophilic pneumonias have been reported in the context of dupilumab treatments [83, 88].

Surgery

It is important that the allergist and sinus surgeon collaborate on the appropriate treatment approach. Sinonasal surgery (polypectomy, functional endoscopic sinus surgery, and/or ethmoidectomy) is indicated in patients with severe or uncontrolled symptoms despite pharmacological treatment. It has been reported that endoscopic sinus surgery improves nasal symptoms, quality of life, nasal endoscopy, and computerized tomography scan scores [1]. It may also reduce bronchial symptoms and the requirement for asthma medications.

AERD patients do not experience such a good response to surgical interventions and are more likely to undergo repeated interventions compared to NSAIDs-tolerant subjects [1]. ASA therapy has shown to be effective in preventing NP progression rather than causing polyp regression, and therefore it is recommended 4-6 weeks after sinus surgery [76].

Conclusion

Patients with AERD present with a variety of clinical features although these patients tend to suffer from severe asthma and CRSwNP. They are affected by chronic type 2 eosinophilic inflammation with the overproduction of cys-LTs in the upper and lower airways. An improved understanding of underlying pathogenesis of AERD will aid in diagnostic evaluations and new therapeutic strategies for improving clinical outcomes. Due to phenotypic heterogeneity of AERD, efforts are focused on establishing precision medicine strategies tailored to individual phenotypes/endotypes with potential biomarkers.

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Chapter 15

Pharmacological Treatment of Asthma

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Abstract

Asthma is one of the most prevalent diseases in the world, therefore knowing and optimizing the available treatments is of great importance in medical practice. It is a syndrome that is notable for its variability, both in its clinical expression and underlying pathophysiology.

Asthma treatment has remained practically unchanged at the end of the 20th century and the beginning of the 21st century, however, there have been major changes in the last decade. The appearance of new chemically synthesized molecules, new inhaled combinations of these molecules, as well as the appearance of biological drugs for the most severe forms of asthma, make the treatment of asthma an exciting area that is worth studying adequately.

Keywords: asthma, ICS, severe asthma, LABA, LAMA

Introduction

Asthma is the most common chronic respiratory disease, affecting more than 300 million people worldwide [1]. Asthma is a syndrome that is notable for its variability, both in its clinical expression and underlying pathophysiology. The classification of asthma severity in the guidelines establishes levels based on symptoms, use of rescue therapy, lung function, and treatment needed to achieve adequate disease control. With this classification, similar treatment

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is proposed for all patients in each level, without taking into account the heterogeneity of individuals in each severity level [1, 2].

In the treatment of asthma, we differentiate between rescue treatment and maintenance treatment. In the acute episode, the objectives are to reverse the immediate airflow obstruction, and for this we will use short-acting inhaled beta-agonists (SABA) [3]. Maintenance therapy, on the other hand, encompasses a prolonged period with the aim of reducing inflammation, symptoms, number of exacerbations and improving lung function [4]. The cornerstone of asthma treatment over the past 50 years has been, and continues to be, inhaled glucocorticoids and, in some circumstances, oral glucocorticoids.

Until recent years, treatments have been universally applied to all patients with asthma, but the diversity of the disease means that responses to treatment differ. The identification of inflammatory endotypes has brought us closer to precision medicine, especially in severe asthma [5]. Corticosteroids are very effective drugs, but their nonspecific mechanism of action, based on the antiinflammatory effect, has not been shown to have a significant long-term impact on the course of the disease [6]. Treatment will depend on the severity of the asthma, as well as the phenotype, with inhaled corticosteroids (ICS) being the cornerstone of treatment in both adults and children, and other medications such as long acting β agonist (LABA), long acting muscaring anatagonists (LAMA), anti-leukotrienes, oral steroids or biologic drugs can be associated.

We will now explain the different pharmacological groups currently used in asthma, excluding biologic treatments, which occupy a chapter of their own in this work.

Maintenance Treatments

Inhaled Corticosteroids (ICS)

Inhaled corticosteroids play a fundamental role in the maintenance treatment of asthma, since they reduce airway inflammation, produce a reduction in bronchial hyperresponsiveness, as well as in the symptomatology, frequency, and severity of exacerbations, also improving pulmonary function and the quality of life of asthmatic patients [7].

They are the treatment of choice for persistent asthma regardless of its severity, and their dose can be gradually escalated according to the degree of severity, considering that the dose-response curve of ICS is relatively flat (Figure 1), so that very high doses will only increase the adverse effects without a parallel increase in benefit, being more indicated to associate another maintenance treatment such as a LABA [8].

In monotherapy, they are indicated in steps 2 (low dose) and 3 (medium dose) of Global Initiative for Asthma (GINA) [1]. The doses equivalence of inhaled corticosteroids between active drugs are shown in Table 1, but it should be borne in mind that the bioequivalence of mometasone furoate depends on the inhalation device (Table 2).

The adverse effects of high-doses of systemic corticosteroids therapy range from skin fragility to osteoporosis, adrenal axis disruption or cataracts [9]. Despite this, we should keep in mind that, in patients with severe asthma, it is sometimes necessary to use these doses of ICS to reduce the use of oral corticosteroids and the number of flare-ups, the profile of ICS being

safer than that of oral corticosteroids [10]. This option should be considered particularly in patients with elevated eosinophils and/or FENO levels.

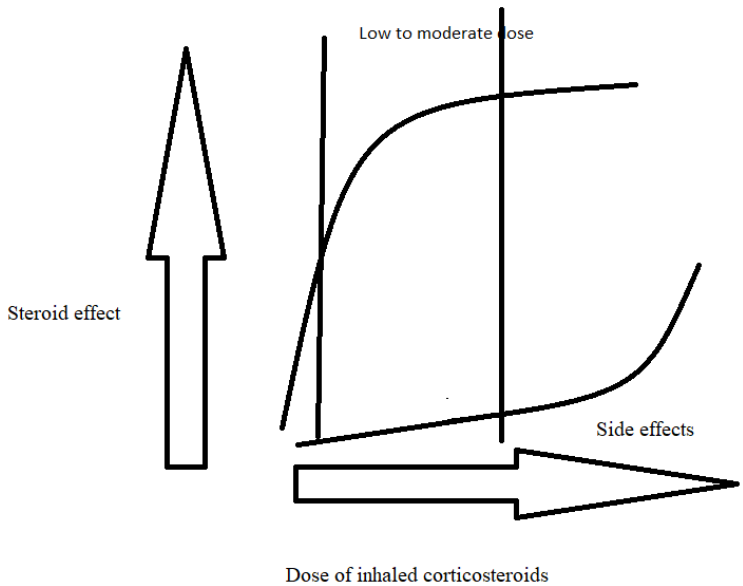

Figure 1. Dose-response effect of inhaled corticosteroids.

Table 1. Dose equivalence of inhaled corticosteroids between active drugs

Inhaled corticosteroid	Adults and adolescents			Children 6–11 years		
	Low	Medium	High	Low	Medium	High
Beclometasone dipropionate (CFC)*	200–500	>500–1000	>1000	100–200	>200–400	>400
Beclometasone dipropionate (HFA)	100–200	>200–400	>400	50-100	>100-200	>200
Budesonide (DPI)	200–400	>400–800	>800	100–200	>200–400	>400
Budesonide (nebules)				250–500	>500–1000	>1000
Ciclesonide (HFA)	80–160	>160–320	>320	80	>80-160	>160
Fluticasone furoate (DPI)	100	n.a.	200	n.a.	n.a.	n.a.
Fluticasone propionate(DPI)	100–250	>250–500	>500	100–200	>200–400	>400
Fluticasone propionate (HFA)	100–250	>250–500	>500	100–200	>200–500	>500
Mometasone furoate	110–220	>220–440	>440	110	≥220–<440	≥440
Triamcinolone acetonide	400–1000	>1000–2000	>2000	400–800	>800–1200	>1200

CFC: chlorofluorocarbon propellant; DPI: dry powder inhaler; HFA: hydrofluoroalkane propellant. *Included for comparison with older literature.

ICS differ in potency and bioavailability after inhalation, with fluticasone being the ICS with the highest lipophilic activity, activity that reflects pulmonary retention time, and thus being considered to have long-lasting antiinflammatory action [11]. In addition, it has higher volume of distribution levels (greater amount of drug in tissue and a smaller amount in plasma), a slower systemic clearance rate than other ICS [12], properties that help maintain its low systemic availability, and, together with its high relative potency, presents a favorable pharmacokinetic and pharmacodynamic risk benefit profile [13].

Mometasone furoate (MF) is the first ICS approved for once-daily dosing in the US and was approved for patients 12 years and older in 2005 and for children 4 to 11 years of age in 2008. Is a synthetic, 17-heterocyclic corticosteroid with a very high affinity for the gluco-corticoid receptor. *In vitro* studies showed that the relative receptor affinity for MF is 2,200

compared with 1,000 for dexamethasone and 1,800 for fluticasone propionate. Also, dissociation of the MF-receptor complex was faster than that observed for fluticasone, allowing for faster redistribution of the drug from lung tissue into the plasma [14,15]. Budesonide has a high ratio of topical antiinflammatory to systemic activity and is one of the most extensively used inhaled glucocorticoids. Budesonide decreases airway hyperresponsiveness and reduces the number of inflammatory cells and mediators present in the airways of patients with asthma. After inhalation of nebulized budesonide, absorption is rapid. Data suggest that plasma concentrations of budesonide are similar in adults and children after inhalation of the same nominal dose from a nebulizer [16].

Beclomethasone dipropionate was the first corticosteroid successfully used for the topical treatment of asthma. This drug first became available in 1972 in a pMDI, using chlorofluorocarbons (CFCs) as a propellant. Beclomethasone dipropionate is a prodrug with a weak binding affinity for the glucocorticoid receptor. It is hydrolyzed by esterases to an active metabolite, beclomethasone-17-monopropionate (17-BMP). The main effect of inhaled beclomethasone dipropionate emanates from 17-BMP, since the affinity of 17-BMP for glucocorticoid receptors is approximately 25 times higher than that of beclomethasone dipropionate [17]. The evidence on long-term maintenance treatment with regular dose ICS therapy (budesonide < 400 mg/day or equivalent) is consistent in showing no increased risk of systemic side effects (growth stunting, fractures, cataracts, adrenal suppression, reduced bone density,) as compared to placebo.

Table 2. Mometasone furoate equivalence between inhalation devices

MF dose level	MF (Twisthaler®)	IND/MF (Breezhaler®)	IND/GLY/MF (Breezhaler®)
Low	200 µg	80 µg	Not investigated
Medium	400 µg	160 µg	80 µg
High	800 µg	320 µg	160 µg

ICS/LABAs

Long-acting bronchodilators (LABAs) constitute the inhaled drugs of choice to associate with ICS at low or medium doses when there is poor symptomatology control, since they complement the effects of corticosteroids through interaction with glucocorticoid signal transduction [18]. In addition, due to their β-2-agonist action they trigger bronchial smooth muscle relaxation, decrease muscle contractility and produce a modulating effect on the release of mast cell and basophil mediators, postulating their antiinflammatory effect after sustained use, as well as a clear bronchodilator effect.

The use of LABA in monotherapy without the concomitant use of an ICS is contraindicated since it is less effective than combined treatment and there are doubts about its safety [19].

Within LABAs, according to their duration and β-2-agonist capacity we distinguish four drugs for inhaled use:

- *Formoterol (FF)*: Onset of action between 3-5 minutes and an effect up to 12 hours. It is its rapid onset of action and its efficacy in clinical trials that offers it the possibility

of also being used as a rescue drug in patients who use it as a background drug [20], a therapy known as SMART or MART.

- *Salmeterol (SAL)*: Slow onset of action after 20-30 minutes and an effect of up to 12 hours [21].
- *Indacaterol (IND)*: Indacaterol is administered by inhalation through the Breezhaler® device. The speed of bronchodilation is like that with salbutamol (about five minutes) and longer (24 hours) than that with traditional LABA. The fast onset of action provides immediate relief of symptoms, and its constant 24-hour bronchodilation facilitates lung emptying, thereby decreasing trapped gas and pulmonary hyperinflation [22].
- *Vilanterol (VIL)*: This is a long-acting beta2-agonist that binds to the beta2-adrenoceptor on the airway smooth muscle, producing bronchodilation. It has a rapid onset of action in experimental models and a 24-hour duration of bronchodilation effects in patients with asthma [23].

Greater benefit has been reported from the association of LABA with ICS at low doses than with increasing doses of ICS, and this is due to the synergistic effect created between the two. However, inhaled therapy with LABA is not free of side effects, the main ones being tachycardia, tremor and hypokalemia. Inhalation devices with fixed combinations of both increase therapeutic adherence and efficacy, as they can maximize the potential for drug co-deposition. They currently have indication as steps 2 to 5 maintenance therapy [1].

A key feature of airway smooth muscle cells in asthma is the low level of CCAAT-enhancer binding protein (CEBP) expression. It is believed that many of the pathological and clinical features of asthma, including inflammation, remodeling, and airway hyper-responsiveness, could be explained by this deficit. In combination, LABAs and ICS can simultaneously activate glucocorticoid receptors and CEBP, suppressing smooth muscle mass proliferation [24].

Long-Acting Muscaring Anatagonists (LAMA)

LAMA are other drugs for the maintenance treatment of asthma, which thanks to their good safety profile with minimal adverse effects and efficacy in terms of bronchodilation, have gained an important role positioning themselves, according to GINA strategy document, ahead of oral corticosteroids or biologic drugs to optimize the control of patients with severe asthma [1].

Drug interaction studies suggest a synergy of effects between LAMAs and ICSs and/or LABAs, even within the triple ICS/LABA/LAMA combination [25].

Given their effects in terms of bronchodilation, reduction of exacerbations and improvement of lung function, the addition of a LAMA should be considered preferentially for patients with persistent airflow limitation after bronchodilation, regardless of blood eosinophils and/or FENO levels [25].

Regarding its adverse effects, upper respiratory tract infections are the most frequently reported adverse events; and less frequently the side effects typically associated with anticholinergic drugs, such as dry mouth and urinary retention.

The three LAMAs currently available are:

- *Tiotropium*: Tiotropium bromide is a once-daily, selective bronchodilator, it has been recommended by the European Respiratory Society (ERS) as an adjunct to ICS/LABA in patients with severe uncontrolled asthma regardless of phenotype, as tiotropium has been shown to be at least as effective as salmeterol when added to ICS [26]. Tiotropium bromide can bind all 3 M (muscarinic) receptor subtypes, while selectively inhibiting M1 and M3 subtypes [27]. It is well-established that M3 receptors of the smooth muscle play a key role in asthma by constricting the bronchus and secreting mucus. Currently, tiotropium bromide is available as a mist or a dry powder inhaler. Its most striking advantage is a long duration of bronchodilation, for over 24 h generally. The tiotropium-M3 receptor complex has a half-life of about 35 h [28].
- *Glycopyrronium*: Inhaled glycopyrrolate has a rapid onset of action and is long lasting in the body, with a terminal elimination-phase t½ of 52.5 hours following inhalation. Inhaled glycopyrrolate has a bioavailability of 57%, with 53% absorbed via the lungs. Inhaled glycopyrrolate is absorbed slowly, predominantly unchanged, from the lungs. Furthermore, inhaled glycopyrrolate is eliminated rapidly from the bloodstream [29].
- *Umeclidinium*: The benefits of LAMA umeclidinium (UMEC) on lung function are well established in chronic obstructive pulmonary disease (COPD) and have also been described in patients with asthma and in those with features of both asthma and COPD, however it is not accepted on label for the treatment of asthma, although it is currently under investigation [30,31].

Triple Therapy ICS/LABA/LAMA

The most recent development has been several triple therapy combinations of ICS-LABA-LAMA in a single inhaler (SITT) have been marketed, and the 2021 GINA recommends adding a LAMA in patients aged ≥18 years who, despite being adherent to inhaled LABA combined with medium or high-dose ICS, still experience symptoms or exacerbations [1]. Several studies support its safety and efficacy for patients with asthma uncontrolled with medium to high doses of ICS/LABA [31,32, 33]. Triple therapy (ICS, LABA and LAMA), compared with dual therapy (ICS plus LABA), was significantly associated with fewer severe asthma exacerbations and slightly better asthma control, but there were no significant differences in quality of life or most adverse events.

TRIMARAN study showed 23% fewer severe exacerbations with beclomethasone dipropinionate (BDP)/formoterol fumarate (FF)/ glycopyrronium (GLY) versus BDP/FF and a 23.7% reduction in the annual rate of days on systemic steroids [33].

IRIDIUM [34] compared the effects of once-daily SITT (medium or high-dose mometasone MF, IND and GLY vs. either once-daily ICS-LABA (medium or high-dose MF–IND), delivered via a single device, multi-dose dry powder inhaler, Breezhaler®, or twice-daily high-dose fluticasone–salmeterol (FP-SLM) in patients with uncontrolled asthma delivered via a different single device, multi-dose DPI (Diskus®). At week 26 both medium and high-dose SITT were associated with greater improvement in trough FEV1; ACQ-7 score was not different in SITT vs. the equivalent MF/IND once-daily dose, but it was better than the combination of FP/SLM twice-daily; SITT did not significantly reduce the annualized rate of

moderate or severe exacerbations vs. equivalent MF/IND once-daily doses, but it did vs. twice-daily FP-SLM; and, adverse events were similar across groups.

CAPTAIN study [31] compared the safety and efficacy of fluticasone furoate/umeclidinium/ vilanterol vs. fluticasone/vilanterol, all delivered once daily through a DPI (Ellipta®). At week 24, the change from baseline in trough FEV1 was significantly higher with SITT; overall, SITT did not significantly reduce exacerbation rates, but higher ICS doses had a greater effect on exacerbations in patients with biomarkers of type-2 airway inflammation (high blood eosinophil or exhaled nitric oxide values), and a similar trend was observed for FEV1 changes from baseline; there was no clinically relevant impact on asthma control; and was well tolerated.

Leukotriene Receptor Antagonists

An alternative for asthma that is not controlled with low to medium dose ICS monotherapy is to add a leukotriene receptor antagonist, given its bronchodilator and antiinflammatory effect [1]. The cysteinyl leukotrienes (LT) C_4, D_4, and E_4 are involved in modulating airway inflammation and remodeling by either direct or indirect effects via enhancing inflammatory cytokine cascades. LTE_4 mediates activation of various inflammatory cells, including mast cells, eosinophils, T helper 2 cells and group 2 innate lymphoid cells, through cysteinyl leukotriene receptor (CysLTR) type 1 or an indirect receptor (purinergic receptor P_2Y_{12}). Leukotriene receptor antagonists (LTRAs) are approved in the treatment guidelines of asthma and widely prescribed in real practice. However, it is not certain whether they are effective in attenuating airway remodeling found in chronic asthmatic airways [35]. Although it is true that the effect is lower at low doses of ICS, a decrease in symptoms and exacerbations has been demonstrated, with improvement in pulmonary function, in association with inhaled corticosteroids or other combined therapies [36]. Within this group, on label zafirlukast and montelukast are available, montelukast offers the advantage of being administered orally in a single daily dose, with minimal side effects.

Studies show that the association of Montelukast with ICS, given its additive effect, is comparable in the long term with the association ICS-LABA, being inferior to the latter in the short term [36].

Systemic Corticosteroids

Add-on low-dosage oral corticosteroids (OCS) (before the introduction of targeted biologics) is recommended for patients with asthma that is not controlled by medium to high-dosage ICS plus controller medications [1]. Systemic corticosteroids (SCS) use to manage uncontrolled asthma and its associated healthcare burden may account for important health-related adverse effects. For patients with severe asthma who are not eligible for the currently available biologic treatments, the 2019 GINA guidelines recommend that several other strategies be considered before maintenance OCS/SCS [1]**.** Overall, OCS/SCS are commonly used for asthma management and are more frequently used in patients with severe asthma than in those with mild disease. Compared with no use, long-term and repeated short-term oral/systemic

corticosteroid use are associated with an increased risk of acute and chronic adverse events, even when doses are low. Greater oral/systemic corticosteroid exposure is also associated with increased costs and healthcare resource use [37].

Allergen Immunotherapy (AIT)

When biologically standardized extracts are used and in properly selected sensitized patients, this treatment has shown a beneficial effect in reducing symptoms, rescue, and maintenance medication and bronchial hyperresponsiveness (both specific and non-specific) [38]. In addition, AIT prevents the development of new sensitizations and asthma in children with rhinitis [39-40].

Rescue Treatments

Short-Acting Beta2 Agonists (SABAs)

As rescue treatment in step 1 to 5 of GINA. Inhaled SABAs (salbutamol or terbutaline) are the most effective and rapid bronchodilator drugs in the treatment of asthma attacks. In the treatment of asthmatic crisis salbutamol is used at a dose of 200 to 400 μg (2 to 4 inhalations) with an inhalation chamber [41]. GINA recommends that asthma in adults and adolescents should not be treated solely with SABA, because of the risks of SABA-only treatment and SABA overuse, and evidence for benefit of ICS. Large trials show that as-needed combination ICS–formoterol reduces severe exacerbations by ⩾ 60% in mild asthma compared with SABA alone, with similar exacerbation, symptom, lung function, and inflammatory outcomes as daily ICS plus as-needed SABA [42]. Also, SABA or ICS–formoterol is also recommended before exercise if needed to prevent exercise-induced bronchoconstriction [1].

Short-Acting Muscarinic Antagonist (SAMA)

Muscarinic receptor 3 (M3) when stimulated by the parasympathetic neurotransmitter acetylcholine inhibits airway smooth muscle relaxation induced by beta2 agonists. Activation of the M3 also causes submucosal glands to release mucus and play a role in airway remodeling and inflammation. Ipratropium is a SAMA with airway effects of about 6 h. The use of ipratropium bromide simultaneously with SABA in the initial phase of moderate or severe seizures is associated with a greater increase in lung function (estimated by FEV1 or PEF) and a decrease in hospital admissions, compared to the use of SABA alone [43]. The use of combined multiple doses of a SABA + SAMA has been called the “first-line” therapy for severe asthma exacerbation attacks in the emergency room.

ICS/SABA

As rescue treatment in step 1 and 2 of GINA [1]. The association salbutamol/beclomethasone dipropionate can be used on demand. However, these indications are not considered in the drug label. Moreover, there are no studies that have analyzed their cost-benefit.

ICS/LABA

As rescue treatment in steps 1 to 5 of GINA. The budesonide/formoterol combination can also be used on demand. In a randomized study in adult patients with asthma, in which approximately half had intermittent asthma and in which an open design was used to reflect clinical practice, it was observed that the use of budesonide/formoterol on demand was superior to salbutamol on demand in the prevention of exacerbations [20]. In a small study in patients with intermittent asthma and elevated exhaled nitric oxide fraction (FeNO), comparing budesonide/formoterol and formoterol, both on demand, the combination demonstrated a greater reduction in FeNO levels [44].

Conclusion

Treatment of asthma varies according to severity from short-acting bronchodilators on demand to high-dose ICS/LABA combination. In addition, leukotriene receptor antagonists and LAMAs may be added depending on the absence of control. Within the inhaled corticosteroids mometasone and fluticasone furoate are corticosteroid options to consider in addition to the already classic fluticasone propionate, beclomethasone, and budesonide. The latest generation LABAs (Vilanterol, Indacaterol) allow single daily administration as the main asset to improve therapeutic adherence. Triple therapy ICS/LABA/LAMA is the latest therapeutic novelty in asthma that allows improving lung function and reducing exacerbations.

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Chapter 16

Immunotherapy for Asthma

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Abstract

Allergen immunotherapy (AIT) consists of the administration of repeated doses of an allergen against which the patient has developed specific IgE whenever its exposure causes respiratory symptoms in order to induce some immunological changes. This fact turned AIT in the unique disease-modifying treatment for allergic asthma which can reduce asthma symptoms and the need for medications, the risk of asthma exacerbations and improvement of quality of life with a long-lasting effect after its cessation. AIT is indicated in patients with persistent allergic asthma, excluding severe asthma, whenever it is possible to demonstrate the clinical relevance of the allergen. Patients with mild asthma may benefit of AIT whenever it accompanies moderate or severe rhinitis with a well-demonstrated allergic component. The most common routes of AIT administration are the sublingual route (SLIT) and the subcutaneous route (SCIT).

Keywords: immunotherapy, asthma, biomarkers, indications, contraindications, precision medicine, component resolved diagnosis

Introduction: Allergen Immunotherapy as Treatment for Respiratory Allergy

Allergen immunotherapy (AIT) consists of administering repeated doses of an allergen against which the patient has developed specific immunoglobulin (Ig)E whenever exposure to it causes respiratory symptoms, to induce immunological changes to increase exposure tolerance and

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decrease clinical manifestations [1, 2]. This therapeutic modality was first proposed empirically in 1911 to treat naso-ocular symptoms due to pollen exposure [3, 4]. For over 100 years, empiricism dominated this therapeutic field, particularly until the discovery, in the late 1960s, of IgE, the main immunoglobulin responsible for allergic diseases [5]. The current situation is, however, very different, with extensive study of the immunological mechanisms of allergic diseases and a profound knowledge of how the diseases are modified by immunotherapy [6-9]. Allergen immunotherapy differs from other therapeutic options for asthma; whereas conventional pharmacotherapy has been designed to control symptoms and inflammation, AIT can modify the disease trajectory by changing the regulation of T-cell and B-cell IgE responses and suppression of effector cells [10-12]. This approach is exclusive to AIT, providing long-term clinical benefits with symptom remission even after discontinuation [7, 11, 12].

Since AIT was first recommended, most clinical trials have focused on its efficacy for controlling the symptoms of allergic rhinitis, with or without allergic conjunctivitis; however, clinicians soon observed that immunotherapy improved bronchial symptoms in patients who also had asthma [13-17]. It is only recently that the efficacy of AIT in asthma has been recognised and recommended in asthma guidelines [18, 19].

The role of AIT in treating asthma is directly related to the debatable association between asthma and atopy [20]. The problem lies in defining atopy as a predisposition and not as a disease in itself. The prevalence of atopy, identified as elevated total IgE or the presence of specific IgE against common environmental allergens, can reach 80% for individuals with asthma. The demonstration of atopy can have a predictive value in children: positive skin tests against environmental allergens at 18 months of age correlates with the presence of asthma at 5 years, suggesting a relevant role for allergic sensitisation in the future development of asthma in children who had not previously presented evidence of pulmonary involvement [21]. The Inner City Asthma Consortium, which focused on the allergic sensitisation profile against cockroach in 10-year-old children, concluded that higher IgE levels were associated with asthma and rhinitis [22], supporting the same hypothesis. Although the prevalence of atopy is lower in adult-onset asthma, figures differ from one series to another. Thus, while Busse et al. in the United States found little difference in the presence of atopy between individuals younger (72%) and older (63%) than 40 years with asthma, a recently published study performed in the Scandinavian countries found that only 20% of the population who presented with asthma after the age of 40 years could be considered atopic [23, 24]. These figures are important when assessing the indication of AIT as a therapeutic option for patients with asthma.

Any inhaled allergen can potentially cause asthma; however, not every patient with asthma and sensitisation to allergens experiences asthma symptoms upon exposure to the specific allergen. In patients with seasonal sensitisation to pollens, for example, a clinical history is typically sufficient to establish the clinical relevance of the sensitisation; however, the role of perennial allergens is more controversial and difficult to define [25]. In these cases, although bronchial challenge tests could help identify candidate patients for AIT, the tests have several disadvantages, as discussed later [25]. In recent years, special attention has been paid to mite allergy, and its role as a relevant cause of asthma in sensitised individuals is recognised [26, 27]. In a recent study, De Blay's group in Strasbourg demonstrated the specificity of the bronchial response in an exposure chamber. In this study, a group of patients with asthma and mite sensitisation and another group without sensitisation underwent controlled exposure to Der p 1 and placebo in a challenge chamber. No patients reacted to the placebo, and only

patients with mite sensitisation showed decreases in forced expiratory volume in 1 second. The result, although expected, is of great value because it demonstrates the specific effect of sensitisation and the indisputable role of allergy in developing symptoms in sensitised patients [28]. Similar specificity should be expected against pollens, as has been demonstrated against grass pollen and birch pollen in another study that also included an exposure chamber for patients with rhinitis and/or bronchial asthma who were administered an active vaccine as placebo [29]. That study, however, analysed objective parameters in rhinitis, not in asthma, and the inclusion of patients with asthma served only as a treatment safety parameter. It can therefore be concluded that the causal relationship between allergy and asthma is real and that, from a theoretical point of view, a treatment that specifically acts on allergic sensitisation could therefore improve asthma.

Principal Routes of Allergen Immunotherapy Administration

This chapter's objective does not include delving into the various types of extracts available and the ways in which they can be administered; however, a brief summary would be useful to interpret the results of studies that will be presented later.

Most of the available allergen-based AIT products are derived from complex allergen sources (e.g., pollen from one or more grasses, tree pollen, house dust mite), which contain numerous proteins that can act as potential allergens. The relative expression of these proteins in the allergenic source can vary due to environmental factors; consequently, the biological activity of one product could change with respect to products from different companies and even within different batches of the same AIT product. These variations are allowed by the European Pharmacopoeia [30]. To address this problem, product-specific biological standardisation is routinely employed by manufacturers and expressed as arbitrary in-house units, making it difficult to compare among manufacturers [31, 32]. The quantification of allergens as a way to minimise changes in the potency of extracts is a matter of debate. Typically, only the main protein components are quantified because of their supposed clinical relevance; thus, minor allergens might be underrepresented or overexpressed depending on the batch.

In general, the two most common administration routes for AIT are sublingual (SLIT) and subcutaneous (SCIT). Although other routes are already being tested, such as intralymphatic and epicutaneous, the studies on these are limited and will not be discussed in this chapter.

Extracts administered by the SCIT route are basically depot extracts that have been physically modified to delay their absorption by means of adjuvants. These adjuvants can, in some cases, act as enhancers of the immune response through various mechanisms [33].

As far as the allergen is concerned, the extracts can basically be of 2 types: native or natural extracts and allergoid or polymerised extracts that have been chemically modified to reduce their allergenicity while maintaining their antigenicity.

The Spanish Society of Clinical Allergology and Immunology (*Sociedad Española de Alergología e Inmunología Clínica*) Immunotherapy Committee makes available to the scientific community a complete manual of all available products in the Spanish market for AIT. In this manual, it is possible to check the quantification of dominant allergens in the products that have it quantified, in addition to a bibliography that supports each product. Before

prescribing an immunotherapy product, this guide should be consulted to investigate the selected product's characteristics.[1]

Indications of Allergen Immunotherapy in Allergic Asthma

If AIT is to be considered as a treatment option for allergic asthma, it must undergo the same developmental steps as other anti-asthmatic drugs.

Following the Global Initiative for Asthma (GINA) and the Spanish Asthma Management Guidelines (GEMA) 5.1, class 2 to 4 well-controlled asthma appears to be the most likely to develop a good response to AIT [18, 19]. Nevertheless, a recent study performed to investigate the efficacy of the house dust mite SLIT in patients with partially controlled asthma has broadened the spectrum of candidate patients [34]. Specifically, a mite tablet product for SLIT has proven efficacious in reducing the frequency and severity of asthma exacerbations in patients with partially controlled asthma [34]. The European Medicines Agency and these guidelines endorse the use of well-standardised extracts, avoiding complex allergen mixtures, particularly of non-related allergens [35, 18, 19]. In patients in stage 1 (intermittent asthma) with concomitant rhinitis, the prescription of immunotherapy is justified for the treatment of rhinitis, provided it is moderate to severe. At this point, it should be remembered that the Allergic Rhinitis and its Impact on Asthma guidelines recommend immunotherapy in patients with allergic rhinitis and asthma, always assessing the potential risk of adverse reactions due to the presence of both conditions in the same patient [36]. According to GINA and GEMA, severe asthma should not be treated with AIT [18, 19].

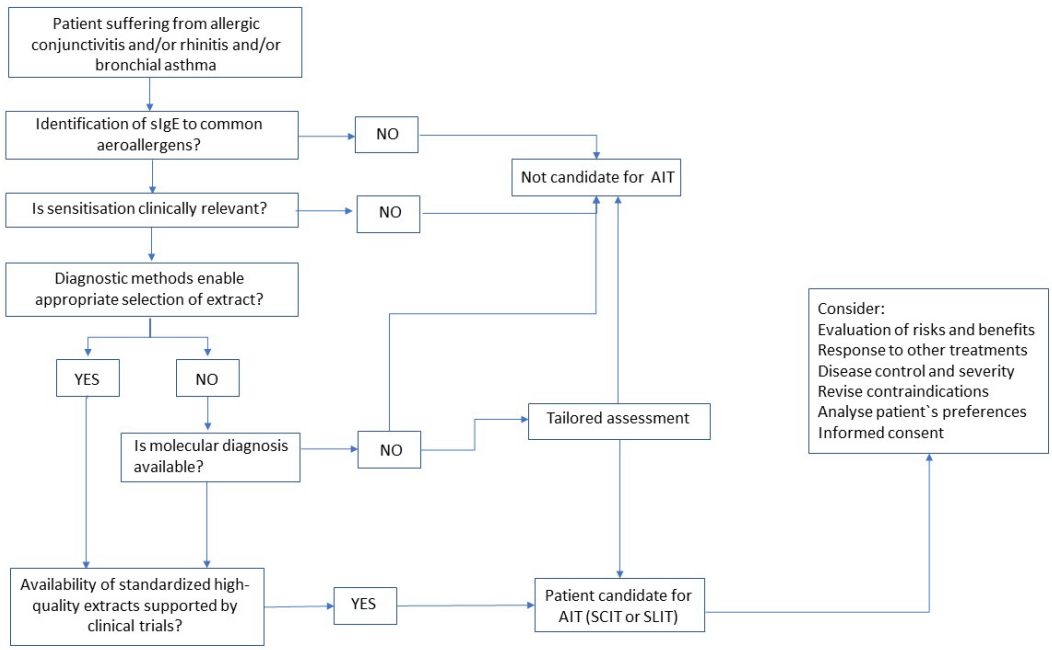
Once asthma severity and control have been established, the main problem before prescribing AIT is recognising the clinical relevance, which is typically performed through a detailed medical history. As previously stated, the task could be easy in cases of sensitisation to seasonal allergens but more difficult if perennial allergens are suspected. Bronchial challenge tests could be the solution to investigate an allergen's capacity to induce an asthmatic response, but it is not recommended in routine clinical practice because it consumes significant time and resources [25].

In patients with asthma sensitised to house dust mites who experience concomitant rhinitis, a nasal allergen challenge could be performed instead because of the good relationship between a positive response and a positive bronchial challenge test with the same allergen [25]; however, this is not the case for cat allergy. Although the implementation of bronchial allergen tests in the asthma clinic would support a more accurate characterisation of patients with asthma, real-world evidence is of utmost importance to move the field forward.

In 2014, the Spanish group QUASAR published a diagnostic algorithm that can be useful to clinicians in their decision to prescribe immunotherapy (Figure 1) [37].

In summary, immunotherapy is indicated for patients with persistent allergic asthma, excluding severe asthma, whenever the clinical relevance of the allergen to which the patient is sensitised can be demonstrated. In intermittent asthma, immunotherapy is indicated when it accompanies moderate to severe rhinitis with a well-demonstrated allergic component.

[1] www.seaic.org.



Modified from: Dávila I, Navarro A, Domínguez-Ortega J, Alonso A, Antolín-Amérigo D, Diéguez MC et al. QUASAR Group; QUality Administration of SLIT in Allergic Rhinitis. SLIT: indications, follow-up, and management. *J Investig Allergol Clin Immunol*. 2014;24 Suppl 1:1-35 [37].

Figure 1. Proposed algorithm to select candidates for allergen immunotherapy (AIT).

Contraindications of Allergen Immunotherapy

A series of AIT contraindications have been developed and published by international academies and national societies of allergology and clinical immunology according to experts' opinion based on the supposed immunological mechanisms that could be altered with vaccination or on the possibility of developing adverse effects [38-42].

In 2019 and within the framework of the European Academy of Allergology and Clinical Immunology (EAACI), the results of a systematic review on the contraindications of specific immunotherapy with allergens were published [43]. This document specifies that, regarding immunotherapy with aeroallergens for respiratory disease, the possible contraindications vary depending on whether the administration route is subcutaneous or sublingual. The type of underlying respiratory disease (rhinitis and/or bronchial asthma) could be relevant insofar as one or another administration route is not considered equally safe depending on the severity of the patient's respiratory disease [43].

Table 1 shows the identified absolute and relative contraindications for AIT for respiratory allergy [adapted from 43], a number of which could change from absolute to relative over time, given that they depend on the degree of disease control, which could change accordingly. There is a gap in the evidence associating AIT with harmful effects in autoimmune or neoplastic disorders. In a large national study in Denmark, patients treated with SCIT had a lower incidence of autoimmunity compared with those undergoing conventional treatment [44]. The German AIT guidelines took a rational approach and focused on the severity of the autoimmune

disease and the relative or absolute contraindication of AIT according to the vital prognosis of the specific disease (for instance, systemic lupus erythematosus could have a very different prognosis than Hashimoto's thyroiditis) [45]. It appears that, in this field of medicine as in many others, generalisations do not apply to each individual patient, and common sense must be employed to make decision-making more straightforward.

Table 1. Absolute and relative contraindications of AIT

Absolute Contraindications	Relative Contraindications
Uncontrolled asthma	Partially controlled asthma
Autoimmune disorders in active forms	Autoimmune disorders in remission
Malignant neoplasias	Treatment with β- blockers (including ocular drops)
AIDS	Pregnancy (build-up phase)
Psychiatric and/or mental disorders	Children (≤ 5 years)

Modified from: Pitsios C, Demoly P, Bilò MB, Gerth van Wijk R, Pfaar O, Sturm G, et al. Clinical contraindications to allergen immunotherapy: an EAACI position paper. *Allergy*. 2015;70:897-909 [38].

Risk of Systemic Reactions during Allergen Immunotherapy

Between September 2012 and February 2014, the European Survey on Adverse Systemic Reactions in Allergen Immunotherapy study was performed in Spain, France and Germany and prospectively included 4,316 patients who had started AIT for rhinitis and/or bronchial asthma to register all the systemic reactions experienced. The total number of treatments included was 4,363, given that a number of the patients underwent more than one type of AIT.

The AIT prescription was performed under normal clinical practice conditions, and doctors selected the type of treatment, route and schedule according to their experience and knowledge. Ninety patients (2.1% of the total) presented at least one systemic reaction (total of 109 systemic reactions). Most (75.8%) of the systemic reactions occurred during the up-dosing phase. In terms of severity, most of them (71.6%) were mild, and only 3.7% were classified as severe; 88% of the reactions occurred with SCIT. The independent risk factors associated with a higher incidence of systemic reactions were classified into 2 large groups: those related to the patient and those related to the extract and administration schedule. The former included (odds ratio [95% CI]) the absence of symptomatic treatment (1.707 [1.008–2.892], P = 0.047), the diagnosis of asthma (1.74 [1.05–2.88], P = 0.03) and sensitisation to epithelia (1.93 [1.21–3.09], P = 0.006) or to pollen (1.16 [1.03–1.30], P = 0.012), even if the extract was from another allergenic source.

Factors dependent on the immunotherapy itself included the use of native or natural extracts (2.74 [1.61–4.87], P = 0.001), and cluster administration schedules (4.18 [1.21–14.37], P = 0.023). Having experienced a previous episode of anaphylaxis also increased the risk of systemic reaction (17.35 [1.91–157.28], P = 0.01). The authors suggested a formula to be applied prior to prescribing immunotherapy so that the risk could be modified depending on each of the terms in the equation. It was not possible to identify similar risk factors for SLIT due to the small number of reactions detected in this type of immunotherapy [46, 47].

Allergen Immunotherapy Biomarkers

In recent years, the search for one or more biomarkers for objective and measurable data to predict the response to AIT has intensified [6, 7, 8, 48-53]. Due to the complex interaction between the genetic component, environmental exposure and inflammation involved in allergy and bronchial asthma, identification of such markers is a difficult task. The pathophysiological response can ostensibly vary from one patient with asthma to another, thus defining various endotypes and phenotypes with possibly different responses to treatments. Thus, cellular, biochemical and molecular changes that occur in patients with allergy could be measured in blood and secretions and behave as potential biomarkers. Moreover, this approach cannot dismiss the well-known interaction between innate and acquired immune systems in developing the allergic disease. In this chapter, we will briefly analyse biomarkers for asthma and biomarkers for AIT, trying to establish a connection between them.

Biomarkers in Asthma

Asthma is a complex and heterogeneous disease. With the introduction of biological drugs, attempts were made to create a simple classification in which each of them could be framed. Thus, the classification was conceived based on the pattern of cytokines produced by their T cells, which have been divided into Th2-high and non-Th2 or Th2-low [18, 19].

Biomarkers in Specific Immunotherapy

In Vitro Biomarkers

Four consecutive cellular and humoral changes can be identified during AIT. First, there is the decreased mast cell and basophil activity that requires higher doses of the allergen to elicit degranulation. Second, the generation of allergen-specific Treg and Breg cells is promoted. Third, there is a decrease in specific IgE levels and an increase in specific IgG_4 levels. Lastly, there is a reduction in tissue mast cells and eosinophils [54]. In 2017, the EAACI published a task force document dealing with this topic. Biomarkers were grouped into 7 domains: 1) total and specific IgE; 2) IgG-subclasses (sIgG1, sIgG4 including SIgE/IgG4 ratio); 3) serum inhibitory activity for IgE; 4) basophil activation tests; 5) measurement of cytokines; 6) cellular markers (T regulatory cells, B regulatory cells and dendritic cells); and 7) *in vivo* biomarkers (including provocation tests) [14]. Only the most commonly used biomarkers will be analysed in this chapter.

IgE and IgG

Both total and specific IgE levels rise in the early phases of treatment and subsequently decrease. An elevation of specific IgE against the responsible allergen with which the patient is being treated in the first weeks of follow-up predicts a favourable response to SLIT [55]. At

the same time, the baseline specific IgE level appears to be related to the response after patient treatment [56]. Given that patients with a specific IgE of approximately 10 kU/L or higher before starting AIT respond better than those with a lower specific IgE, this parameter could be useful for selecting the response [57].

The relationship between specific IgE and total IgE could be of value in monitoring AIT, because it represents the percentage of the total patient IgE that is directed against a specific allergenic source. The studies that support this idea use symptom scales to assess patient efficacy [56].

In addition to IgE, both IgG1 and IgG4 are being used as biomarkers in AIT. These immunoglobulins experience a gradual increase during treatment; however, their relationship with clinical improvement is not always evident. The explanation for the latter comes from the mechanism by which IgG4 exerts its action. IgG4 is capable of blocking IgE binding to allergens and preventing IgE-facilitated allergen presentation. Recent functional studies have confirmed this hypothesis using enzyme-linked immunosorbent assay techniques combined with flow cytometry. The main limitation of IgG4 is its complexity, which means that it cannot be used in routine clinical practice, given that it is available in only a few centres [58].

Cellular Changes

The appearance of regulatory cells is a potential biomarker of AIT efficacy. Regulatory cells induced by immunotherapy secrete interleukin (IL)-35, which promotes IL-10 production from $CD19^+$ B cells, Bregs and Tregs. These regulatory cells persist for long periods, which could explain the long-term effect of AIT once administration has stopped. Recent studies on these regulatory cells and on the molecules they express, such as glycoprotein-A repetitions predominant and special AT-rich sequence-binding protein 1, have made it possible to demonstrate the relationship with eosinophilic inflammation due to the interaction of these molecules with IL-5 expression in the early stages of type 2 cell differentiation [48].

Basophil Activation Tests

The incubation with the allergen of basophils coated with specific IgE provokes their degranulation and the expression of CD63 and CD203c, which can be detected. A decrease in the expression of these molecules has been demonstrated in patients treated with AIT [59]. Unfortunately, no direct relationship between basophils and clinical improvement has been confirmed.

Serum Cytokines

These do not appear to be useful because their levels in blood are very low, and it is difficult to detect changes with treatment.

In Vivo Biomarkers

Skin Prick Test Reactivity

This is a common endpoint in AIT clinical trials. Although a reduction in skin reactivity is typically reported, its correlation with clinical scores is poor, and it is not recommended in daily clinical practice as a tool to follow-up AIT efficacy [60].

Controlled Exposure Tests

Considered the gold standard in allergy diagnosis, controlled exposure tests are indispensable in certain clinical trials to prove the efficacy of a specific AIT product, particularly in finding the allergen's appropriate dose [35]. The most accurate method is the environmental chamber exposure, in which conditions of natural exposure to allergens are recreated [25, 60].

Allergen Immunotherapy as an Example of Precision Medicine in Bronchial Asthma

Allergy diagnoses have long been based on the positive results of skin prick tests with allergen extracts and/or serum-specific IgE against the suspected allergen sources. Nevertheless, allergen sources contain many different proteins, and only a few of them might behave as allergens. The identification of allergenic proteins in an allergen source has driven major advances in allergy diagnosis through the detection of specific IgE to every single molecule and has helped distinguish between genuine allergens (allergens involved in symptoms) and cross-reactive allergens (allergens not involved in a primary sensitisation). Such a diagnostic approach is known as a "component resolved diagnosis" [61] or "molecular allergy diagnosis" [62], which is aimed at mapping a patient's allergen sensitisation profile, using purified natural or recombinant molecules. As a result, there have been changes in the prescription of AIT. Several studies have shown that the composition chosen by the prescribing physician varies when the diagnosis is based on identifying specific IgE against complete allergenic sources versus the diagnosis based on recognising IgE against molecular components. Although clinical experience supports the selection based on a deeper understanding of the patient's pattern of allergic sensitisation, the superior efficacy of the vaccine selected through molecular allergy diagnosis remains to be seen. For now, molecular allergy diagnosis helps identify patients with the highest risk of systemic adverse reactions during AIT [63-68].

In recent years, the assessment of treatable traits has gained importance, traits that include allergic sensitisation, which could be especially important in the form of severe asthma in which a synergy could be sought between the effect of certain new biological drugs directed at cytokines or their receptors and AIT. Although severe asthma is considered a contraindication for immunotherapy, treatment with a biological drug that achieves better control of the disease would facilitate the administration of subsequent immunotherapy. Thus, a drug that does not change the natural history of the disease but does improve its control would have a synergistic effect with another drug (immunotherapy), which can achieve long-term modification through the immunological changes it induces. This theoretical approach requires confirmation in relevant studies or clinical trials that demonstrate this synergy [69].

Clinical Trials in Allergen Immunotherapy

At present, AIT is supported by the accumulated data from large, double-blind, placebo-controlled clinical trials, a large number of non-interventional studies, systematic reviews and meta-analyses [70], and real-world evidence [71-74]. Despite the definitive value of positive results in clinical trials, a number of questions arise when dealing with the design of studies on AIT in asthma.

What Is the Best Outcome?

One of the main limitations when interpreting the results of clinical trials in bronchial asthma is the inconsistent definition of "response." The fact that asthma is a variable and heterogeneous disease makes it difficult to reach a consensus in this regard. As far as possible, the definition of response should be based on objective parameters such as pulmonary function tests, markers of inflammation and exacerbation rate per year. In fact, the exacerbation rate is the most common outcome used in clinical trials evaluating the efficacy of biologic drugs; however, it also implies a certain subjective assessment of the patient's condition [75-76]. To date, only one study has focused on the effects of AIT in preventing asthma exacerbations [34], and the positive results achieved in that study launched SLIT with mites as a therapeutic option for patients with moderate, partially controlled asthma in the GINA guideline [19]. However, what is the situation for patients who do not experience frequent asthma exacerbations? Is AIT also suitable for them? In 2016, Demoly et al. published an interesting review focused on the effects of AIT in reducing asthma treatment and observed that patients who are most likely to improve in terms of reduction of anti-asthmatic medication are those with moderate asthma in GINA stages 3 or 4 [77].

For intermittent and mild asthma, the effects sought with AIT could be the prevention of disease progression or even the emergence of asthma in patients with exclusive nasal symptoms.

Although clinical trials focused on exacerbation rates provide extraordinary value to the treatment, other outcomes such as changes in symptom and medication scores and changes in the natural history of the disease should be taken into account.

Placebo Effect on Allergen Immunotherapy

The EAACI recently published an extensive review on the placebo effect in AIT and possible ways to minimise it. The use of placebo in clinical trials can pose an ethical problem in certain cases because, for prolonged treatments such as those required in the field of AIT, it implies leaving a group of patients without a potentially effective treatment [46]. However, the European Medicines Agency recommends the use of a placebo to ensure that the effects achieved are due to the active ingredient under analysis [35]. The placebo effect exists in all medical specialties, but the solution differs depending on the study drug's characteristics.

The main alternatives presented in this document for AIT are as follows [46]:

Modification of the Nature of the Placebo

The administration of AIT products is usually accompanied by certain local effects that usually do not go unnoticed by either the patient or investigator and include reactions at the administration site in the form of itching with/without erythema or inflammation. Maintaining blinding in a study can be difficult when some of the patients report these reactions. The inclusion in the placebo of substances attempting to reproduce these effects was a technique used in the past but not currently allowed. An alternative is the use of what is called "active placebo" in which the placebo is replaced by a different allergen against which the patient is sensitised but is not clinically relevant [78]. This approach is not possible in monosensitised patients.

Elimination of the Placebo Arm

Eliminating the placebo arm would allow only the monitoring of products already marketed but not the registration of a new one.

Application of Digital Systems (mHealth) and Big Data

Digital systems could be useful for verifying the causal relationship between symptoms and exposure to allergens and pollutants. A number of these studies are already being performed with this type of application to determine the interaction of these factors.

Cost-Efficacy Studies of AIT in Asthma

As stated earlier in this chapter, AIT exerts an immunomodulatory effect that facilitates the persistence of the clinical benefit beyond the treatment period; thus, studies on cost-efficacy should consider not only the benefits of AIT during administration but also the interference in the natural history of the disease.

In 2008, Brüggenjürgen et al. performed one of the most complete studies to date on this subject in Germany, using the Markov calculation model to compare drug treatment and the combination of AIT and drugs in asthma, with a follow-up of 15 years. Both direct and indirect costs were considered, and the patients' age when AIT was established was analysed. The authors found a benefit for AIT in patients with asthma, in contrast to patients with rhinitis, for whom the cost-efficacy analysis was negative for AIT [79]. Other authors have performed a similar approach with specific AIT products such as SCIT with a high-dose allergoid extract of mites (Acaroid®) and SLIT with a mite tablet (Acarizax®), reaching the same conclusions [80].

Conclusion

AIT is a unique disease-modifying treatment for allergic asthma that can reduce asthma symptoms and the need for medications, lower the risk of asthma exacerbations and improve quality of life, with a long-lasting effect after its cessation thanks to the induction of changes in the immunological response to allergens.

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Chapter 17

Biological Treatments for Asthma

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Abstract

The development of targeted therapies for patients with severe asthma (SA) has revolutionized the treatment of these patients because, previously, treatment options for these patients were limited, and some of them had unacceptable side effects. In approximately half of SA patients, there is a type 2 inflammation characterized by the release of IL-4, IL-5, and IL-13 and associated with eosinophilic and allergic phenotypes. Biologic therapies target specific inflammatory pathways involved in the pathogenesis of asthma.

In addition to anti-IgE therapy, three anti–IL-5 biologicals and one anti–IL-4R biological have recently emerged as promising treatments for T2 asthma, with variable effects in reducing asthma exacerbations, improving lung function, reducing the adverse effects of oral corticosteroid use, and improving the quality of life and asthma control. Also, tezepelumab (anti-TSLP) is the only monoclonal antibody that has been approved by the US Food and Drug Administration for the treatment of SA with no phenotype or biomarker limitation.

We present a review of biologicals approved for the treatment of SA or in development, emphasizing the main phase 3 clinical trials and real-life studies and suggesting a decision algorithm.

Keywords: asthma, biologic treatment, T2 inflammation, phenotype, endotype, severe asthma, biomarkers, corticosteroids

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Introduction

Asthma is a chronic inflammatory airways disorder associated with bronchial hyper-responsiveness and variable airflow limitation. Although most patients can achieve disease control with standard controller therapy, approximately 10% of adults suffer from SA, which remains uncontrolled despite proper adherence to standard treatments. In addition, some treatment options for these patients, such as oral corticosteroids (OCS), produce unacceptable side effects. Targeted therapy with biological drugs is usually an effective and safe alternative for SA patients.

The European Respiratory Society/American Thoracic Society consensus defined SA as an inadequately controlled asthma despite receiving high-intensity treatment with inhaled corticosteroids (ICS) and additional controllers (including oral corticosteroid [OCS]) for at least six months per year or by loss of asthma control on the attempt to reduce the high-intensity treatment [1]. Differential diagnoses should be discarded, comorbidities treated, persistent triggers eliminated, and patient adherence optimized before adding further treatments. Before considering treatment with biologicals, determining the phenotype of patients is mandatory. Phenotyping is described elsewhere in the textbook.

Six approved biologics are available for the management of SA, targeting IgE (omalizumab); interleukin (IL) receptor alpha chain IL-4 (IL-4RA), thus blocking IL-4 and IL-13 signaling (dupilumab); IL-5 (mepolizumab, reslizumab); IL-5 receptor alpha chain (IL-5Rα) (benralizumab); and thymic stromal lymphopoietin (TSLP) (tezepelumab). Currently, tezepelumab is only approved by the FDA in patients aged 12 years and older, but not by the EMA.

Table 1 shows the indications and doses of the biologics used to treat SA, and Table 2 shows the markers of efficacy of biologics in SA.

Table 1. Biologic agents for the treatment of severe asthma

Biologic agent	Patient's age	Indication	Route of administration and dose	Forms	Safety
Omalizumab (anti-IgE)	≥ 6	Moderate to severe persistent asthma, positive allergy testing, uncomplete control with an ICS and IgE: 30 and 1500 kU/L Other indications: chronic idiopathic urticaria, chronic rhinosinusitis with nasal polyps.	SC. Every 2 to 4 weeks, according to body weight and pretreatment level of serum total IgE	Prefilled syringe	Serum sickness, hypereosinophilic conditions (e.g., EGPA), abrupt discontinuation of OCS; black-box warning for anaphylaxis.
Mepolizumab (anti-IL5)	≥ 6	Additional treatment in adult patients, adolescents, and children from 6 years of age with severe refractory eosinophilic asthma. Peripheral blood eosinophilia ≥ 150/μL at the beginning of treatment or ≥ 300/μL in the last 12 months.	6-11 years: 40 mg every 4 weeks. ≥12 years: 100 mg every 4 weeks	Prefilled syringe, autoinjector pen	It rarely causes hypersensitivity reactions; it can cause Zoster activation.

Biologic agent	Patient's age	Indication	Route of administration and dose	Forms	Safety
Benralizumab (anti-IL5Rα)	≥ 18 (EMA) ≥ 12 (FDA)	Add-on maintenance treatment in adult patients with severe eosinophilic asthma uncontrolled despite high-dose inhaled corticosteroids and long-acting β-agonists. Greater efficacy and efficiency in patients with eosinophilia ≥300/μL in peripheral blood.	≥12 years: 30 mg, sc. Every 4 weeks, the first 3 doses, then every 8 weeks.	Prefilled syringe, autoinjector pen	Rarely causes hypersensitivity reactions.
Reslizumab (anti-IL5)	≥ 18	>18 years of age with severe eosinophilic asthma unresponsive to other GINA step 4-5 therapies. AEC≥ 400 cells/μl.	3 mg/kg every 4 weeks.	IV infusion	Black box warning: approx. 0.3% risk of anaphylaxis in clinical trials.
Dupilumab (anti-IL4Rα)	≥ 6 (FDA) ≥ 12 (EMA)	Add on maintenance treatment for severe asthma with type 2 inflammation characterized by raised blood eosinophils and/or increased FeNO, which are inadequately controlled with high dose ICS plus another medicinal product for maintenance treatment.	SC, Every 2 weeks	Prefilled syringe, autoinjector pen	Rarely causes hypersensitivity reactions; higher incidence of injection site reactions (up to 18%) and hypereosinophilia (4-14%).
Tezepelumab (anti-TSLP)	≥ 12 (only FDA approved)	Add-on maintenance treatment of patients with severe asthma without phenotype (e.g., allergic or eosinophilic) or with limiting biomarkers.	210 mg every 4 weeks	SC	Pharyngitis, arthralgia, back pain.

EMA: European Medicines Agency. FDA: Food and Drug Administration; OCS: oral glucocorticosteroids; EGPA eosinophilic granulomatosis with polyangiitis SC: subcutaneous, IV: intravenous.

Table 2. Markers of the efficacy of biologics in severe asthma: Results of phase III trials and a Cochrane review evaluating 25 RCTs for omalizumab

	Asthma exacerbation (% reduction in active treatment)	Lung Function (ml improvement in FEV1-mean difference vs. placebo)	Corticosteroid Weaning (% active vs. placebo)	Quality of Life (ACQ) (mean difference vs. placebo)
Omalizumab (Cochrane review evaluating 25 RCTs)	Reduces by 25%	Small effect on FEV1	Decreases use of ICS, but no data that it helps with OCS weaning	Minor improvement
Mepolizumab MENSA/SIRIUS	53/38	98/114	NA/50 vs. 0	0.44/0.52
Reslizumab Castro *et al.*	50-59	90-126	NA	0.2-0.27
Benralizumab CALIMA/SIROCCO /ZONDA	28/51/55	116/159/112	NA/NA/ 75 vs. 25	0.1-0.29/0.55

Table 2. (Continued)

	Asthma exacerbation (% reduction in active treatment)	Lung Function (ml improvement in FEV1-mean difference vs. placebo)	Corticosteroid Weaning (% active vs. placebo)	Quality of Life (ACQ) (mean difference vs. placebo)
Dupilumab QUEST/VENTURE	46-47.7/59.3	130-140/220	NA/70.1 vs. 41.9	0.31-0.34/0.47
Tezepelumab NAVIGATOR SOURCE	56 and 41*	130	Ongoing	0.33

*Patients with blood eosinophil counts of less than 300 per microliter at baseline.
NA: not applicable, ICS: inhaled corticosteroid; OCS oral corticosteroid; RCTs: randomized control trials.

Biologics

Anti-IgE

Omalizumab

Mechanism of Action

Omalizumab is a humanized anti-IgE IgG1kappa monoclonal antibody and was the first biological approval to treat asthma. Omalizumab precludes IgE from binding to high-affinity receptor (FcεRI) found on mast cells and basophils, dampening the release of proinflammatory mediators and blunting the downstream allergic response. Due to the reduction of free IgE levels, omalizumab also down-regulates the expression of FcεRI on mast cells, further increasing its effect. In addition, clinical studies have shown that omalizumab also reduces exacerbations during peak viral seasons, which has been related to an enhancement in IFN-α production in response to rhinovirus, probably blunted through an IgE-involved mechanism [2, 3].

Efficacy

Omalizumab has been used to treat severe allergic asthma for almost 20 years showing favorable outcomes in several randomized control trials (RCTs) [4, 5, 6, 7, 8] and real-life trials.

A Cochrane review evaluating 25 RCTs in patients with moderate to severe and allergic asthma found that omalizumab, compared with placebo, reduced asthma exacerbations by approximately 25%, reduced hospitalizations, and ICS dose [9]. However, minor improvements in quality of life and lung function were found with omalizumab. Most of the studies conducted with omalizumab have selected patients with moderate-severe asthma, and very few studies have studied the effect of omalizumab in patients with severe asthma [10, 11]. In a randomized trial, omalizumab was assessed in 850 patients with severe asthma (aged 12 to 75 years). A 25% reduction in asthma exacerbations was noted in the omalizumab group compared with placebo. No reduction in exacerbations was noted in the subgroup taking daily oral glucocorticoids. However, the lack of effect in this subgroup may be due to insufficient sample size [12].

In a recent meta-analysis of 86 observational studies (real-world effectiveness), Bousquets *et al.* found improvement with omalizumab in Global Evaluation of Treatment Effectiveness

(GETE) (good/excellent in 82% of patients at 12 months) in forced expiratory volume in 1 second (FEV1) (250 mL at 12 months), Asthma Control Questionnaire score (-1.13 at 12 months), the annualized rate of severe exacerbation (RR 0.45) and proportion of patients receiving OCS (RR 0.59) [13]. Also, in a recent real-life retrospective study, omalizumab improved non-allergic severe asthma patients [14]. Nevertheless, these results should be confirmed.

Unlike other biological treatments for severe asthma, there has been a development of omalizumab in pediatric patients (≥ 6 years), including RCTs with pooled *post hoc* analyses, systematic reviews, real-life studies, and clinical case series. RCTs demonstrated a significant reduction in asthma exacerbations, a sparing effect of ICS, and improved quality of life [15, 16, 17, 18].

Predictors of response to omalizumab have been analyzed in a few studies with limited results. In a *post hoc* analysis of the EXTRA clinical trial, reductions in asthma exacerbations were more significant in patients with elevated FeNO levels, blood eosinophils, and serum periostin levels than in subjects with low levels (low biomarker subgroup) [19]. Nevertheless, it cannot be discarded that the effects were probably associated with an increased risk of exacerbations in these patients. Notwithstanding, several analyses have not shown reliable predicting biomarkers, particularly total serum IgE, perennial allergen sensitization, patient weight, or pre-treatment blood eosinophil count [20, 21, 22].

Indications and Safety

Omalizumab is approved for subcutaneous administration in patients six years of age or older with moderate-to-severe persistent allergic asthma, symptoms inadequately controlled by ICS, positive skin-prick test or allergen-specific IgE to a perennial aeroallergen, and a total serum IgE level between 30 and 1,500 kU/L which is used in combination with weight to calculate the dose and frequency of administration.

An initial trial of at least four months should be conducted to evaluate the clinical response to omalizumab. Treatment should be continued indefinitely if a patient has a favorable response, as supported by the XPORT (Xolair Persistence of Response after Long-Term Therapy) trial. In most patients, the withdrawal of omalizumab after prolonged treatment reverses the effect on IgE and basophils that correlates with a deterioration of asthma control [23].

Omalizumab has a notable safety and tolerability profile, being the most frequent adverse events local reactions confined to drug injection sites. Headache, nausea, or fatigue have also been reported. The global pattern of adverse events related to omalizumab is similar to that occurring in placebo-treated patients [24].

Although omalizumab is directed against IgE, counterintuitive anaphylactic and anaphylactoid events have been sporadically described. Anaphylaxis occurs in 0.1 to 0.2% of patients, most frequently with one of the first three doses [25]. Even if IgE is the antibody involved in immune protection against parasitic infestations, these infections are rare during biologic therapy with omalizumab. Only patients living in or moving to regions where these parasitic infections are endemic should be advised about this potential risk [24].

Concerning the use of omalizumab during pregnancy, the observational study EXPECT, which included 250 asthmatics pregnant women [26], showed no increased frequency of congenital anomalies in infants whose mothers had been treated with omalizumab during

pregnancy. In any case, it is not recommended to initiate during pregnancy due to the risk of anaphylaxis.

Omalizumab has been proven efficacious against comorbid conditions sometimes seen in patients with severe asthma, such as chronic spontaneous urticarial [27] and chronic rhinosinusitis with nasal polyposis (CRSwNP) [28].

Anti-IL5

Some patients with moderate to severe asthma have an eosinophilic phenotype characterized by increased sputum eosinophils (>2%) and/or blood eosinophils despite treatment with corticosteroids and are more susceptible to frequent exacerbations [29]. IL-5 is the main cytokine involved in eosinophil recruitment, activation, and survival. By inhibiting this pathway, anti-IL-5 biologics reduce eosinophilic airway inflammation.

Mepolizumab and reslizumab, targeting the ligand interleukin-5, and benralizumab, which depletes eosinophils targeting the interleukin-5 receptor alpha (IL-5RA) and induce antibody depending cellular cytotoxicity induced by NK cells, are approved biologic agents for the treatment of patients with severe eosinophilic asthma [30].

Mepolizumab

Mechanism of Action

Mepolizumab is an IgG1k monoclonal antibody that binds to IL-5, blocking its binding to IL5RA expressed on the eosinophil's cell surface and preventing its effects, essential for proliferation, maturation and activation, and migration of these cells.

Efficacy

Initial studies with mepolizumab selected moderate asthma patients without evidence of eosinophilic airway inflammation and failed to benefit [31, 32], but subsequent trials demonstrated a significant reduction in exacerbations among patients with severe eosinophilic asthma. Mepolizumab reduced asthma exacerbations by 50% and improved lung function in a phase 3 trial (MENSA study [33]). The efficacy became apparent at a blood eosinophil count of more than 150 cells/uL and increased progressively with counts above this [34]. SIRIUS trial confirmed a significant OCS-sparing effect [35]: mepolizumab led to a 50% reduction in OCS dose in patients with eosinophilic asthma receiving OCS versus no reduction in the placebo group. This corticosteroid-sparing effect occurred while maintaining the effects of exacerbation reduction (32%) and improving asthma control.

The effect of mepolizumab on lung function has been less consistent. Some trials improved FEV1 (MENSA and SIRIUS), while one of the more extensive trials, the phase 2b, DREAM trial [36], showed no significant change in FEV1 with mepolizumab. A Cochrane review concluded that patients with eosinophilic asthma treated with mepolizumab had a 50% reduction in asthma exacerbations and an increase in FEV1 of 110 mL compared to placebo [37].

Mepolizumab also resulted in a clinically and statistically significant improvement in quality of life (QOL) as measured by the St. George's Respiratory Questionnaire [38]. Lack of clinical response to omalizumab did not predict failure or response to mepolizumab.

In real-life studies, mepolizumab treatment has been shown to reduce exacerbations and hospitalizations, improve asthma control, and reduce the OCS burden [39, 40, 41]. REDES was a real-life study conducted in Spain that evaluated the effectiveness and safety of mepolizumab in severe eosinophilic asthma that incorporates a prespecified stratification by blood eosinophil counts for the analysis of results. This study confirmed the effectiveness of mepolizumab in reducing clinically significant exacerbations, improving lung function, and decreasing OCS dependence and mean OCS dose at 12 months, regardless of baseline eosinophil counts as stated by the authors [42]. REDES included a prespecified stratification by blood eosinophil counts, but only 7.9% of the study population had counts below 150 eosinophils/μL.

Indications and Safety

Mepolizumab is approved for adults and children up to 6 years with severe asthma with an eosinophilic phenotype (although efficacy data in patients under 18 years of age are limited) [43]. Although the US Food and Drug Administration has not established a required blood absolute eosinophil count (AECs) for its use, RCTs have suggested a benefit for patients with a count as low as 150 eosinophils/μL, particularly in patients receiving chronic OCS. The efficacy of mepolizumab appears evident at blood eosinophil counts above 150 cells/uL and increases progressively with higher counts. Clinical response should be seen within four months, and mepolizumab treatment should be continued indefinitely if a clinical response is achieved.

Mepolizumab has been shown to have a similar safety profile to placebo. A herpes zoster vaccine (preferably recombinant) should be given four weeks before drug initiation in people 50 years of age or older.

Mepolizumab has also been approved for treating eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome) and hypereosinophilic syndrome at 300 mg every four weeks [44, 45]. In addition, mepolizumab has been approved to treat chronic sinusitis and nasal polyposis (CRScPN) [46, 47].

Reslizumab

Mechanism of Action

Reslizumab is a humanized IgG4k monoclonal antibody that binds to IL-5, thus preventing IL-5 from binding to its receptor on eosinophils, reducing eosinophils and, consequently, eosinophilic inflammation.

Efficacy

In patients with AECs≥400 cells/μL, reslizumab has been shown to reduce asthma exacerbations (50% to 59%) and improve lung function (a 90-126 mL improvement in FEV1) and a significant improvement in symptoms) [48]. No clinical trial has demonstrated an OCS-sparing.

Reslizumab lacks evidence of benefit in patients with eosinophils below 400 cells/μL. Some reports have demonstrated that IV weight-adjusted to reslizumab can rescue patients'

failures upon treatment with mepolizumab fixed-dose [49], attributed to a higher dose, the intravenous administration, or immune complex formation.

In a *post hoc* analysis, Bruselle et al. [50] found that relative to placebo, reslizumab induced a more significant reduction in asthma exacerbations and a superior improvement in lung function in patients with late versus early-onset asthma. That likely reflects the higher prevalence of type 2 airway inflammation in these patients.

Reslizumab has proven to reduce exacerbations and health care resource utilization and improve asthma control and quality of life in a real-world setting [51].

In addition, an open-label extension study showed that benefits in lung function were maintained over two years of uninterrupted treatment with reslizumab (3mg/kg q4w) [52]. In a real-life study, Pérez de Llano et al. [53] observed that reslizumab achieved total asthma control in approximately half of the patients with severe asthma. Treatment also demonstrated a statistically significant decrease in exacerbations, OCS doses, and symptom improvement.

Indications and Safety

Reslizumab is approved as an add-on treatment for patients 18 years of age and older with severe eosinophilic asthma (AEC≥400 cells/μL) and is available only by intravenous injection. Reslizumab is well tolerated, with adverse events like those in the placebo group. The most frequently reported adverse events were respiratory infections, headache, and worsening asthma [54]. The FDA gave a black-box warning because some cases of anaphylaxis occurred during the RCTs.

Benralizumab

Mechanism of Action

Benralizumab is a humanized IgG1k directed against the alpha chain of IL5R. The Fc portion lacks fucose residues, so the IgG receptors of natural killer cells recognize it, thus initiating the apoptosis of cells that present IL5RA, basically eosinophils and their precursors and part of the basophils. The clinical significance of the differences in the mechanism of action between benralizumab and other anti-IL-5 antibodies is unknown.

Efficacy

There are 3 phase III studies with benralizumab: SIROCCO [55] and CALIMA [56], which evaluated the effect of benralizumab on exacerbations, and ZONDA [57], which analyzed the reduction in OCS use. In the SIROCCO and CALIMA studies, benralizumab showed statistically significant reductions in exacerbation rate of 51% and 28%, respectively, and an improvement in prebronchodilator FEV1 greater than 100 mL compared with placebo. Both results were in the population with baseline eosinophil levels ≥300/μl. In both studies, benralizumab showed improvements in total asthma symptom scores and the Asthma Control Questionnaire (ACQ-6). ZONDA showed a 75% reduction in OCS dosage versus 25% with placebo.

BORA, a phase III extension study, demonstrated that the efficacy and safety profile of benralizumab was maintained after an additional year of treatment for patients who completed SIROCCO, CALIMA, or ZONDA and chose to continue long-term treatment [58]. The open-label MELTEMI extension study [59] confirmed the findings of SIROCCO, CALIMA,

ZONDA, and BORA trials, proving that long-term eosinophil depletion was not associated with an increase in severe infections and the decrease in eosinophil levels and rates of asthma exacerbations described in previous studies were maintained over the long term. These results further reaffirm the long-term safety and efficacy of benralizumab in achieving and sustaining asthma control over time among patients treated for up to 5 years.

In a real-life cost-effectiveness study, Padilla-Galo et al. [60] showed a clear improvement in asthma control and lung function, as well as a reduction in severe exacerbations, emergency room visits, OCS use, and optimized inhaled corticosteroid (ICS) in patients with eosinophilic asthma refractory to benralizumab treatment for one year.

Finally, in a *post hoc* analysis, Menzies-Gow et al. [61] analyzed whether clinical remission (defined as zero exacerbations, zero OCS use, ACQ-6 score ≤ 0.75, and pre-bronchodilator FEV1 increase ≥ 100 mL after 6 or 12 months) could be achieved upon benralizumab treatment, finding that, overall, 15–23% of patients achieved clinical remission in 6 months, and approximately 15% achieved remission within 12 months.

Indications and Safety

Benralizumab has been approved for >12 years old asthmatic patients with uncontrolled eosinophilic asthma (AEC >300 cells/uL). At least a 4-month trial is recommended to assess response, although if exacerbations are an essential objective, it should be extended to 12 months.

Benralizumab is generally well-tolerated and has a safety profile similar to other anti-IL-5 blockers, but it can rarely induce hypersensitivity reactions [62].

Although benralizumab is not yet approved for nasal polyp treatment, two phase III trials have been developed: OSTRO [63] and ORCHID [64]. The results of OSTRO have been published, showing that benralizumab, when added to baseline treatment, significantly reduced nasal polyp score and nasal obstruction. Improvements in Sinonasal Outcome Test 22 score at week 40 and time to first NP surgery and/or SCS use for NP were not statistically significant between treatment groups. Nominal significance was obtained for improvement in difficulty in the sense of smell score at week 40. ORCHID is expected to be read out in the second half of 2023.

Clinical trials are undergoing to evaluate the efficacy of benralizumab compared to mepolizumab in treating eosinophilic granulomatosis with polyangiitis [65].

Anti–IL-4/IL-13

Dupilumab

Mechanism of Action

Dupilumab is a fully human IgG4k monoclonal antibody that targets IL4RA and blocks the signaling of both IL-4 and IL-13, two critical T2 cytokines.

Efficacy

The clinical development program for dupilumab in asthma comprised three studies: two of 24 and 52 weeks (DRI12544 and LIBERTY ASTHMA QUEST), whose primary endpoints were

the annualized rate of severe asthma exacerbations and the absolute change from baseline in FEV1 before bronchodilator use [66, 67] and the 24-week LIBERTY ASTHMA VENTURE that evaluated the effect of dupilumab in reducing SOC use [68]. Selected patients had moderate-severe asthma regardless of baseline levels of type 2 biomarkers. In the trial population, prespecified subgroup analyses were performed based on baseline levels of blood eosinophils and FeNO.

The phase III QUEST study compared two dupilumab doses (200 mg and 300 mg) in an unselected population. Dupilumab demonstrated a statistically significant reduction in the annual rate of exacerbations compared to placebo in the intention-to-treat-population (47.7% reduction for dupilumab 200 mg; 46% reduction with dupilumab 300 mg); improvement in FEV1 (140 ml improvement with dupilumab 200 mg over placebo and 130 ml improvement with dupilumab 300 mg over placebo).

In the Phase III VENTURE study, dupilumab produced a statistically significant overall discontinuation of oral glucocorticoid use compared to placebo (70.1% with dupilumab 300 mg vs. 41.9% in the placebo group). In QUEST and VENTURE, it was subsequently demonstrated that efficacy was statistically significant only in patients with elevated FeNO (≥25 ppb and/or blood eosinophil counts ≥150/μL). Prespecified subgroup analyses showed that better responded to treatment subgroups of patients with baseline blood eosinophil levels ≥ 300/μL and/or FeNO ≥ 50 ppb and in those patients with combined elevation of eosinophils ≥150/μL and FeNO ≥ 25 ppb.

Most of the patients who participated in the DRI12544, QUEST, and VENTURE studies were selected for TRAVERSE's open-label extension study [69]. Patients received 300 mg of dupilumab for up to 148 weeks of treatment. The safety profile of dupilumab in TRAVERSE was consistent with the safety profile observed in pivotal asthma studies of up to 52 weeks of treatment. Efficacy, measured as a secondary endpoint, was similar to the results observed in the pivotal studies and was maintained for up to 96 weeks.

In phase 3 pivotal study in 6-11year-old children (VOYAGE), dupilumab reduced exacerbations, increased FEV1, and lowered FeNO levels [70].

In a real-life retrospective cohort study involving adults with severe asthma (mainly oral glucocorticoid–dependent), add-on therapy with dupilumab significantly improved asthma control and lung function and reduced oral steroids use and exacerbations rate [71].

Indications and Safety

Dupilumab has been approved as additional maintenance therapy in adults and adolescents aged 12 years and older with severe asthma with type 2 inflammation characterized by elevated blood eosinophils and/or elevated FeNO, who are not adequately controlled with high-dose ICS in combination with another drug for maintenance therapy.

The most frequent adverse events are injection-site reactions. Hypereosinophilia (AEC≥1500 cells per microliter) can appear in 4-25% of patients and persist after six months in 14%. Although dupilumab-induced hypereosinophilia is usually asymptomatic, cases of eosinophilic granulomatosis with polyangiitis have been rarely reported. In contrast with the studies of dupilumab in atopic dermatitis, no meaningful differences in adverse events of conjunctivitis and oral herpes were found between dupilumab and placebo groups.

Dupilumab is approved to treat other type-2-high diseases that can coexist with severe asthma: atopic dermatitis [72] and CRSwNPCRS [73].

Anti-Epithelial Cytokine Antibodies

The epithelium can no longer be considered a mere barrier, but it has a predominant role in the inception and maintenance of immune response. When external agents (allergens, viruses, pollutants) produce epithelial damage, the epithelium activates and releases the so-called alarmins; TSLP (thymic stromal lymphopoietin), IL-25, and IL-33. In turn, alarmins activate multiple cells initiating and enhancing downstream inflammation. In recent times, alarmins have been one of the targets in developing new anti-asthma biologics, with the rationale that interfering upstream in the inflammatory cascade might improve asthma outcomes in a broader patient population. Thus, anti-TSLP (tezepelumab), anti-IL-33 (itepekimab), and astegolimab, an anti-suppressor of tumorigenicity 2 (ST2), have been developed.

Tezepelumab

Mechanism of Action

Tezepelumab is an IgG2λ monoclonal antibody that binds to TSLP, precluding it from interacting with its heterodimeric receptor, thus blocking the effects of this alarmin. In asthmatic patients, TSLP levels have been shown to correlate with airway obstruction, disease severity, and glucocorticoid resistance [74, 75].

Efficacy

NAVIGATOR was a phase 3 trial (a phase 3 RCT) involving adolescents and adults with severe uncontrolled asthma, in which add-on monthly therapy with tezepelumab (210 mg administered subcutaneously) significantly reduced the annualized asthma exacerbation rate in patients with T2-high and T2-low asthma [76]. Tezepelumab also improved lung function, asthma control, and quality of life; blood eosinophil counts, FeNO, and IgE were reduced. This study confirmed the phase 2b PATHWAY trial [77].

CASCADE [78] was a phase 2 mechanistic bronchoscopy trial evaluating the effect of tezepelumab on airway inflammatory cells, remodeling, and hyperresponsiveness in patients with moderate-to-severe uncontrolled asthma. A significant reduction of eosinophils in the airway submucosa was found, although neutrophils, mast cells, or T cells were not significatively reduced, nor were differences in reticular basement membrane thickness and epithelial integrity. A reduction of bronchial hyperresponsiveness to mannitol by Tezepelumab was observed.

SOURCE was a phase 3 trial that evaluated the effect of tezepelumab in OCS reduction in OCS-dependent asthma, irrespective of blood eosinophil county [79]. The primary objective, i.e., the categorized percentage reduction from baseline in daily oral corticosteroid dose at week 48 without losing asthma control, was not fulfilled. However, an improvement was observed in participants with baseline blood eosinophil counts of at least 150 cells per μL.

Finally, DESTINATION is a phase 3, multicentre, randomized, double-blind, placebo-controlled, parallel-group, ongoing trial aimed to evaluate the long-term safety and tolerability of tezepelumab in adults and adolescents with severe, uncontrolled asthma [80].

Indications and Safety

In December 2021, the FDA approved tezepelumab as an add-on maintenance treatment for adults and pediatric patients 12 years of age or older who have severe asthma, without phenotype or biomarker limitation.

In pivotal studies, the most common adverse events described for tezepelumab were nasopharyngitis, upper respiratory tract infection, and headache.

Itepekimab

In a proof-of-concept, phase 2 trial, patients were randomized to receive 300 mg of itepekimab (a monoclonal antibody directed against IL-33), 300 mg of itepekimab plus 300 mg dupilumab, 300 mg of dupilumab, or placebo; all of them administered fortnightly and subcutaneously [81]. Itepekimab showed efficacy in asthma control and FEV1, quality of life, and was the only active treatment that produced a reduction of eosinophils. The combined treatment of itepekimab and dupilumab did not provide additional benefits to those of individual treatments.

Astegolimab

In a phase 2b trial, astegolimab (an anti-ST2 receptor) administered subcutaneously every four weeks reduced exacerbations compared with placebo at the doses of 70 mg or 490 mg, but not 210 mg, without improving lung function [82].

Indirect Treatment Comparisons among Biologics

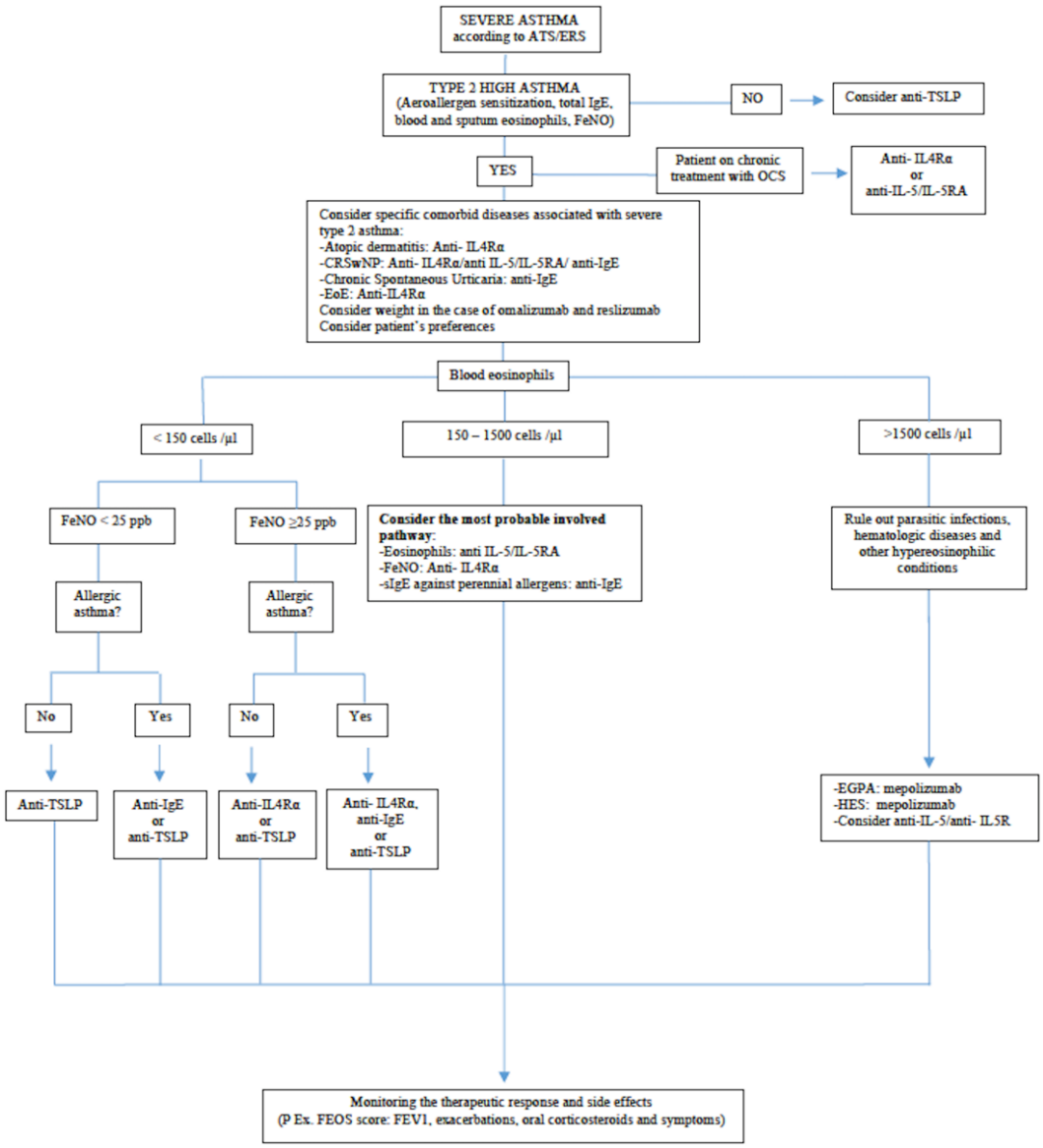
As there are no head-to-head comparative studies with biologics in the treatment of asthma, indirect treatment comparisons using different approaches have been performed [83]. The main problem of this type of studies is the different inclusion and exclusion criteria. Maybe a comparison of efficacy matching blood eosinophil counts could be valid. According to Pavord et al. [83], in patients with a baseline peripheral blood count of 300 eosinophils/μL or higher, improvement of exacerbations seems similar, whereas dupilumab tended to be associated with greater improvement in FEV1.

Selection of Biologic for Severe Uncontrolled Asthma

In the absence of head-to-head clinical trials, several algorithms for selecting biologics in severe asthma patients have been published [84, 85, 86]. There has been a clear evolution from anti-IgE predominance to more complex models and, lately, to algorithms based on the purportedly more relevant pathway. A new algorithm for selecting biologics for the treatment of SA is proposed (Figure 1).

Anycase, in the selection of a biologic for the treatment of asthma, several steps should be considered:

1. Reassurance that the patient has a diagnosis of severe uncontrolled asthma.
2. Ensuring that the patient is adherent to treatment and his/her inhalation technique is adequate.



OCS: oral corticosteroid; CRSwNP: chronic rhinosinusitis with nasal polyps; EoE: Eosinophilic esophagitis; EGPA: Eosinophilic granulomatosis with polyangiitis; HES: hypereosinophilic syndrome.

Figure 1. Algorithm for biologic selection in severe uncontrolled asthma.

3. Check whether any factors are susceptible to deteriorating asthma, such as allergen exposure, tobacco, irritants, obesity, gastroesophageal reflux, or others. Weight should be considered for omalizumab and reslizumab; also, consider the pharmacoeconomic point of view.
4. Demonstration of a T2 mechanism (>150 eosinophils/µL, FeNO ≥ 20 ppb or presence of clinically relevant allergy. If values are close to thresholds, repeated checking is

recommended). If the patient has non-T2 asthma, consider tezepelumab if the goal to improve is exacerbations.

5. Evaluate possible factors capable of influencing the selection of the biologic. Thus, if the patient had nasal polyposis, consider dupilumab, omalizumab, or mepolizumab; if the patient has chronic spontaneous urticaria, consider omalizumab; for concomitant atopic dermatitis, consider dupilumab; for oral corticodependent patients, consider dupilumab, mepolizumab or benralizumab.
6. Check what the possible dominant pathway in the patient is. That is the most challenging part. It is based on three biomarkers: *eosinophils*, *FeNO*, and *allergen sensitization*. If eosinophils are dominant, the suggested choice is anti-IL5/IL5RA (there is no precise data to select among them). If FeNO is dominant, the suggested choice is anti-IL4RA. If allergy is dominant, the suggested choice is anti-IgE. For non-T2 asthma, tezepelumab is the only biologic that has shown efficacy in reducing exacerbation. Tezepelumab could be used in the three mentioned situations. If no one is dominant, any possible biologics can be chosen. Anycase, for a patient with more than 1500 eosinophils/μL, the choice must be IL5/IL5RA.
7. Review at 4 months evaluating OCS reduction, ACT, and lung function. For exacerbations, one year of treatment is recommended. Recently a quantification method has been suggested (FEOS) [87], although it has not been validated. If no improvement is seen, switching the biologic should be considered.

Conclusion

Biologic therapy is revolutionizing the treatment of SA and other comorbid conditions. Anti-IgE, anti-IL-5/IL-5R, and anti-IL-4RA are biological agents that are effective therapy for treating T2 SA. These therapies have significantly decreased exacerbation rates and improved lung function and the quality of life in moderate to severe asthma. Tezepelumab (anti-TSLP) is the only biological approved for treating severe asthma without phenotype or biomarker limitation. The selection of a particular biologic should be based on a holistic approach considering all the facets of both patient and disease.

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Chapter 18

Clinical Pharmacogenomics in Asthma Therapy

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Abstract

Asthma represents one of the most prevalent chronic respiratory illnesses. The goal of asthma treatments is to minimize symptoms, ameliorate patients' quality of life, prevent adverse outcomes and improve lung function. The pharmacological options used for asthma treatment are classified into three main categories: controller medications, reliever drugs, and add-on biological therapies directed against specific eosinophilic or allergic targets.

In this chapter, we analyze the multiple genetic markers that determine the response to asthma therapies and evaluate the potential clinical relevance of these genetic determinants to identify specific and potentially actionable pharmacogenetic profiles. In this respect, genetic studies have provided a large insight by searching pharmacogenetic biomarkers to predict the responsiveness to asthma therapy. However, the evidence of the SNVs associated with asthma treatment response is not still sufficiently conclusive to be translated to clinical practice to optimize drug asthma treatment. Future efforts are still needed to implement personalized therapeutics strategies and achieve better outcomes.

Keywords: asthma treatment, personalized medicine, pharmacogenomics, corticosteroids, anti-leukotrienes, beta-agonists, biological therapies

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Introduction

Asthma represents the second most prevalent chronic respiratory illness, affecting almost 300 million people. The estimated incidence is around 43 million people worldwide, being the second leading cause of death among chronic respiratory diseases [1, 2]. Global Initiative for Asthma (GINA) defines this illness as a heterogeneous disease that involves a history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough with variably expiratory airflow limitation [3].

The goal of asthma treatments is to minimize symptoms, ameliorate patients' quality of life, prevent adverse outcomes and improve lung function. Several asthma guidelines have been elaborated to facilitate asthma management [3, 4].

The pharmacological options used for asthma treatment are classified into 3 main categories:

1. *Controller medications*: These drugs aim to reduce airway inflammation, control symptoms, decrease the occurrence of exacerbations and prevent pulmonary dysfunction.
2. *Reliever drugs*: These are prescribed as needed for relief of symptoms or to prevent exercise-induced bronchoconstriction.
3. *Add-on biological therapies directed against specific eosinophilic or allergic targets*: They are mainly prescribed for patients with persistent symptoms or severe asthma [3].

Despite the development of new treatment strategies, 5% to 10% of asthma patients manifest uncontrolled episodes and exacerbations [5]. Also, clinical trials have demonstrated a large variation in bronchodilator response and wide heterogeneity in improvement in lung function upon asthma therapy [6]. This variability might be due to heterogeneous factors, such as lack of adherence to treatments or different comorbidities including obesity or chronic rhinosinusitis [3]. Moreover, extensive differences in asthma prevalence have been identified among ethnic groups [7]. These wide differences suggest a role of genetic background in the asthma treatment response. In fact, several studies have demonstrated a large genetic contribution to asthma susceptibility and diverse loci have been associated with childhood-onset asthma or certain asthma phenotypes [8].

In this chapter, we analyze the multiple genetic markers that determine the response to asthma therapies and evaluate the potential clinical relevance of these genetic determinants to identify specific pharmacogenetic profiles (Table 1). Consequently, this might help to implement personalized therapeutic strategies and achieve better outcomes.

Table 1. Summary of pharmacogenetics factors involved in asthma therapy response

Gene	SNV	Population, *n*	Major clinical findings associated with the minor allele	Ref.
Inhaled corticosteroids				
CRHR1	rs242941	USA, Caucasians, n= 781	Positive treatment response and improved FEV1	[9]
	rs242941	USA, adults and children, n = 129	Decreased FEV1% predicted	[10]

Gene	SNV	Population, *n*	Major clinical findings associated with the minor allele	Ref.
	rs242941	Netherlands, n=164	No association with improved FEV1	[11]
	rs242941	Turkey, children, n= 82	No association with improved FEV1	[12]
	rs242941	USA, children, n=311	No association with improved FEV1	[13]
	rs1876828	USA, Caucasians, n= 336	Positive treatment response and improved FEV1	[9]
	rs1876828	USA, adults and children, n = 129	Improvement of FEV1% predicted	[10]
	rs1876828	Netherlands, n=164	No association with improved FEV1	[11]
	rs1876828	Turkey, children, n= 82	No association with improved FEV1	[12]
STIP1	rs4980524	USA, adults, n = 439	Lower FEV1 and FEV1%pred	[14]
	rs6591838	USA, adults, n = 439	Lower FEV1 and FEV1%pred	[14]
	rs1011219	USA, adults, n = 439	Lower FEV1 and FEV1%pred	[14]
	rs2236647	USA, adults, n = 439	Lower FEV1 and FEV1%pred	[14]
	rs2236647	Tunisian, adults, n = 230	No association with FEV1%pred	[15]
	rs2236648	USA, adults, n = 439	Lower FEV1 and FEV1%pred	[14]
	rs2236648	Tunisian, adults, n = 230	No association with FEV1%pred	[15]
NR3C1	rs4142327	Turkey, children, n= 82	Improvement in FEV1	[12]
DUSP1	rs881152	Multi-ethnic, n= 646	Greater bronchodilator response	[16]
HDAC1	rs17411981	Korean, adults and children, n= 105	Lower improvement in FEV1	[17]
CYP3A4	rs35599367	USA, children, n= 734	Improvement in asthma control score	[18]
CYP3A5	rs776746	USA, children, n= 64	Improvement in asthma control score	[19]
TBX21	rs2240017	Multi-ethnic, children, n= 195	Improvement in PC20	[20]
TBX21	rs2240017	Korean, adults, n= 53	Worse control of symptoms	[21]
TBX21	rs2240017	Turkey, children, n= 82	No association with improved FEV1	[12]
TBX21	rs9910408	Slovenian, adults, n = 208	Poorer improvement in FEV1	[22]
GLCCI1	rs37972	USA, adults, n = 844, children, n =219	Decreased lung function in response to ICSs	[23]
	rs37972	Chinese, adults, n = 182	Poorer improvement in FEV1 in response to ICSs	[24]
	rs3797	Tunisian, adults, n = 230	Poorer improvement in FEV1 in response to ICSs	[15]
	rs37973	Chinese, adults, n = 182	No association with FEV1	[24]
	rs37973	Chinese, adults, n = 418	Diminished clinical response to ICSs	[25]
	rs37973	Japanese, adults, n = 224	Associated with a decline in FEV1	[26]
	rs37973	Slovenian, adults, n = 208	Better treatment response to ICSs	[27]
	rs37973	USA, adults and adolescents, n = 1924	No FEV1 changes associated	[28]
	rs37973	Tunisian, adults, n = 230	Poorer improvement in FEV1 in response to ICSs	[15]
T gene	rs1134481	USA, Caucasians adults and children, n = 418	Worse FEV1 values	[29]
	rs1134481	Netherlands, adults, n= 597 Multi-ethnic, adults, n = 9842	Increased frequent exacerbation risk	[30]

Table 1. (Continued)

Gene	SNV	Population, *n*	Major clinical findings associated with the minor allele	Ref.
	rs2305089	USA, Caucasians adults and children, n = 418	Worse FEV1 values	[29]
	rs3099266	USA, Caucasians adults and children, n = 418	Worse FEV1 values	[29]
FBXL7	rs10044254	USA, white children, n= 124 USA, white children, n= 77	Increased asthma symptom scores	[31]
APOBEC3B-APOBEC3C	rs5995653	Multi-ethnic, children, n = 1347 European, children, n= 1697	Improvement in FEV1	[32]
ORMDL3	rs2872507	Slovenian, children, n= 311	Improvement in FEV1	[33]
ORMDL3	rs72821893	Netherlands, children, n= 110	A reduction in FEV1%pred	[34]
VEGFA	rs2146323	Slovenian, children, n= 40	Greater improvement in FEV1	[35]
VEGFA	rs3025039	Chinese, children, n= 128	Smaller change in FEV1	[36]
FCER2	rs28364072	Netherlands, children, n= 1325	Increased risk of asthma-related hospital visits	[37]
FCER2	rs28364072	USA, children, n= 311	Worse lung function response after ICSs therapy	[13]
FCER2	rs28364072	USA, children, n= 311	Severe exacerbations	[38]
Anti-leukotriene agents				
ALOX5	Microsatellite located in the Sp1-binding domain	USA, adults, n= 221	Worse FEV1 response	[39]
	Microsatellite located in the Sp1-binding domain Microsatellite located in the Sp1-binding domain	Spain, adults and adolescents, n= 61	More asthma exacerbations and poorer improvement of FEV1	[40]
	Microsatellite located in the Sp1-binding domain	Multi-ethnic, adults, and adolescents n= 174	Improvement in peak expiratory flow	[41]
	Microsatellite located in the Sp1-binding domain	UK, adults, n= 52	No association with bronchodilator response	[42]
	Microsatellite located in the Sp1-binding domain	USA, adults, n= 252	Lesser risk of exacerbations	[43]
	Microsatellite located in the Sp1-binding domain	USA, children, and adolescents, n= 270	Poor control of asthma and reduced lung function	[44]
	rs2115819	USA, adults, n= 61	higher FEV_1 response to montelukast	[43]
	rs2115819	USA, adults, n= 577	Better response to montelukast and zileuton	[45]
	rs2115819	Caucasian, adults, n= 189	No association with changes in FEV1 after montelukast therapy	[10]
	rs2115819	Asian, adults, n= 52	No association with changes in FEV1 after montelukast therapy	[46]
	rs4987105	Multi-ethnic, adults, and adolescents n= 174	Improvement in the peak expiratory flow	[41]
	rs4986832	Multi-ethnic, adults, and adolescents n= 174	Improvement in the peak expiratory flow	[41]
LTA4H	rs2660845	USA, adults, n= 61	Increase risk of asthma exacerbations	[43]
	rs2660845	Japan, adults, n= 62	Poor response to montelukast	[46]
	rs2660845	European, children and adults, n= 523	Increased risk of asthma exacerbations	[47]
	rs2540491	Puerto Rico and Mexico, children and adolescents, n= 649	Increase in FEV1 associated with leukotriene modifier therapy	[48]

Gene	SNV	Population, *n*	Major clinical findings associated with the minor allele	Ref.
		Puerto Rico and Mexico, children and adolescents, n= 649	Increase in FEV1 associated to leukotriene modifier therapy	[48]
LTC4S	rs730012	UK, adults, n= 23	Improvement in FEV1 and forced vital capacity with zafirlukast treatment	[49]
	rs730012	USA, adults and adolescents, n= 12	Better response to montelukast	[50]
	rs730012	USA, adults, n= 61	Decreased risk of asthma exacerbation	[43]
	rs730012	Japan, adults, n= 349	Better FEV1 response to pranlukast	[51]
	rs272431	USA, adults, n= 577	Improved lung function to zileuton	[45]
ABCC1	rs119774	USA, adults, n= 61	An increase in % predicted FEV1 after montelukast therapy	[43]
	rs119774	USA, adults, n= 577	Improved FEV1 after zileuton therapy	[45]
	rs215066	USA, adults, n= 577	Improved FEV1 after zileuton therapy	[45]
SLCO2B1	rs12422149	USA, adults, n= 489	Lower montelukast plasma concentrations	[52]
	rs12422149	USA, adolescents, n= 26	Lower montelukast plasma concentrations	[53]
	rs12422149	Caucasian, adults, n= 16	No effects on montelukast plasma levels	[54]
	rs12422149	Asian, adults, n= 24	No effects on montelukast plasma levels	[55]
	rs12422149	Chinese, children, n= 50	Higher clearance of montelukast	[56]
CYSLTR1	rs773347588	Korea, adults, n= 89	Anti-leukotriene longer requirements for management of aspirin-intolerant asthma patients	[57]
CYSLTR2	rs912277	Multi-ethnic, adults, and adolescents n= 174	Improvement in peak expiratory flow after treatment with montelukast	[41]
	rs912278	Multi-ethnic, adults, and adolescents n= 174	Improvement in peak expiratory flow after treatment with montelukast	[41]
MLLT3	rs6475448	Multi-ethnic, adults, and children n= 317	Increased ΔFEV1 in response to montelukast	[58]
MRPP3	rs12436663	Multi-ethnic, adults, n= 526	Significant reduction in ΔFEV1 in response to zileuton	[59]
Beta-agonists				
ADRB2	rs1042713	USA, adults, n= 190	Decrease in peak expiratory flow in response to salbutamol	[60]
	rs1042713	New Zealand, adults, n= 157	Increased risk of asthma exacerbations with the use of salbutamol	[61]
	rs1042713	USA, adults, n= 78	Lower morning peak expiratory flow rate during salbutamol treatment	[62]
	rs1042713	Italian, children, n= 100	Lower FEV1 values in response to fenoterol	[63]
	rs1042713	USA, adults, and adolescents, n= 174	No association with response to salmeterol	[64]
	rs1042713	USA, adults, and adolescents, n= 2630	No association with response to salmeterol	[65]

Table 1. (Continued)

Gene	SNV	Population, *n*	Major clinical findings associated with the minor allele	Ref.
	rs1042713	USA, adults, n= 87	No association with response to salmeterol	[66]
	rs1042713	USA, adults, and adolescents, n= 544	No association with response to salmeterol	[67]
	rs1800888			[68]
	rs1800888	USA, adults, and adolescents, n= 174	No association with response to salmeterol	[64]
	rs1800888	USA, adults, and adolescents, n= 544	No association with response to salmeterol	[67]
	rs1800888	India, adults, n= 398	Less response to Salbutamol in patients with persistent severe asthma	[69]
	rs1800888	USA, adults, n= 659	Increased risk of asthma exacerbation in response to LABA	[70]
ADCY9	rs2230739	USA, children, n = 436	Improvement in FEV1 in response to salbutamol associated with budesonide	[71]
	rs2230739	Korea, adults, n = 86	Improvement in FEV1 in response to LABA associated with ICSs	[72]
ARG1	rs2781659	USA, adults and children, n= 962	Lower bronchodilator response	[73]
	rs2781659-rs2781663-rs2781665-rs60389358	USA, adults, n= 96	Lower bronchodilator response	[74]
ARG2	rs17249437 rs3742879	Netherlands, adults, n= 200	Lower lung function	[75]
CRHR2	rs73294475	USA, Latino children, n= 1782	Better bronchodilator response	[76]
THRB	rs892940	USA, children = 607 and adults, (n= 435 and n= 155)	Better bronchodilator response	[77]
SPATS2L	rs295137	USA, adults, n= 1644	Better bronchodilator response	[78]
	rs295137	USA, children and adults, n= 604	Better bronchodilator response	[79]
Biologic agents				
IL4RA	rs8832	USA, non-Hispanic, adults, n= 407	Increased risk of asthma exacerbation in response to pitrakinra	[80]

Adapted from García-Menaya et al. [81].

Drugs Used in Asthma Treatment

Inhaled Corticosteroids (ICSs)

ICSs constitute the cornerstone of asthma treatment by exerting a range of antiinflammatory effects, improving pulmonary capacity, and reducing the risk of asthma exacerbations [3]. ICSs mechanism of action is through binding to the intracellular glucocorticoid receptor. This interaction mediates the upregulation of antiinflammatory genes and genes encoding β_2-adrenergic receptors [82]. Throughout the last years, pharmacogenetic studies have examined

the effects on ICSs response of genetic variants in genes involved in functional pathways, activity regulators, and pharmacokinetics of corticosteroids [81].

Firstly, we summarize the main evidence from studies on genes involved in ICSs functional pathways. Corticotropin-releasing hormone receptor 1 (CRHR1) is activated by the corticotropin-releasing hormone to modulate the release of adrenocorticotropic hormone and regulate endogenous corticosteroid levels. CRHR1 is encoded by the *CRHR1* gene [83]. Thus, the intronic single nucleotide variations (SNVs), rs242941 and rs1876828, in the *CRHR1* gene have been associated with positive treatment response and improved forced expiratory volume in 1 second (FEV1) [9]. An additional study also correlated these SNVs, rs242941, and rs1876828, with a decreased and an increased percent predictive FEV1 (FEV1%pred), respectively [10]. However, 3 additional studies were unable to corroborate this evidence [14-16]. Therefore, overall data are inconclusive so far and further studies are required.

The steroid binding, trafficking, and turnover of the glucocorticoid receptor (GR) is regulated by a multiprotein hsp90/hsp70-based chaperone system where the stress-induced phosphoprotein 1 (STIP1) modulates the chaperone activity of the hsp90/hsp70 complex [84]. Several intronic SNVs in the *STIP1* gene, rs4980524, rs6591838, rs2236647, rs2236648, have been associated with the baseline FEV1 and FEV1%pred. Also, rs6591838 and rs2236647 correlated with the lung function response after 4 weeks, whereas rs6591838 and rs1011219 were associated after 8 weeks of ICSs therapy [14]. However, the rs2236647 and rs2236648 variants were not found associated with a change in FEV1%pred after 12 weeks of ICSs therapy in a Tunisian cohort [15]. Additionally, the nuclear receptor subfamily 3 group C member 1 (*NR3C1*) gene encodes the basis for the different glucocorticoid isoforms [85]. Keskin et al. correlated the intronic *NR3C1* rs4142327 with an improvement in FEV1 in children treated with ICSs [12]. Also, additional variants in genes related to corticosteroid pathways have been explored. Thus, the protein kinase dual-specificity phosphatase 1 (DUSP1) reduces the expression and production of proinflammatory cytokines whereas histone deacetylase (HDAC1) removes acetyl groups from histones to regulate inflammatory gene expression [86, 87]. In this regard, the *DUSP1* rs881152 and *HDAC1* rs17411981 variants have been associated with a greater bronchodilator response or a lower improvement in FEV_1, respectively [16, 17]. Nevertheless, none of these SNVs in *STIP1* and *NR3C1*, or *HDAC1* genes have been replicated in independent studies so far.

Regarding genetic variations in the pharmacokinetics of ICSs, CYP3A4, CYP3A5, and CYP3A7 are the predominant isoforms which metabolize the most frequently prescribed ICSs [88]. Variants in the genes coding for these isoforms may affect ICSs metabolism and generate variability in response to these drugs. Two independent studies have explored this issue so far. The rs35599367 variant, which is the signature SNV for the *CYP3A4*22* allele, and causes a splice defect, is related to a decreased CYP3A4 activity, and the rs776746 variant, which is the signature allele for *CYP3A5*3*, and causes a splice defect, encode a nonfunctional CYP3A5 and have been associated with a significant improvement in asthma control scores [18, 19].

Additionally, a heterogeneous group of studies has been focused on variants in genes related to the regulation of ICSs activity. *TBX21* encodes for transcription factor T-bet, which is responsible for T helper cells regulation and has been implicated in the pathogenesis of asthma [89]. The nonsynonymous variant *TBX21* rs2240017 has been associated with a significant decrease in airway responsiveness in children [20]. Nevertheless, this evidence showed discrepancies with the study carried out by Ye et al., which demonstrated that adult patients who carried the mutant allele showed a worse symptom control [21]. Additionally,

Keskin et al. did not find a significant association between rs2240017 and FEV_1 change in children [12]. These findings suggest that further investigations are required to determine the role of *TBX21* rs2240017 as a genetic predictor of response to ICSs. More recently, the intronic variant rs9910408, which is placed in the *TBX21* 5´ region has been associated with response to ICSs treatment. Particularly, individuals carrying the mutant allele showed a poorer improvement in bronchial hyperresponsiveness and FEV_1 [22]. However, the functional consequences of this SNV are unknown.

Genome-wide association studies (GWAS) have been conducted to detect pharmacogenetic variants related to ICSs response. These GWAS have identified 9 new loci predicting the symptomatic response to these drugs. These loci comprise SNPs within the genes *GLCCI1, T, FBXL7, CMTR1, APOBEC3, THSD4, HIVEP2, ROBO2,* and *ALLC* [32-39, 90-92]. The SNV rs37972, which is placed in the promoter region of the glucocorticoids-induced transcript 1 (*GLCCI1*) was significantly associated with a decreased response to ICSs therapy [23]. This polymorphism has no known functional effect. However, this variant is in complete linkage disequilibrium with the rs37973 SNV. This latter has been associated with a reduced expression of *GLCCI1* [23]. The association of the SNVs rs37972 and rs37973 with the decreased response to ICSs was also corroborated by independent studies carried out in Asian populations [40-42]. However, these findings were not confirmed in different studies [27, 28]. Afterward, *Salhi* et al. corroborated the association between rs37973, but not rs37972, and a worse response to ICSs after 12 weeks of treatment [15] whereas contradictory results were reported for the rs37973 variant [30]. Therefore, further studies to elucidate the role of *GLCCI1* variants are warranted.

Regarding the *T* gene locus, 3 SNVs were detected. The SNVs rs1134481 and rs3099266, which are located in the gene flanking regions, and the missense variant rs2305089 (Gly177Asp) [29]. Individuals carrying the mutant allele of any of the mentioned SNVs had a worse FEV1 response [29]. Recently, the SNV rs1134481 minor allele was also associated with an increased frequency of exacerbation [30]. The *T* gene encodes a transcription factor that may interact with the gene encoding the glucocorticoid receptor *NR3C1* [29]. Therefore, this evidence might contribute to understanding the mechanistic basis of the ICSs variability. Furthermore, the intronic variant *FBXL7* rs10044254 has been associated with a decreased expression of *FBXL7,* and individuals carrying the rs10044254 minor allele in homozygosity showed an increase in asthma symptom scores in pediatric patients but not in adult patients [31]. Nevertheless, no further studies have tried to confirm these promising findings. Recently, Hernández-Pacheco et al. performed a GWAS in a population comprised of admixed children with asthma treated with ICSs. This study identified an association between the minor allele of rs5995653 and an improvement in FEV1 [32]. This SNV is placed in the intergenic region of *APOBEC3B* and *APOBEC3C*. The encoded proteins APOBEC3B and APOBEC3C play a role as restrictors of viral infections and regulate the expression of genes involved in several cellular processes in the lung [32]. Finally, a genome-wide interaction study evaluated the interaction of genetic variation and age on ICSs response and found associated two intronic SNVs *THSD4* rs3463160 and *HIVEP2* rs2328386 [93]. Nonetheless, the functional effects of both SNVs remain unknown and additional studies are warranted to elucidate their role in responsiveness to ICSs.

Two variants of the Orosomucoid like-3 (OM1-like protein 3; *ORMDL3*) have shown an association with the response to ICSs. This gene encodes a protein that mediates the synthesis of sphingolipids which exerts a pivotal role in synthesizing inflammatory proteins [94].

Children who were carrying the minor allele of the *ORMDL3* intergenic variant rs2872507 in homozygosity demonstrated an improvement in FEV1 [33]. Moreover, the SNV rs72821893 was linked to ICSs treatment response in asthmatic children [34]. More recently, a GWAS has identified several genetic variants of *ORMDL3*, none identified in the association studies cited, as markers of asthma susceptibility in children [93]. However, although variants in *ORMDL3* may play a role in the underlying mechanism and susceptibility to asthma, the lack of evidence of functional effects for these two SNVs requires further studies to unveil the role of these SNVs in the interindividual variations in response to ICSs.

The SNV *VEGFA* rs2146323 has been associated with response to ICSs therapy in children. Asthma patients who were homozygous for the minor allele had a greater improvement in the FEV1 [35]. *VEGFA* gene encodes the vascular endothelial growth factor (VEGFA), one main angiogenesis regulator which is elevated in asthma patients [35]. Therefore, it is reasonable to assume that SNVs in *VEGFA* may affect the response to ICSs. However, this variant is in a noncoding region, and its functional consequences remain unknown. Recently, Wan et al. identified an association between the *VEGFA* rs3025039 and changes in FEV1/FVC in asthmatic children [36]. However, this preliminary work requires additional replication studies.

The most consistent significant association between SNVs and ICSs response involved the SNV *FCER2* rs28364072. This gene encodes the Fc fragment of IgE receptor II (CD23). The rs28364072 variant is related to a decreased *FCER2* expression and has a negative impact on the normal negative feedback in the regulation of IgE synthesis [38]. The rs28364072 SNV has been associated with poor ICSs response in 2 follow-up studies including children with asthma where symptoms were measured by an asthma control questionnaire [37] or exacerbations and FEV1 response [13, 38]. Therefore, overall findings reflect that the rs28364072 variant might be a promising biomarker to predict the ICSs response in asthma patients.

Anti-Leukotriene Agents

Anti-leukotrienes agents are prescribed for patients with moderate to severe persistent asthma, they are considered as an adjuvant therapy to ICSs in step 3 or 2 of the GINA guideline [3]. Anti-leukotrienes agents' interplay with the leukotriene pathway to reduce inflammatory processes and bronchoconstriction. These comprise 2 groups: leukotriene receptor antagonists (LTRAs) which prevent leukotrienes from binding to the cysteinyl leukotriene receptor 1 (CysLTR1); and leukotriene inhibitors which block the 5-lipoxygenase (5-LO) enzyme. LTRAs are the most commonly anti-leukotriene agents prescribed for asthma patients [6]. In recent years, numerous pharmacogenetic studies have analyzed the effect of genetic variants on the variability of anti-leukotriene agents' responsiveness; being the response to LTRAs the most common effect assessed [6, 81].

The 5-LO enzyme is encoded by the *ALOX5* gene. This enzyme catalyzes the arachidonic acid biotransformation to 5-hydroperoxy-eicosatetraenoic acid (5-HPETE), and leukotriene A4 (LTA4). The latter is subsequently converted to LTE4 and LTD4, which interact with leukotriene receptors to trigger the bronchoconstriction response [6, 81, 95]. Four *ALOX5* genetic variants have been associated with altered response to LTRA treatment. Firstly, an *ALOX5* microsatellite located in the Sp1-binding domain. Drazen et al. demonstrated that patients carrying 5 tandem repeats (the most frequent allele) in homozygosity or heterozygosity

showed a greater improvement in FEV1 in response to leukotriene inhibitors, as compared to patients with the double mutant genotype [39]. Likewise, Telleria et al. reported that patients treated with montelukast, an LTRA drug, and carrying 5 tandem repeats in homozygosity or heterozygosity showed a decrease in the number of asthma exacerbations, an improvement in FEV1, and lower use of beta2 agonists [40] as compared to non-carriers. In contrast, Klotsman et al. demonstrated that patients carrying non-5 repeats in homozygosity showed an improvement in the peak expiratory flow (PEF) [41]. Additionally, Lima et al. described a decreased risk of exacerbations associated with asthmatic patients treated with montelukast carrying this variant allele [43]. Nevertheless, Fowler et al. did not corroborate this association in patients receiving montelukast [42]. More recently, Mougey et al. analyzed an asthmatic pediatric cohort and concluded that individuals carrying at least one variant allele in *ALOX5* presented a poor control of asthma and an increased leukotriene production [44]. Overall, these findings are not conclusive and further research is warranted.

Additionally, patients carrying the intronic *ALOX5* rs2115819 minor allele in homozygosity have been found associated with higher FEV1 response to montelukast [43, 45] and the 5-LO inhibitor zileuton [45]. However, subsequent studies did not find an association between this variant and changes in FEV1 with montelukast [10, 46]. Furthermore, Klotsman et al. demonstrated that patients carrying the *ALOX5* rs4987105 and/or rs4986832 minor variants showed an improvement in the PEF in response to montelukast [41]. Further studies are needed to corroborate these findings.

The leukotriene A4 hydrolase *(LTA4H)* gene encodes the LTA4H enzyme, which generates the pro-inflammatory leukotriene LTB4 from LTA4 [39]. Different SNVs in *LTA4H* have been associated with an increased risk of asthma and atopy susceptibility [81, 96]. Lima et al. reported that patients treated with montelukast and carrying the minor allele of the upstream variant *LTA4H* rs2660845 showed an increased probability of enduring asthma exacerbations [43]. This evidence was also observed in a small population of Asian patients [46]. More recently, Maroteau et al. also reported the association between the G allele of rs2660845 and the increased risk of experiencing asthma exacerbations [47]. However, the functional effect of this association remains to be elucidated. Tcheurekdjian et al. analyzed the effect of SNVs in *LTA4H* on the drug-drug interaction between leukotriene modifiers and albuterol in 2 Latin American populations [48]. This study revealed that individual heterozygotes and homozygotes for the minor allele at rs2540491 and heterozygotes for the major allele at rs2540487 showed a significant increase in percent change in FEV1 associated with leukotriene modifier administration after albuterol intake in the Puerto Ricans population [48]. This finding was not, however, replicated in the Mexican population [48]. Therefore, this putative association requires further independent studies.

Leukotriene C4 synthase (LTC4S) promotes the biosynthesis of cys-leukotrienes (cysLTs) by conjugating reduced glutathione at the C-6 position of LTA4 to form LTC4. Most of the studies aimed to elucidate the role of the *LTC4S* SNVs in the responsiveness to anti-leukotrienes agents in asthmatic patients have been focused on the upstream variant rs730012 [81]. Thus, patients carrying the minor allele rs730012C in heterozygosity or homozygosity showed a higher LTC4 production. Also, when these individuals were treated with the LTRA zafirlukast demonstrated a significant improvement in FEV1 and the forced vital capacity compared to carriers of the rs730012A allele in homozygosity [49]. The better response to LTRAs in patients carrying the minor allele rs730012C was also replicated in three independent studies evaluating different outcomes, such as changes in the fraction of exhaled nitric oxide

[43, 50, 51]. Additionally, the intronic variant *LTC4S* rs272431 has been associated with an improved lung function to the leukotriene inhibitors zileuton [45]. However, the functional consequences of this SNV are unknown.

The role of variants in genes involved in the leukotrienes' transport has also been investigated [81]. LTC4 is transported by the multidrug resistance-associated protein 1 (MRP1). MRP1 is encoded by the *ABCC1* gene [97]. Thus, Asano et al. reported that individuals carrying the minor allele of the intronic variant *ABCC1* rs119774 showed a significant increase in the predicted FEV1 when they were treated with an LTRA [43]. A subsequent study by Tantisira et al. corroborated this finding in patients receiving zileuton and demonstrated an additional association of the variant *ABCC1* rs215066 with lung function response [45]. These promising findings warrant further research to unveil the role of *ABCC1* SNVs. Besides, variants in the *SLCO2B1* were also investigated [81]. *SLCO2B1* encodes the organic anion transporting polypeptides 2B1 (OATP2B1). Both proteins, OATP2B1 and OATP2A2 have been identified as montelukast transporters [52]. Mougey et al. reported that patients carrying the non-synonymous variant *SLCO2B1* rs12422149 minor allele presented lower plasma concentrations of montelukast and poor response to treatment [52]. These findings were corroborated in a subsequent study involving adolescent patients [53]. Nonetheless, 2 independent studies on healthy individuals did not replicate the functional effect of rs12422149 in montelukast distribution [54, 55]. Nonetheless, Li et al. showed that asthmatic children carrying the minor allele of rs12422149 had a significantly higher montelukast clearance [56]. Thereby, these controversial results require further investigation.

The physiological action of cysLTs is mediated through the interaction with diverse receptors, including CysLTR1 and CysLTR2. Both receptors are encoded by *CysLTR1* and *CysLTR2* genes and have also been associated with asthma and atopy [98, 99].

Despite that it has been suggested that SNVs in *CysLTR1* might contribute to explaining the variable clinical response associated with anti-leukotrienes drugs, few studies have been successfully exploring this issue [81, 98]. The most promising evidence is the *CysLTR1* rs773347588 variant. Kim et al. reported that patients carrying the rs773347588 minor allele showed higher expression levels than those carrying the homozygous wild-type genotype. However, this is an intronic variant with a low allelic frequency [57]. Concerning *CysLTR2* variants, the most remarkable evidence is the nonsynonymous variant *CysLTR2* rs41347648. The occurrence of this variant is associated with a diminished potency of LTD4 [100]. Additionally, the SNVs *CysLTR2* rs912277 and rs912278 have been associated with an improvement in the response to montelukast therapy. Asthma patients carrying the *CysLTR2* rs912277 and rs912278 minor variants presented an 18-25% improvement in PEF [41]. However, further independent studies are required to corroborate this evidence.

More recently, Dahlin et al. have published two GWAS evaluating the impact of genetic variants in the clinical response to montelukast and zileuton [58, 59]. None of these GWAS corroborated the association with the genetic variants identified in previous candidate gene studies. The first study demonstrated that patients carrying the minor variant of *MLLT3* rs6475448 in homozygosity presented an increased ΔFEV1 in response to montelukast [58]. The latter study included a cohort of patients receiving zileuton and 2 additional cohorts of patients under montelukast therapy. The combined analyses demonstrated that individuals following zileuton treatment who were homozygous for the variant *MRPP3* rs12436663

showed a significant reduction in mean ΔFEV1 [59]. However, it is uncertain the role of both genes in the impact of the responsiveness to the anti-leukotrienes drugs.

Beta-Agonists

Beta-agonists comprise the most widely prescribed bronchodilator drugs for asthma treatment, they are classified into 3 classes as follows: Short-acting beta-agonists (SABAs). These are characterized by showing short half-lives and are used as rapid relievers. Long-acting beta-agonists (LABAs) and ultra-long-acting beta-agonists (ultra-LABAs). LABAs and ultra-LABAs show a longer duration of action, thus providing sustained symptomatic relief [81, 101].

These drugs are accompanied by ICSs to diminish the risk of adverse reactions associated with beta-agonists-only treatment [3]. Therefore, identifying the risk biomarkers which may contribute to the occurrence of adverse reactions associated with beta-agonist is a mainstay to develop genotype-guided treatment leading to better and safe outcomes.

Variants in the *ADRB2* gene are the most studied SNVs on the pharmacogenetics of the beta-agonists response [81]. *ADRB2* encodes the beta2-adrenergic receptor, which is involved in the bronchodilation process [102]. Three non-synonymous SNVs have been found associated with the response to these bronchodilators. These SNVs are as follows: rs1042713, rs1042714, and rs1800888. Although, the findings are not conclusive [103]. Concerning *ADRB2* rs1042713, three studies demonstrated that patients carrying the minor allele of rs1042713 in homozygosis showed a higher risk of suffering asthma exacerbations in response to the SABA salbutamol [60–62]. Also, homozygous patients for this SNV, during treatment with regularly scheduled salbutamol, showed lower values in PEF rate in comparison with those receiving salbutamol only as a rescue medication [60]. Additionally, this finding was also observed in long-term treatment with salmeterol [62]. Recently, Hikino et al. carried out a meta-analysis to evaluate the association between rs1042713 and the FEV1% after salbutamol treatment. The overall results did not find differences between FEV1% and rs1042713 genotypes [104]. However, subgroup analyses identified two significant associations. Firstly, an association with asthmatic patients without comorbidities, and secondly, being more questionable, with asthmatic patients where methacholine provocation was not conducted [104]. Additionally, Scaparrotta et al. reported that individuals carrying the rs1042713 minor allele showed lower FEV1 values in asthmatic Caucasian children treated with SABA [63].

The role of rs1042713 in the response to LABA or LABA and ICSs combination therapies has been extensively explored. However, the overall findings reported indicate that this SNV is not associated with increased asthma exacerbations or difficulties to control asthma [64-67, 81]. Nonetheless, diverse independent meta-analyses have attempted to shed light on this question. Thus, Turner et al. reported that the rs1042713 variant is associated with an increased risk of asthma exacerbations in asthmatic children treated with LABA or a combined therapy [105]. Subsequently, this finding was also corroborated in children, and not found in adults [106]. Thus, this controversial evidence warrants additional studies to confirm or discard these associations.

The *ADRB2* rs1042714 variant causes the amino acid substitution of Gln27Glu. The minor variant alters the region binding conformation on the structure of the beta2-adrenergic receptor which results in variable response to agonists [107]. Several studies have assessed the effect of

rs1042714 on changes in lung function in response to beta-agonists [106, 108]. However, the overall findings are inconclusive to determine the role of this variant in the responsiveness to beta-agonists. Finally, the nonsynonymous *ADRB2* rs1800888 variant is associated with an altered beta2-adrenergic receptor function [68]. Several independent studies have assessed the relationship between this variant and the occurrence of asthma exacerbations in patients treated with beta-agonists. The findings are controversial and failed to find a robust association [64, 67, 69, 70]. Further research is needed to determine the relationship between the rs180888 polymorphism and beta-agonists response.

The ADRB2 activation stimulates the enzyme adenyl cyclase, which catalyzes the generation of cyclic adenosine monophosphate to trigger the transduction signal. This enzyme is encoded by the *ADCY9* gene. The nonsynonymous *ADCY9* rs2230739 minor variant confers a loss of function [109]. Tantisira et al. reported that asthmatic children carrying the minor allele showed a significant improvement in FEV1 when they were co-administrated with budesonide and the SABA salbutamol [71]. This evidence was confirmed by Kim et al. [72]. In addition, they reported an interaction between the SNVs *ADCY9* rs2230739 and the *ADRB2* rs1800888 which demonstrated an additive effect of EFV1 values in response to LABA [72]. These promising findings warrant further studies to confirm this evidence.

Moreover, the association between SNVs in *ARG1* and *ARG2* genes and responsiveness to bronchodilators have also been explored. Both genes encode the isoenzymes arginase 1, and arginase 2 which may regulate the extrahepatic nitric oxide levels in the pathogenesis of inflammation [110]. The SNV rs2781659, located in the *ARG1* regulatory region, has been weakly associated with decreased adjusted bronchodilator response [73]. Subsequently, the authors analyzed the association between the haplotypes composed of the SNVs (rs2781659, rs2781663, rs2781665, and rs60389358) and the response to bronchodilators in 3 asthma trials [74]. The minor haplotype (rs2781659 G, rs2781663 A, rs2781665 T, and rs60389358 T) was associated with lower bronchodilator response in all 3 trials. In concordance with this evidence, this haplotype showed a lower expression in cells transfected [74]. Regarding SNVs in *ARG2*, the variants rs17249437 and rs3742879 have been correlated with lower lung function, more severe airway obstruction, and increased airway hyperresponsiveness. Also, *ARG1* and *ARG2* were associated with lower beta2-agonists reversibility in adult asthma patients [75]. However, this evidence requires further investigation. To our knowledge, findings for *ARG1* variants are promising and require additional independent studies.

In addition, diverse studies have explored the role of SNVs in the *CRHR2* gene in the responsiveness to beta-agonists [81]. As far as our concern, most of the SNVs analyzed are located in the *CRHR2* upstream region and did not show any statistically significant association with bronchodilator response [81]. The most promising candidate is the rare variant rs73294475, which has been associated with a better bronchodilator response. However, this finding requires independent studies to confirm this association [76].

The differential expression of transcription factors in asthma pathophysiology and their role in the Beta-agonists response have been previously explored [111]. Based on this, Duan et al. investigated the association of SNVs in 98 transcription factor genes with bronchodilator response. The authors identified an association between the SNV rs892940, which is located in the *THRB* gene promotor, and the response to beta-agonists [77]. *THRB* gene encodes the β subunit of the thyroid hormone receptor and its expression has been shown to cause altered response to Beta-agonists *in vitro* [111]. This promising evidence requires additional studies.

Himes et al. performed a GWAS in asthma patients and the most promising evidence was a putative association between bronchodilator response and rs295137 [78]. This SNV is in the *SPATSL2* gene region and might influence gene transcription. However, the statistical significance is insufficient for a GWAS study [78, 81]. Additionally, Sordillo et al. corroborated the association between individuals carrying the *SPATSL2* rs295137 minor allele and an increase in bronchodilator response [79]. Interestingly, this effect was progressively reduced for every year of aging [79, 108]. The rationale behind this association might be the fact that *SPATSL2* may regulate the expression of ADRB2 [78, 108]. Although this evidence is promising, additional replication studies are required.

Biological Agents

A better understanding of the underlying mechanism of the pathophysiology of asthma has contributed to the development of new therapies options, such as biological agents. These treatments are targeted against inflammatory mediators that exert a key role in the pathogenesis of asthma. These include a sort of agents directed against IgE and different cytokines expressed by Th2 cells and mast cells [112]. The first biologic agent approved for the asthma treatment was omalizumab, a humanized anti-IgE monoclonal antibody [112]. Afterward, different anti-monoclonal antibodies have been developed for asthma treatment. These therapies are recommended in patients suffering persistent symptoms, or severe uncontrolled asthma which requires a corticosteroids chronic treatment [3]. However, it is recommended their use in patients with a positive evaluation for responsiveness biomarkers, including genetic evidence [113]. Nevertheless, these pharmacogenetic studies are scarce. Thus, Slager et al. reported that asthmatic patients carrying the *IL4RA* rs8832 common allele in homozygosity and receiving pitrakinra, a recombinant anti-IL-4R agent, demonstrated a lower rate of asthma exacerbations and an improvement in life quality [80]. These findings let to demonstrate the pitrakinra efficacy, which had been shown negative results in a previous clinical trial [114]. More recently, Condreay et al. performed a GWAS to analyze the genetic variants associated with the response to mepolizumab therapy [115]. Although no variants reached the GWAS *P*-value threshold, 6 SNVs showed a suggestive *P*-value. However, these SNVs are all intergenic and mapped in chromosome 6 and chromosome 9; where the nearest genes do not seem to be related to asthma therapy [115].

Future Directions

Genetic studies have provided a large insight searching pharmacogenetic biomarkers to predict the responsiveness to asthma therapy. However, the evidence of the SNVs associated with asthma treatment response is not still sufficiently conclusive to optimize drug asthma treatment. Thereby, it remains a proportion of variability in the asthma treatment response to be ascertained. Future investigations need to corroborate the most promising evidence and explore additional candidate genes. Additionally, copy number variations have been described in *ALOX5* and *LTC4S* genes. Nevertheless, the putative role of these structural variants in the responsiveness to anti-leukotrienes agents remains to be explored [81, 116, 117]. Likewise,

CYP3A4 and *CYP3A5* SNVs have been associated with variability in response to ICSs [81], the role of variations in genes involved in beta-agonists metabolism warrants to be analyzed. Since CYP3A5 represents the main CYP3A isoform in the lung, and certain asthma medications may be susceptible to pre-systemic metabolism, variants in these genes require further exploration [81, 118].

Furthermore, epigenomics, transcriptomics, and metabolomics techniques may contribute to exploring the responsiveness of asthma treatments. Thus, recently 2 epigenome-wide association studies have analyzed the consequences of DNA methylation patterns in peripheral blood cells on the response to ICSs [119, 120]. The authors reported that the hypomethylation of the CpG site cg00066816, near the *IL12B* gene, was associated with the absence of severe exacerbations in European asthmatic children [119]. Also, the hypermethylation of the CpG site cg27254601, near the *BOLA2* gene, was associated with an improvement in FEV1 followed by ICSs therapy [120]. Additionally, diverse transcriptomic studies have identified a profile of co-regulated genes associated with an improvement in lung function and LTRA or ICSs treatments [121]. Concerning metabolomics studies, they may contribute to characterizing the asthma treatment response and the role of genetic variants in the variability of the response to asthma therapy [121]. To conclude, in recent years, pharmacogenomic studies have shed light contributing to designing a panel of biomarkers that are associated with asthma therapy response. Nonetheless, further efforts are required to corroborate this evidence and to include ethnically diverse populations. Finally, an integrative approach including different omics techniques may contribute to unveiling new insights on the responsiveness to asthma treatments.

Conclusion

Genetic studies have provided a large insight in searching pharmacogenetic biomarkers to predict the responsiveness to asthma therapy, although the evidence of the SNVs associated with asthma treatment response is not still sufficiently conclusive to optimize drug asthma treatment. The most promising associations should be validated and replicated before these can be translated into clinical practice.

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Index

A

access, 69, 70, 115, 117, 203, 280
acetyl salicylic acid (ASA), 217, 218, 223, 226, 227, 228, 229, 230, 231
acetylcholine, 85, 86, 244
acid, 41, 98, 151, 174, 217, 218, 223, 293
acute asthma, 72, 125, 126, 163, 170, 178, 179, 247
adaptive immune response, 21, 22, 23, 27, 61, 196, 197
adaptive immunity, 21, 22, 24, 29, 220
adenosine, 25, 37, 49, 85, 297
adhesion, 22, 28, 230
adolescents, 4, 6, 13, 14, 49, 50, 75, 90, 147, 150, 190, 234, 244, 266, 274, 275, 283, 287, 288, 289, 290
adulthood, 61, 105, 110, 157, 162
adults, 1, 4, 6, 7, 8, 11, 12, 13, 14, 15, 21, 27, 30, 50, 51, 52, 61, 72, 73, 75, 85, 88, 89, 91, 94, 96, 99, 109, 110, 111, 112, 118, 119, 120, 122, 123, 129, 130, 136, 156, 157, 164, 170, 171, 178, 179, 183, 187, 189, 212, 218, 219, 227, 235, 238, 240, 244, 245, 246, 247, 250, 260, 261, 266, 267, 271, 274, 275, 276, 279, 280, 281, 282, 283, 286, 287, 288, 289, 290, 291, 292, 296, 297, 301, 305
adverse effects, 157, 160, 207, 208, 209, 230, 238, 241, 243, 253, 265
adverse event, 208, 211, 241, 242, 243, 244, 269, 272, 274, 276
adrenocorticotropic hormone (ACTH), 174, 175, 176, 177, 179
aerosols, 85, 101, 125, 126, 127, 131, 134
age, ix, 4, 5, 6, 7, 11, 12, 13, 14, 19, 48, 75, 79, 81, 83, 98, 99, 117, 119, 129, 133, 140, 160, 170, 171, 172, 204, 239, 250, 259, 266, 267, 269, 271, 272, 276, 292, 304
agonist, 88, 108, 133, 238, 240, 241, 246, 247, 282, 296, 303
air pollution, ix, 36, 41, 45, 46, 51, 60, 127, 131, 134, 135, 146, 150, 151, 156, 162, 163, 164, 165, 170, 171, 177
air quality, 73, 149, 150, 154, 163
airflow, ix, 2, 13, 31, 62, 72, 77, 80, 81, 83, 84, 85, 88, 109, 114, 115, 121, 205, 238, 241, 266, 286
airflow obstruction, 2, 13, 62, 72, 80, 84, 85, 121, 205, 238
airway epithelial cells, 18, 22, 27, 111, 177, 179, 213, 234
airway hyperresponsiveness, 62, 84, 86, 120, 122, 178, 183, 184, 233, 240, 297
airway inflammation, ix, 19, 27, 30, 31, 35, 37, 49, 67, 80, 83, 93, 94, 97, 105, 115, 116, 120, 161, 164, 169, 171, 177, 183, 185, 225, 229, 231, 232, 234, 238, 243, 270, 272, 286
airway remodelling, 110, 112, 113, 117, 185, 233
airways, 2, 21, 24, 28, 29, 34, 60, 62, 67, 69, 70, 80, 81, 84, 85, 86, 87, 90, 96, 97, 110, 111, 112, 113, 114, 115, 120, 121, 122, 125, 126, 129, 130, 131, 132, 167, 181, 182, 183, 184, 185, 187, 188, 189, 190, 193, 194, 196, 198, 203, 208, 211, 217, 218, 219, 221, 223, 224, 225, 227, 230, 232, 233, 234, 240, 266
algorithm, 77, 78, 252, 253, 265, 276
allele, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 302
allergen challenge, 188, 226, 234, 252, 261, 305
allergen immunotherapy (AIT), 30, 31, 32, 63, 70, 71, 73, 127, 176, 177, 190, 191, 244, 247, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263
allergenic pollen, 34, 46, 49, 137, 140, 147, 148, 151
allergic asthma, ix, 2, 17, 18, 19, 20, 23, 24, 28, 29, 31, 55, 60, 61, 67, 72, 73, 93, 96, 97, 112, 117, 120, 126, 132, 134, 150, 160, 168, 177, 178, 185, 187, 195, 219, 220, 221, 225, 231, 233, 236, 249, 252, 259, 260, 261, 263, 268, 269, 278, 279, 305
allergic bronchopulmonary aspergillosis (ABPA), ix, 66, 70, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215
allergic inflammation, 22, 25, 26, 28, 30, 111, 112, 160, 168, 178

allergic reaction, 26, 56, 60, 69
allergic rhinitis, 33, 39, 40, 48, 52, 62, 72, 83, 86, 111, 116, 118, 126, 127, 129, 131, 132, 133, 137, 138, 147, 150, 162, 181, 183, 185, 186, 187, 188, 189, 190, 191, 225, 233, 234, 235, 247, 250, 252, 260, 261, 262, 263, 279
allergic sensitisation, 250, 251, 257
allergy, 5, 12, 14, 32, 33, 34, 39, 40, 41, 44, 45, 48, 49, 50, 51, 52, 55, 60, 63, 70, 71, 72, 73, 74, 75, 91, 100, 104, 105, 111, 126, 127, 129, 130, 131, 132, 133, 134, 135, 138, 146, 147, 148, 150, 153, 159, 162, 163, 164, 165, 167, 168, 181, 183, 186, 187, 188, 189, 190, 208, 226, 231, 233, 247, 250, 252, 253, 255, 257, 260, 261, 262, 263, 266, 277, 278
Alt a 1, 55, 64, 66, 67, 70, 73, 74, 75
alters, 118, 157, 160, 296, 303
alveoli, 21, 153, 182, 194
amino acid, 41, 43, 47, 174, 296
amylase, 59, 95, 98
anaphylaxis, 254, 266, 267, 270, 272, 280
animal dander, 19, 23, 184
anti-asthma, 130, 155, 252, 258, 275, 302
antibodies, 17, 24, 26, 31, 32, 51, 93, 94, 96, 97, 98, 100, 101, 103, 104, 113, 117, 169, 174, 176, 177, 179, 186, 194, 196, 199, 201, 204, 207, 209, 210, 211, 213, 214, 215, 231, 233, 260, 265, 268, 269, 270, 271, 272, 273, 275, 276, 278, 279, 280, 281, 298
antifungal therapy, 194, 207, 210, 211
antigen, 26, 32, 36, 64, 74, 84, 87, 97, 111, 112, 158, 164, 168, 169, 178, 197, 199, 201, 262
antihistamines, 63, 186, 188, 229
antiinflammatory drugs (NSAIDs), 3, 90, 110, 111, 189, 217, 218, 227, 228, 229, 230, 231, 234, 305
anti-leukotrienes, 238, 285, 293, 294, 295, 296, 298
antioxidant, 158, 159, 160
apoptosis, 22, 171, 220, 272
ARG1, 234, 290, 297, 303
Asia, 6, 31, 42, 44, 135, 136
aspergillosis, ix, 66, 193, 194, 195, 196, 204, 212, 213, 214
Aspergillus, 64, 66, 67, 68, 69, 95, 111, 120, 145, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 208, 209, 210, 212, 213
Aspergillus fumigatus (*A. fumigatus*), 64, 66, 67, 68, 111, 120, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 210, 212, 213
aspirin, viii, ix, 85, 90, 91, 120, 186, 217, 218, 226, 227, 230, 232, 234, 235, 236, 289, 302
aspirin-exacerbated respiratory disease (AERD), ix, 90, 120, 186, 217, 218, 219, 220, 221, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 235
assessment, 77, 82, 88, 96, 100, 105, 114, 115, 117, 118, 174, 175, 177, 214, 257, 258, 262
asthma attacks, 6, 14, 39, 69, 126, 127, 128, 129, 131, 132, 134, 145, 169, 170, 171, 177, 218, 244
asthma death, 12, 55, 69
asthmatic children, 12, 75, 157, 162, 219, 293, 295, 296, 297, 299, 301
asthmatic symptoms, 6, 96, 199, 201, 218
asymptomatic, 19, 84, 87, 207, 211, 226, 274
athletes, 86, 90, 153
atmosphere, 46, 64, 66, 125, 126, 131, 137, 138, 139, 142, 152, 153
atopic asthma, 73, 157, 231, 305
atopic dermatitis, 32, 66, 72, 209, 274, 278, 282
atopy, 12, 13, 18, 30, 61, 72, 78, 83, 110, 119, 147, 154, 163, 164, 186, 187, 225, 250, 260, 294, 295
autoimmune disease, 116, 254, 261
avoidance, 55, 62, 99, 101, 169, 217, 218, 219, 226, 229

B

bacteria, 3, 19, 167, 176, 184, 190
barriers, 117, 150, 162
base, 4, 13, 62, 63, 83, 94, 95, 97, 101, 103, 104, 178, 203, 246, 251
basement membrane, 21, 183, 185, 220, 275
basophils, 17, 18, 19, 23, 24, 26, 29, 32, 39, 97, 111, 161, 165, 188, 197, 209, 223, 240, 255, 256, 262, 268, 269, 272
beneficial effect, 187, 231, 244
benefits, 117, 174, 242, 250, 259, 263, 272, 276
beta-agonists, 247, 285, 289, 296, 297, 299
biologic treatment, 238, 243, 265
biological activity, 58, 63, 67, 251
biological therapies, 11, 96, 117, 285, 286
biologics, ix, 101, 102, 109, 116, 181, 187, 188, 209, 210, 232, 243, 266, 267, 268, 270, 275, 276, 278, 283, 305
biomarkers, 18, 22, 30, 31, 101, 102, 103, 104, 105, 108, 110, 114, 115, 116, 117, 119, 122, 127, 177, 194, 203, 218, 224, 225, 226, 231, 232, 233, 234, 236, 243, 249, 255, 256, 257, 260, 262, 263, 265, 267, 269, 274, 276, 278, 279, 285, 293, 296, 298, 299
biopsy, 27, 116, 204, 224
blood, 19, 24, 26, 28, 29, 32, 83, 87, 97, 110, 194, 198, 199, 201, 209, 219, 224, 231, 234,

241, 243, 255, 256, 266, 267, 268, 269, 270, 271, 274, 275, 276, 279, 281
bone, 24, 185, 240
breathing, 84, 86, 117, 128, 133, 170, 183, 184
bronchial asthma, 2, 4, 5, 28, 46, 63, 138, 149, 150, 151, 152, 153, 154, 156, 162, 212, 217, 234, 251, 253, 254, 255, 258, 263
bronchial epithelial cells, 22, 26, 37, 233
bronchial hyperresponsiveness, 2, 63, 98, 99, 110, 123, 157, 238, 244, 275, 292
bronchiectasis, 79, 186, 193, 194, 195, 198, 199, 200, 201, 202, 203, 205, 206, 207
bronchiolitis, 170, 174, 177
bronchitis, 83, 86, 90, 116, 154, 184, 189, 195
bronchoconstriction, 80, 83, 87, 90, 91, 113, 115, 132, 184, 223, 244, 286, 293
bronchodilator, 81, 85, 88, 99, 114, 170, 234, 240, 242, 243, 244, 273, 274, 286, 287, 288, 290, 291, 296, 297, 298, 302, 303, 304
bronchoscopy, 27, 122, 199, 201, 206, 275
bronchospasm, 85, 169, 177, 209, 219

C

calcium, 40, 43, 45, 48, 53
carbon dioxide (CO_2), 74, 137, 139, 147
Caucasians, 286, 287, 288
causal relationship, 99, 251, 259
cell death, 24, 193, 195, 198, 212
challenges, 91, 98, 103, 104, 135, 183, 187, 224, 227, 232, 235
chemical, 59, 60, 67, 74, 96, 98, 99, 101, 104, 157, 158, 159, 160, 164, 223
chemokines, 18, 19, 23, 27, 219
chest radiography, 199, 206, 210
childhood, 2, 5, 13, 19, 34, 61, 71, 97, 110, 119, 121, 150, 157, 162, 163, 164, 165, 189, 260, 286, 301, 304, 306
children, 1, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 32, 35, 40, 41, 49, 50, 61, 67, 69, 73, 75, 81, 83, 84, 85, 88, 89, 90, 109, 119, 122, 129, 133, 135, 147, 150, 151, 153, 156, 157, 162, 163, 164, 165, 168, 170, 171, 174, 178, 183, 187, 189, 190, 219, 233, 234, 238,239, 244, 246, 247, 250, 254, 260, 262, 263, 266, 271, 274, 279, 280, 282, 286, 287, 288, 289, 290, 291, 292, 293, 295, 296, 297, 299, 300, 301, 302, 303, 304, 305, 331
China, 39, 44, 47, 52, 133, 135, 163, 179
chronic diseases, 11, 117, 154, 190
chronic obstructive pulmonary disease (COPD), 15, 79, 80, 83, 86, 96, 133, 167, 171, 172, 177, 178, 179, 182, 185, 188, 190, 195, 214, 242, 246, 247
chronic rhinosinusitis with recurrent nasal polyps (CRSwNPs), 218, 222, 226, 227
cities, 1, 38, 41, 46, 125, 126, 129, 131, 150, 151, 153, 155, 156, 160, 161
classification, 57, 96, 187, 213, 237, 255
climate, ix, 1, 37, 38, 41, 45, 50, 69, 72, 127, 129, 132, 134, 135, 137, 138, 139, 142, 145, 146, 147, 148, 156, 160, 162
climate change, ix, 1, 38, 41, 45, 50, 72, 127, 129, 132, 134, 135, 137, 138, 139, 145, 146, 147, 148, 160, 162
clinical symptoms, 48, 51, 63, 163
clinical syndrome, 172, 179, 195, 218
clinical trials, 27, 188, 208, 210, 211, 231, 240, 250, 257, 258, 261, 265, 267, 276, 286
coding, 160, 291, 305
colonization, 19, 30, 193, 194, 195, 203, 207, 208, 212
commercial, 42, 62, 73, 104
community, 49, 91, 109, 251
comorbidity, 171, 172, 177, 189
complement, 30, 219, 240
composition, 19, 41, 49, 73, 153, 198, 257
compounds, 28, 33, 35, 49, 95, 158, 208
computed tomography, 113, 199, 200, 205, 206, 213
conjunctivitis, 34, 97, 226, 274
consensus, 27, 71, 91, 103, 104, 105, 108, 117, 186, 187, 199, 212, 246, 258, 260, 261, 262, 263, 266
consumption, 108, 149, 152, 153, 155, 156, 208
contamination, 70, 74, 145, 153, 159, 161
contraindications, 84, 85, 227, 228, 249, 253, 254, 257, 261
controversial, 62, 110, 116, 184, 187, 207, 230, 250, 295, 296, 297
correlation, 60, 141, 145, 161, 177, 185, 220, 221, 224, 225, 257
corticosteroids (CS), 2, 18, 63, 72, 73, 83, 101, 107, 108, 109, 111, 114, 116, 119, 122, 126, 130, 132, 163, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 179, 194, 199, 207, 208, 210, 211, 214, 219, 224, 229, 230, 233, 234, 238, 239, 240, 241, 243, 245, 246, 247, 265, 266, 267, 270, 280, 281, 282, 283, 285, 286, 290, 291, 298, 300, 301, 304, 305, 306
cortisol, 174, 175, 176, 177
cost, 12, 13, 63, 73, 119, 245, 259, 273, 281
cough, ix, 2, 77, 78, 79, 86, 90, 169, 184, 193, 195, 199, 286
COVID-19, 12, 15, 90, 167, 169, 170, 171, 172, 174, 175, 176, 177, 178, 179
crop, 37, 49, 148

cystic fibrosis, 66, 79, 86, 186, 193, 194, 195, 199, 201, 207, 212, 214, 215
cytokines, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 61, 67, 111, 112, 113, 116, 117, 121, 150, 151, 158, 159, 168, 177, 194, 196, 197, 198, 219, 220, 221, 230, 233, 255, 257, 273, 291, 298
cytometry, 29, 35, 256
cytoplasm, 38, 125, 131, 159, 220, 225

D

danger, 22, 29, 132
death, 2, 12, 15, 24, 55, 69, 109, 117, 125, 127, 128, 129, 133, 134, 145, 150, 163, 193, 195, 198, 212, 219, 286
defence, 113, 185, 186
deficiency, 26, 27, 28, 186
deficit, 160, 189, 241
dendritic cells (DCs), 17, 18, 19, 21, 22, 23, 26, 27, 29, 31, 36, 37, 97, 196, 197, 220, 255
deposition, 25, 182, 241
Der p 1, 47, 55, 56, 59, 62, 71, 250
dermatitis, 32, 66, 72, 78, 94
desensitization, 70, 217, 218, 227, 230, 232, 235, 236
detection, 30, 41, 100, 102, 206, 257, 260
diagnosis, vii, 1, 2, 4, 5, 6, 7, 8, 14, 47, 51, 55, 61, 62, 69, 70, 72, 75, 77, 78, 79, 80, 83, 84, 85, 89, 90, 91, 93, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 107, 109, 114, 116, 118, 119, 120, 122, 133, 149, 153, 156, 169, 189, 190, 194, 198, 199, 201, 203, 204, 205, 206, 211, 212, 213, 214, 217, 218, 226, 227, 228, 232, 235, 236, 254, 257, 262, 263, 266, 276, 301
diagnostic criteria, 199, 201, 203, 206, 212, 213, 218
diisocyanates, 3, 95, 99
diseases, 2, 5, 11, 12, 13, 21, 27, 30, 31, 32, 51, 56, 63, 64, 66, 71, 72, 73, 78, 80, 84, 94, 103, 111, 117, 119, 120, 122, 133, 135, 138, 146, 147, 149, 150, 151, 152, 154, 156, 157, 158, 160, 162, 164, 167, 172, 177, 185, 188, 189, 198, 203, 205, 209, 213, 233, 236, 237, 250, 260, 261, 262, 274, 286, 300, 304, 306
disorder, 1, 2, 29, 89, 93, 122, 266
distribution, 60, 66, 94, 115, 229, 239, 295
diversity, 167, 179, 238
DNA, 157, 159, 160, 193, 195, 198, 212, 219, 299, 306
dosage, 73, 126, 132, 243, 272, 281
dosing, 232, 239, 254
drugs, 2, 3, 32, 63, 110, 111, 115, 116, 123, 126, 130, 156, 158, 164, 174, 184, 189, 207, 208, 211, 215, 217, 218, 223, 228, 229, 231, 233, 234, 235, 237, 238, 239, 240, 241, 244, 245, 246, 247, 252, 255, 257, 258, 259, 265, 266, 267, 269, 271, 274, 280, 281, 285, 286, 290, 291, 292, 294, 295, 296, 298, 299, 302, 303, 304, 305, 306
dyspnea, 2, 13, 78, 79, 88, 90

E

eczema, 5, 13, 60, 83, 116, 147
editors, 14, 30, 31, 32, 49, 162, 163
education, 63, 97, 101, 102, 107, 116, 117
ELISA, 39, 101, 139, 204
emergency, 15, 34, 49, 125, 126, 127, 128, 129, 130, 133, 134, 149, 150, 151, 152, 153, 156, 162, 163, 179, 218, 230, 244, 273
encoding, 158, 160, 290, 292
endophenotypes, 181, 182, 185, 186, 188
endoscopy, 218, 227, 231
endotypes, 18, 30, 103, 107, 108, 110, 112, 113, 115, 118, 119, 122, 211, 217, 219, 224, 232, 233, 238, 255, 265
energy, 116, 151, 154
environment, 21, 52, 57, 61, 66, 80, 89, 101, 112, 116, 133, 134, 146, 157, 160, 163, 194, 195, 208
environmental exposure, 1, 2, 60, 71, 100, 185, 210, 255, 261
environmental factors, 5, 21, 113, 127, 128, 135, 150, 160, 171, 251
enzyme, 3, 17, 28, 34, 36, 39, 59, 95, 98, 102, 105, 139, 158, 159, 160, 176, 177, 204, 223, 225, 256, 293, 294, 297, 304, 305
eosinophil, 18, 19, 22, 27, 28, 29, 97, 110, 111, 113, 120, 168, 171, 175, 194, 197, 198, 199, 200, 201, 202, 203, 207, 209, 212, 217, 219, 220, 224, 225, 231, 233, 234, 243, 268, 269, 270, 271, 272, 273, 274, 275, 276, 279, 280, 281
eosinophilia, 22, 24, 25, 27, 28, 29, 97, 99, 101, 110, 114, 115, 120, 121, 174, 185, 189, 194, 198, 199, 201, 205, 213, 219, 224, 225, 233, 234, 266, 267, 280, 281
eosinophilic extracellular traps (EETs), 194, 198, 210
eosinophilic granuloma, 105, 267, 271, 273, 274
eosinophils, 18, 19, 22, 23, 24, 25, 26, 27, 28, 29, 32, 35, 37, 67, 83, 97, 100, 111, 113, 115, 151, 185, 193, 195, 198, 219, 220, 221, 223, 224, 233, 239, 241, 243, 255, 267, 269, 270, 271, 272, 274, 275, 276, 277, 278, 281, 302

epidemic, 14, 127, 129, 134, 135, 136, 145
epidemiology, vii, ix, 1, 4, 12, 13, 72, 74, 75, 94, 109, 125, 162, 178, 183, 189, 195
epithelia, 3, 29, 64, 95, 254
epithelial cells, 18, 21, 22, 23, 26, 27, 28, 36, 37, 59, 111, 113, 150, 177, 179, 198, 225
epithelium, 17, 18, 21, 22, 23, 29, 31, 61, 66, 67, 81, 113, 121, 168, 183, 184, 197, 198, 212, 219, 220, 221, 225, 232, 233, 275
etiology, 1, 2, 33, 34, 101, 127, 199
Europe, 6, 13, 33, 34, 37, 38, 40, 41, 42, 44, 46, 47, 48, 49, 50, 52, 57, 60, 67, 72, 75, 126, 129, 132, 137, 138, 139, 140, 142, 147, 152, 157, 164, 190, 261
European Community, 4, 6, 13, 14, 72, 75
European Respiratory Society (ERS), 80, 91, 94, 97, 104, 108, 109, 117, 118, 242, 278, 300
evidence, 1, 2, 11, 21, 28, 35, 36, 39, 47, 60, 61, 62, 69, 83, 84, 95, 97, 98, 100, 104, 113, 131, 134, 138, 156, 157, 158, 168, 170, 174, 181, 182, 183, 184, 185, 186, 187, 188, 189, 203, 205, 206, 209, 211, 240, 244, 246, 250, 252, 253, 258, 260, 263, 270, 271,285, 291, 292, 293, 294, 295, 296, 297, 298, 299
evolution, 8, 14, 110, 152, 172, 177, 179, 218, 276
exclusion, 174, 227, 276
exercise, 2, 78, 79, 84, 87, 88, 90, 91, 93, 117, 152, 160, 189, 199, 244, 286
exposure, 2, 17, 19, 25, 34, 35, 38, 41, 42, 43, 45, 46, 47, 49, 50, 51, 52, 55, 56, 60, 62, 63, 64, 69, 70, 71, 72, 73, 80, 89, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 110, 130, 138, 145, 147, 150, 151, 152, 157, 160, 164, 165, 169, 187, 193, 194, 210, 217, 226, 228, 244, 249, 250, 255, 257, 259, 261, 263, 277
extracellular matrix, 22, 113, 198, 203
extracts, 41, 48, 55, 56, 62, 63, 70, 72, 73, 74, 98, 244, 251, 252, 254, 257

F

families, 12, 43, 44, 48, 57, 67
fever, 33, 34, 127, 129, 131, 147, 163, 193, 195, 260
fibrinogen, 25, 198, 203, 221
fibroblasts, 22, 121, 198, 225
fibrosis, 24, 66, 79, 86, 157, 186, 193, 194, 195, 199, 201, 205, 207, 212, 214, 215, 220
flour, 71, 95, 98, 105
flowering period, 38, 40, 46
fluid, 112, 115, 199, 201
food, 32, 40, 41, 50, 51, 57, 61, 70, 84, 85, 95, 148, 186
Food and Drug Administration (FDA), 123, 158, 265, 266, 267, 271, 272, 276
formation, 152, 160, 193, 195, 198, 210, 272
France, 47, 52, 100, 125, 140, 146, 148, 254
fungal infection, 45, 214, 215
fungal spores, 19, 55, 56, 64, 66, 67, 69, 70, 71, 130, 148, 167
fungi, 3, 23, 55, 64, 65, 66, 67, 69, 73, 74, 75, 110, 133, 136, 138, 145, 153, 168, 193, 194, 195, 196, 197, 198, 199, 201, 203, 204, 212, 214, 215

G

gene expression, 22, 61, 121, 156, 177, 291, 301
general practitioner, 91, 125, 126, 130
genes, 19, 61, 113, 158, 159, 160, 179, 290, 291, 292, 295, 296, 297, 298, 299, 305
genetic factors, 2, 112, 304
genetic predisposition, 19, 55, 56, 60, 61, 63, 168
genome, 61, 72, 292, 304, 305
genotype, 294, 295, 296, 302, 303, 304
genus, 44, 46, 48, 66, 194
Germany, 50, 69, 75, 140, 147, 154, 163, 254, 259
germination, 34, 39, 194, 196
global warming, 1, 45, 134, 137, 138, 139, 142, 162, 195
glucocorticoid receptor, 113, 208, 240, 241, 290, 291, 292, 300, 304
glucocorticoids, 79, 81, 113, 121, 170, 171, 194, 205, 206, 207, 208, 214, 238, 240, 241, 268, 274, 275, 280, 281, 282, 290, 291, 292, 300, 301, 304
glutathione, 46, 160, 165, 294
goblet cells, 113, 183, 198, 220
granules, 26, 28, 38, 60, 131, 132, 220
grass pollen, 19, 34, 35, 37, 38, 39, 40, 41, 49, 50, 51, 69, 127, 128, 129, 130, 131, 132, 133, 135, 136, 145, 146, 148, 150, 151, 153, 155, 156, 161, 163, 165, 191, 251, 263
grasses, 19, 33, 34, 35, 37, 38, 39, 40, 41, 49, 50, 51, 69, 127, 128, 129, 130, 131, 132, 133, 135, 136, 145, 146, 148, 150, 151, 153, 155, 156, 161, 163, 165, 171, 191, 251, 262, 263
greenhouse, 95, 137, 139, 140
growth, 24, 25, 28, 34, 45, 46, 51, 62, 70, 109, 112, 139, 150, 162, 194, 199, 201, 240
growth factor, 24, 25, 28, 112, 162
guidelines, 11, 63, 77, 80, 83, 91, 101, 102, 104, 117, 118, 122, 186, 187, 217, 229, 236, 237, 243, 250, 252, 253, 261, 278, 286, 300

H

hay fever, 33, 34, 127, 129, 131, 147, 163, 260
health, 2, 12, 14, 15, 34, 46, 47, 56, 73, 88, 90, 94, 95, 101, 102, 108, 117, 127, 128, 129, 133, 135, 137, 145, 147, 149, 150, 151, 152, 153, 154, 160, 162, 164, 182, 212, 243, 246, 272, 300
health care, 15, 88, 135, 272
heart disease, 79, 153, 156, 167, 261
heterogeneity, 122, 211, 232, 238, 286, 300
high attenuation mucus, 194, 199, 201, 206
histamine, 28, 85, 160, 183
histone, 113, 157, 291, 300, 304
history, 2, 4, 12, 46, 61, 69, 71, 78, 90, 96, 98, 99, 100, 117, 127, 131, 133, 134, 135, 178, 185, 199, 201, 227, 232, 250, 257, 258, 259, 286
homeostasis, 112, 203, 219, 220, 225
homes, 56, 57, 62, 69, 73, 151
hormone, 174, 291, 297, 304
hospital, 1, 4, 8, 9, 11, 14, 15, 17, 33, 55, 69, 72, 77, 93, 95, 107, 110, 125, 126, 127, 128, 129, 130, 133, 134, 135, 137, 145, 149, 167, 174, 181, 193, 195, 212, 217, 230, 244, 265, 285, 288, 331
hospitalization, 14, 15, 34, 49, 75, 134, 218, 230
host, 1, 2, 19, 22, 94, 105, 153, 174, 176, 177, 179, 196, 198, 203
hotels, 61
house dust, 3, 19, 23, 25, 27, 48, 52, 55, 71, 72, 73, 146, 184, 191, 234, 251, 252, 261, 263
house dust mites (HDM), 3, 19, 23, 25, 27, 48, 52, 55, 56, 57, 58, 59, 60, 61, 62, 63, 71, 72, 73, 184, 191, 234, 252, 261
human, 4, 17, 19, 21, 22, 25, 32, 37, 47, 49, 51, 57, 98, 104, 110, 120, 121, 123, 135, 138, 150, 151, 153, 158, 162, 164, 168, 176, 177, 178, 196, 212, 233, 273, 304, 305
human health, 47, 135, 150, 151, 153, 162
humidity, 57, 60, 62, 64, 66, 69, 70, 73, 80, 87, 130, 134, 145, 146, 153
hygiene, 94, 101, 102
hyperinflation, 117, 205, 241
hypermethylation, 160, 165, 299
hypersensitivity, 19, 24, 26, 56, 64, 71, 75, 160, 172, 178, 179, 186, 189, 193, 194, 195, 199, 201, 217, 218, 223, 224, 226, 227, 228, 231, 234, 266, 267, 273
hypothesis, 162, 183, 185, 186, 187, 250, 256

I

identification, 97, 98, 101, 104, 120, 122, 224, 238, 255, 257, 280
identity, 24, 41, 45
IFN, 24, 25, 26, 160, 165, 197, 268
imitation, ix, 31, 77, 88, 109, 114, 115, 241, 265, 278, 286
immune response, 17, 18, 23, 25, 27, 29, 30, 49, 59, 61, 111, 112, 113, 150, 151, 157, 158, 159, 160, 161, 164, 169, 172, 174, 176, 177, 196, 197, 210, 225, 251, 275
immune system, 18, 19, 23, 28, 33, 35, 59, 60, 69, 97, 110, 150, 157, 158, 159, 176, 185, 193, 194, 196, 198, 223, 224, 255
immunity, 21, 22, 24, 29, 30, 31, 59, 152, 169, 211, 233
immunoglobulin E (IgE), 2, 17, 19, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 39, 40, 41, 46, 50, 51, 52, 55, 56, 61, 62, 64, 67, 70, 74, 93, 94, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 111, 112, 117, 119, 133, 150, 158, 160, 169, 174, 175, 176, 177, 185, 186, 187, 188, 189,190, 194, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 209, 210, 211, 213, 214, 215, 224, 225, 231, 249, 250, 255, 256, 257, 260, 262, 265, 266, 268, 269, 275, 276, 278, 279, 293, 298
immunoglobulin G (IgG), 113, 174, 194, 196, 199, 200, 201, 202, 203, 204, 209, 210, 213, 255, 272
immunology, vii, ix, 12, 17, 18, 30, 38, 46, 73, 103, 117, 147, 149, 187, 212, 233, 234, 251, 253, 262, 263, 279, 280, 331
immunomodulatory, 150, 171, 220, 259
immunotherapy, 30, 31, 32, 41, 42, 48, 50, 62, 63, 71, 73, 127, 171, 176, 177, 181, 186, 187, 188, 190, 191, 207, 247, 249, 250, 252, 253, 254, 256, 257, 260, 261, 262, 263
improvements, 170, 268, 272
in vitro, 37, 55, 62, 99, 104, 113, 161, 168, 188, 297
in vivo, 49, 55, 62, 73, 220, 255
incidence, 4, 8, 9, 11, 94, 129, 130, 153, 157, 253, 254, 261, 267, 286, 299
income, 1, 4, 5, 6, 11, 14
India, 195, 204, 212, 290
indications, 84, 85, 116, 170, 187, 227, 241, 245, 249, 250, 252, 253, 261, 266, 267, 269, 271, 272, 273, 274, 276
individuals, 17, 22, 62, 63, 67, 70, 84, 87, 93, 95, 97, 99, 117, 127, 129, 130, 132, 133, 134, 157, 168, 190, 198, 220, 221, 223, 224, 225, 226, 231, 238, 250, 292, 294, 295, 296, 298, 304

induction, 19, 23, 27, 32, 40, 115, 220, 259
industries, 61, 94, 95, 102
infection, 15, 66, 113, 168, 171, 172, 174, 176, 177, 178, 179, 194, 198, 208, 228, 276
inflammation, vii, ix, 17, 18, 19, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 37, 48, 49, 67, 75, 80, 83, 93, 94, 96, 97, 105, 110, 111, 112, 113, 115, 116, 117, 118, 120, 121, 122, 123, 126, 148, 150, 151, 152, 157, 158, 159, 160, 161, 164, 168, 169, 170, 176, 177, 178, 183, 184, 185, 187, 188, 189, 190, 196, 208, 210, 218, 220, 221, 222, 223, 224, 225, 229, 230, 231, 232, 233, 234, 238, 241, 243, 244, 250, 255, 256, 258, 259, 265, 267, 270, 271, 272, 274, 275, 286, 297
inflammatory cells, 99, 110, 111, 231, 235, 240, 243, 275, 282
inflammatory disease, 2, 77, 150, 221
inflammatory mediators, 2, 18, 116, 298
ingestion, 56, 71, 90, 227
inhaled corticosteroids (ICS), 83, 88, 101, 107, 108, 109, 111, 115, 122, 126, 127, 133, 171, 172, 173, 174, 177, 179, 208, 214, 219, 224, 225, 230, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 266, 267, 268, 269, 273, 274, 281, 282, 286, 300, 301, 304, 305, 306
inhaled therapy, 109, 115, 170, 241
inhaler, 81, 109, 116, 242, 246, 247
inhibitor, 59, 117, 215, 223, 294
initiation, 19, 21, 24, 27, 120, 170, 271
injuries, 12, 29, 113, 178, 179, 184, 193, 194
innate immune response, 61, 111, 112, 212
innate lymphoid cells type 2 (ILC2), 17, 19, 22, 24, 27, 111, 112, 113, 196, 197, 219, 220
insects, 33, 34, 56
integrin, 27, 28, 120
integrity, 17, 23, 25, 121, 275
interferons, 22, 27, 162, 168, 233
interleukin 13 (IL-13), 18, 19, 22, 23, 24, 25, 26, 27, 28, 101, 111, 112, 113, 116, 120, 121, 151, 158, 159, 164, 177, 178, 179, 194, 197, 209, 219, 220, 224, 225, 231, 265, 266, 273
interleukin 4 (IL-4), 18, 19, 22, 23, 24, 25, 26, 27, 28, 32, 101, 111, 112, 113, 116, 117, 158, 159, 164, 169, 176, 177, 178, 179, 194, 197, 209, 224, 231, 265, 266, 273, 278, 298, 304
interleukin 5 (IL-5), 18, 19, 23, 24, 25, 27, 28, 101, 111, 112, 117, 120, 151, 158, 159, 177, 194, 197, 209, 219, 220, 221, 224, 231, 234, 256, 265, 266, 270, 271, 272, 273, 278
interleukin 17 (IL-17), 26, 112, 113, 121, 157, 164
intervention, 73, 83, 117, 126, 170, 177
Israel, 30, 118, 123, 282, 283, 303
issues, 74, 94, 133, 150
Italy, 48, 72, 100, 111, 120, 125, 126, 129, 130, 131, 140

J

Japan, 15, 33, 44, 45, 74, 153, 232, 288, 289

L

larynx, 181, 182, 183, 184
lead, 17, 19, 21, 27, 29, 80, 87, 88, 93, 94, 97, 102, 110, 113, 114, 115, 159, 170, 174, 185, 193, 195, 219
leukotriene modifier, 109, 288, 289, 294, 302, 303
leukotrienes, 28, 116, 217, 218, 238, 243, 285, 293, 294, 295, 296, 298, 305
light, 59, 73, 296, 299
locus, 72, 286, 292, 301, 303, 304
long acting muscaring anatagonists (LAMA), 116, 117, 237, 238, 241, 242, 245
long acting β agonist (LABA), 108, 109, 171, 172, 237, 238, 240, 241, 242, 243, 245, 246, 290, 296, 297
longitudinal study, 111, 120, 135
lumen, 21, 219, 220
lung disease, 21, 27, 79, 81, 84, 94, 103, 193, 194, 205, 207
lung function, 13, 35, 49, 55, 61, 63, 69, 73, 81, 83, 87, 88, 91, 101, 109, 110, 116, 117, 118, 119, 149, 150, 156, 157, 169, 171, 177, 207, 211, 225, 237, 238, 241, 242, 244, 245, 265, 268, 270, 271, 272, 273, 274, 275, 276, 278, 285, 286, 287, 288, 289, 290, 291, 295, 297, 299, 300, 306
lymphocytes, 17, 18, 19, 22, 23, 24, 26, 27, 29, 31, 97, 111, 160, 161, 168
lymphoid, 17, 18, 19, 22, 24, 27, 29, 32, 111, 112, 113, 120, 121, 168, 196, 197, 213, 219, 220, 233, 243, 282

M

macrophages, 18, 25, 198, 221
magnesium, 126, 170, 179
majority, 29, 140, 167, 230
management, 5, 11, 13, 72, 73, 91, 93, 96, 97, 101, 102, 103, 104, 105, 108, 109, 115, 116, 117, 118, 120, 121, 122, 129, 135, 170, 179, 181, 182, 183, 186, 187, 188, 190, 208, 211, 213, 214, 217, 218, 229, 232, 234, 235, 243, 245, 246, 253, 260, 261, 266, 286, 289,305

mannitol, 70, 85, 86, 91, 220, 275
manufacturing, 102, 105, 190
mast cell, 17, 18, 19, 23, 24, 25, 26, 28, 29, 32, 39, 97, 111, 185, 186, 198, 209, 219, 220, 221, 240, 243, 255, 268, 275, 298
matrix, 22, 33, 35, 37, 113, 121, 230
matter, 66, 84, 131, 164, 208, 251
measurement, 4, 81, 83, 84, 87, 99, 100, 101, 103, 116, 133, 155, 255
mechanical ventilation, 169, 170, 174, 232
medical, 49, 73, 99, 102, 107, 108, 127, 128, 131, 133, 134, 138, 181, 182, 217, 218, 230, 237, 252, 258, 331
medication, 6, 11, 14, 55, 63, 70, 88, 116, 117, 149, 150, 152, 153, 155, 156, 161, 188, 244, 258, 296
medicine, 30, 61, 63, 97, 104, 105, 108, 116, 119, 170, 181, 188, 232, 238, 249, 254, 263, 285, 305, 306
Mediterranean, 6, 33, 34, 38, 42, 44, 47, 48, 51, 152
memory, 22, 24, 26, 27, 32, 168
meta-analysis, 15, 41, 49, 91, 98, 104, 120, 212, 232, 247, 260, 268, 282, 296, 305
metabolism, 217, 218, 291, 299
metabolites, 36, 37, 198, 246
methylation, 157, 164, 165, 299, 306
methylprednisolone, 174, 194, 208, 214
Mexico, 47, 150, 162, 288, 289
mice, 19, 24, 25, 37, 51, 72, 164, 304
microbiota, 17, 18, 19, 20, 21, 29, 30, 31, 204
microscope, 38, 40, 44, 58
migration, 25, 28, 37, 113, 135, 197, 209, 230, 270
mild asthma, 83, 109, 155, 244, 246, 249, 258
models, 17, 19, 24, 28, 99, 113, 139, 168, 202, 241, 276
modifications, 113, 115, 157, 158, 160
mold, 66, 73, 75, 132, 147, 162, 195
molecular weight, 3, 39, 40, 41, 42, 43, 45, 46, 47, 48, 93, 184
molecules, 22, 26, 36, 50, 97, 98, 108, 111, 112, 113, 160, 220, 237, 256, 257
monoclonal antibody, 24, 176, 177, 209, 210, 214, 265, 268, 270, 271, 273, 275, 276, 278, 281, 298
montelukast, 186, 229, 235, 243, 288, 289, 294, 295, 302, 303
morbidity, 11, 12, 109, 157, 232
mortality, 1, 2, 4, 11, 12, 14, 15, 109, 110, 161, 163, 170, 174, 261
mucin, 113, 193, 194
mucoid, 201, 205, 206, 213
mucosa, 21, 26, 29, 34, 36, 158, 159, 164, 184, 190, 219, 220, 221, 223, 225, 228, 233
mucus, 22, 24, 25, 79, 113, 114, 169, 171, 177, 194, 196, 198, 199, 201, 205, 206, 210, 214, 220, 221, 225, 242, 244
mutant, 198, 212, 291, 292, 294

N

nasal polyp, 80, 110, 120, 185, 217, 218, 229, 234, 235, 236, 266, 270, 271, 273, 277, 278, 280, 281, 282
National Health and Nutrition Examination Survey, 13, 52, 75, 260
near fatal asthma, 125, 126, 129, 133, 232
nebulizer, 85, 86, 240
Netherlands, 287, 288, 290
neutrophils, 18, 26, 37, 115, 121, 198, 230, 275
New Zealand, 13, 14, 133, 136, 157, 289
nitric oxide, 18, 22, 73, 83, 97, 105, 110, 116, 121, 122, 151, 225, 230, 234, 243, 245, 294, 297
nitrogen, 149, 150, 151, 152, 163
nitrogen dioxide, 151, 152, 163
NK (natural killer) cells, 18, 25, 150, 161, 270
nonallergic, 2, 5, 18, 28, 29, 32, 95, 185, 190, 219, 220, 221, 231, 232, 261, 269
non-T2 asthma, 18, 112, 115, 118, 278
North America, 6, 33, 34, 39, 40, 44, 46, 47, 50, 100, 148
NSAIDs, 90, 217, 218, 227, 228, 229, 230, 231, 305
nucleus, 113, 158, 159
nursing, 171, 172, 173, 177
nursing home, 171, 172, 173, 177

O

obesity, 88, 110, 122, 208, 277, 286
obstruction, 2, 13, 35, 62, 72, 79, 80, 81, 84, 85, 87, 90, 99, 113, 114, 118, 121, 130, 183, 223, 225, 226, 233, 273, 275, 297
occupational asthma (OA), ix, 55, 57, 61, 62, 72, 85, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 110, 186
oral corticosteroids (OCS), 2, 111, 112, 115, 117, 179, 208, 224, 230, 238, 241, 243, 266, 267, 268, 269, 270, 271, 272, 273, 275, 277, 278
osteoporosis, 235, 238, 246
outpatient, 45, 46, 170, 174
overlap, 96, 99, 109, 194
overproduction, 217, 218, 229, 232
oxidative stress, 116, 151, 152, 158, 159, 160, 162, 203

ozone, 85, 113, 130, 131, 149, 150, 152, 155, 156, 162, 163

P

parallel, 40, 111, 153, 238, 247, 275, 281, 282, 283
parenchyma, 80, 84, 114, 115
pathogenesis, 2, 19, 21, 26, 28, 29, 33, 41, 45, 95, 97, 111, 120, 197, 198, 211, 232, 265, 291, 297, 298
pathogens, 22, 36, 168, 184
pathology, 11, 96, 138, 167, 195, 196
pathophysiological, 18, 96, 177, 181, 188, 225, 255
pathophysiology, 23, 29, 97, 110, 113, 176, 187, 196, 237, 297, 298, 305
pathways, 32, 59, 97, 103, 111, 112, 113, 116, 117, 118, 121, 160, 164, 169, 174, 176, 177, 178, 179, 181, 183, 188, 190, 197, 209, 223, 224, 231, 233, 265, 270, 276, 278, 291, 293, 302, 303, 304, 305
pattern recognition, 22, 36, 196
peripheral blood, 24, 26, 29, 97, 194, 198, 202, 203, 205, 267, 276, 299
persistent asthma, 109, 122, 178, 219, 238, 246, 247, 266, 279, 282, 293, 303
personalized medicine, 61, 116, 263, 285, 305
pH, 116, 122, 220
pharmacogenetics, 286, 296, 300, 302, 303, 305
pharmacogenomics, viii, ix, 285, 300, 301, 302, 303, 304
pharmacokinetics, 246, 291, 302
pharmacotherapy, 101, 102, 247, 250
phenotypes, 19, 22, 29, 30, 32, 77, 83, 96, 97, 99, 102, 103, 104, 107, 108, 111, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 187, 190, 191, 218, 223, 224, 226, 232, 233, 238, 242, 255, 265, 266, 267, 270, 271, 276, 278, 286, 304
physicians, 90, 95, 116, 117, 118, 126, 128, 228, 230
placebo, 71, 72, 165, 191, 209, 234, 240, 250, 258, 259, 261, 263, 267, 268, 269, 270, 271, 272, 274, 275, 276, 280, 281, 282, 283, 303
plants, 33, 34, 40, 70, 79, 80, 137, 139, 151, 152, 153, 154, 156, 160, 162, 167, 195
plasma cells, 24, 97, 197
pneumonitis, 64, 167, 172, 195
Poland, 38, 41, 100, 140
polarization, 22, 36, 37, 49, 169, 233
pollen allergy, 41, 42, 45, 49, 50, 51, 126, 127, 130, 131, 132, 134, 226
pollen grains, 33, 34, 35, 38, 39, 41, 42, 44, 45, 48, 49, 50, 51, 125, 126, 127, 130, 131, 132, 134, 138, 141, 145, 146, 148, 153, 160
pollen tube, 34, 40, 45
pollens, vii, 3, 19, 23, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 64, 73, 75, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 150, 151, 153, 154, 155, 156, 160, 161, 162, 163, 165, 167, 171, 184, 191, 226, 250, 251, 254, 262, 263
pollination, 33, 34, 40, 49, 52, 156
pollinosis, 33, 39, 44, 49, 50, 51, 126, 129, 132, 137, 138, 139, 140, 144, 147, 148
pollutants, 3, 21, 36, 41, 64, 111, 146, 148, 149, 150, 151, 152, 153, 154, 156, 157, 158, 159, 160, 162, 164, 184, 259, 275
pollution, viii, ix, 1, 45, 46, 51, 60, 78, 127, 131, 134, 135, 137, 138, 146, 149, 150, 151, 153, 154, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 170, 171, 177, 184, 219
polymorphisms, 160, 197, 213, 300, 301, 302, 303, 304, 305
polyp, 78, 80, 87, 110, 120, 229, 231, 236, 273
population, ix, 4, 5, 6, 13, 14, 15, 23, 39, 41, 42, 45, 46, 47, 50, 51, 52, 60, 61, 67, 72, 74, 75, 80, 83, 87, 94, 103, 109, 111, 120, 137, 138, 140, 150, 153, 156, 178, 208, 218, 226, 246, 250, 271, 272, 274, 275, 280, 286, 287, 288, 289, 290, 292, 294, 300, 301,303, 305
precipitins, 194, 199, 201, 204, 205
precision medicine, 30, 63, 97, 104, 105, 119, 188, 232, 238, 249, 306
prednisone, 81, 113, 122, 207, 209, 280
pregnancy, 3, 13, 21, 89, 90, 91, 157, 269, 280
prevention, 4, 62, 71, 96, 102, 103, 105, 126, 127, 134, 146, 164, 179, 245, 247, 258
probability, 83, 88, 98, 198, 294
prognosis, 61, 101, 169, 211, 232, 254
pro-inflammatory, 17, 23, 25, 196, 207, 294
proliferation, 17, 22, 24, 27, 197, 198, 241, 270
promoter, 160, 292, 302
propagation, 167, 185, 196
prostaglandin, 18, 29, 219
protection, 30, 213, 269
protective role, 25, 177, 179
protein family, 43, 45, 47, 59
proteins, 21, 28, 33, 36, 39, 41, 43, 45, 46, 51, 60, 95, 139, 160, 198, 203, 210, 220, 251, 257, 292, 295
public health, 2, 12, 102, 127, 128, 147, 183

Q

quality of life, 1, 2, 12, 93, 110, 121, 157, 183, 187, 188, 229, 231, 238, 242, 249, 259, 265, 268, 269, 271, 272, 275, 276, 278, 285, 286
quantification, 50, 188, 225, 226, 251, 278

R

rain, 39, 64, 126, 127, 128, 129, 134, 145, 151, 153, 154
reactions, 18, 19, 25, 30, 42, 43, 56, 64, 69, 109, 126, 132, 160, 189, 195, 207, 210, 211, 217, 218, 227, 228, 234, 252, 254, 257, 259, 266, 267, 269, 273, 274, 296
reactivity, 39, 40, 41, 42, 45, 46, 47, 51, 59, 67, 73, 74, 85, 98, 187, 190, 204, 220, 224, 228, 235, 257
receptor, 18, 19, 22, 23, 24, 25, 26, 27, 28, 29, 31, 36, 60, 97, 111, 113, 117, 121, 158, 159, 168, 176, 177, 184, 196, 197, 209, 210, 213, 219, 220, 229, 230, 234, 235, 239, 240, 242, 243, 244, 245, 246, 257, 266, 268, 270, 271, 272, 275, 276, 281, 290, 291, 293, 295, 296, 297, 302, 303, 304, 305
recognition, 22, 36, 50, 67, 102, 118, 159, 170, 177, 223
recommendations, 117, 182, 236, 245, 263
reduced lung function, 35, 49, 61, 288, 302
regression, 14, 97, 141, 231
regulatory T cells (Tregs), 18, 25, 112, 160, 168, 256
relapses, 171, 211, 214
relaxation, 170, 240, 244
relevance, 38, 42, 50, 71, 75, 98, 114, 115, 183, 184, 226, 249, 250, 251, 252, 285, 286
relief, 63, 88, 229, 241, 247, 286, 296
remission, 31, 205, 207, 211, 246, 250, 254, 273
remodelling, 110, 112, 113, 117, 282
repair, 18, 25, 176
replication, 176, 234, 293, 298, 303
reproduction, 62, 64, 167
requirements, 69, 73, 161, 201, 211, 231, 279, 289, 302
researchers, 34, 38, 41, 95
resistance, 27, 45, 46, 84, 113, 114, 115, 120, 121, 184, 190, 195, 204, 209, 219, 275, 282, 295, 305
resources, 1, 2, 12, 108, 116, 128, 129, 252
respiratory failure, 171, 174, 206
respiratory syncytial virus, 61, 168, 170, 172, 174, 177, 179
responsiveness, 13, 30, 81, 84, 98, 122, 190, 197, 241, 266, 285, 292, 293, 294, 296, 297, 298, 299, 302, 304
rhinitis, 5, 30, 33, 34, 39, 40, 46, 48, 52, 62, 72, 73, 75, 78, 83, 86, 94, 97, 99, 111, 116, 118, 125, 126, 127, 129, 130, 131, 132, 133, 137, 138, 147, 150, 162, 176, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 218, 225, 233, 234, 235, 244, 247, 249,250, 251, 252, 253, 254, 259, 260, 261, 262, 263, 279
risk, 1, 2, 3, 5, 12, 13, 15, 21, 41, 49, 55, 61, 62, 63, 67, 69, 71, 72, 75, 83, 88, 90, 94, 97, 102, 103, 109, 110, 111, 112, 114, 116, 117, 118, 119, 121, 126, 127, 129, 131, 132, 133, 134, 135, 136, 145, 147, 150, 151, 153, 154, 157, 158, 159, 160, 161, 162, 164, 165, 168, 170, 171, 172, 174, 176, 178, 179, 183, 184, 189, 197, 198, 208, 218, 228, 235, 239, 240, 244, 246, 249, 252, 254, 257, 259, 267, 269, 270, 287, 288, 289, 290, 294, 296, 301, 303
RNA, 167, 174, 233
rural areas, 4, 45, 127, 147, 151, 152, 163

S

safety, 50, 63, 72, 101, 116, 123, 191, 215, 230, 231, 236, 240, 241, 242, 243, 245, 247, 251, 263, 269, 271, 272, 273, 274, 275, 279, 280, 281, 282, 283
salts, 21, 95, 98
SARS-CoV, 171, 172, 174, 176, 177, 179
school, 13, 61, 63, 88, 109, 119, 168, 170
seasonality, 64, 146, 148
secrete, 17, 25, 111, 198, 256
secretion, 22, 24, 25, 113, 196, 220, 225
sensitivity, 62, 71, 74, 89, 96, 98, 129, 201, 203, 204, 228
sensitization, 19, 22, 23, 33, 34, 35, 36, 38, 40, 41, 42, 43, 46, 47, 48, 49, 50, 51, 52, 55, 56, 57, 59, 60, 61, 62, 63, 64, 66, 67, 69, 70, 71, 72, 73, 74, 75, 93, 97, 98, 99, 101, 102, 103, 110, 111, 119, 127, 131, 133, 135, 138, 140, 142, 146, 147, 150, 156, 164, 168, 172, 177, 178, 190, 194, 203, 204, 206, 212, 214, 231, 234, 260, 269, 278
sensors, 69, 158, 159
serum, 24, 28, 30, 46, 98, 99, 100, 121, 186, 187, 194, 198, 199, 200, 201, 202, 203, 204, 205, 207, 209, 210, 211, 213, 224, 225, 226, 255, 257, 262, 266, 269
severe acute respiratory syndrome, 171, 174, 179
severe asthma (SA), ix, 11, 13, 15, 26, 29, 30, 39, 55, 69, 70, 72, 75, 91, 97, 104, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118,

119, 120, 121, 122, 123, 126, 128, 132, 134, 150, 157, 163, 168, 171, 179, 187, 189, 190, 194, 195, 201, 203, 204, 206, 209, 212,214, 215, 218, 228, 231, 232, 233, 234, 236, 237, 238, 241, 242, 243, 244, 245, 249, 252, 257, 263, 265, 266, 267, 268, 269, 270, 271, 272, 274, 276, 278, 279, 280, 281, 282, 283, 286, 290, 300, 303, 305
sex, 5, 7, 8, 9, 11, 25, 83, 94, 99, 110, 171
shock, 59, 131, 132, 134
short-acting inhaled beta-agonists (SABA), 88, 170, 238, 244, 245, 296, 297
shortness of breath, ix, 77, 78, 79, 135, 169, 286
showing, 8, 39, 40, 41, 47, 77, 112, 240, 268, 273, 296
side effects, 111, 116, 208, 240, 241, 243, 265, 266
signalling, 111, 112, 113, 233
signals, 18, 27, 36, 111
signs, 77, 78, 85, 131, 174, 187
sinus, 182, 224, 228, 229, 230, 231, 235
sinusitis, 2, 3, 90, 189, 230, 235, 271
skin, 34, 46, 50, 52, 55, 56, 57, 60, 62, 64, 66, 70, 74, 75, 89, 97, 99, 100, 103, 104, 105, 150, 151, 158, 159, 161, 164, 165, 186, 187, 199, 200, 202, 203, 226, 238, 250, 257, 269
smoking, 13, 83, 110, 118, 119, 157, 189
smooth muscle, 26, 27, 28, 81, 113, 121, 170, 183, 198, 240, 241, 242, 244, 305
smooth muscle cells, 26, 113, 198, 241, 305
sodium, 175, 186, 198, 209
solution, 115, 224, 252, 258
Spain, 1, 2, 4, 6, 7, 8, 12, 13, 14, 15, 17, 33, 42, 46, 49, 50, 52, 55, 67, 72, 75, 77, 93, 100, 137, 140, 146, 148, 149, 154, 163, 167, 171, 177, 193, 217, 237, 249, 254, 263, 265, 271, 285, 288, 299, 331
species, 17, 19, 36, 37, 38, 39, 44, 45, 46, 47, 55, 56, 57, 64, 66, 67, 69, 70, 127, 131, 145, 147, 170, 194, 195, 204, 208
specific inhalation challenge (SIC), 97, 98, 99, 100
spore, 67, 70, 75, 145, 198
sputum, 26, 27, 29, 32, 83, 97, 98, 99, 100, 101, 110, 113, 115, 120, 122, 185, 189, 198, 199, 201, 204, 210, 224, 234, 270, 280
starch granules, 38, 131, 132
state, 6, 12, 14, 15, 18, 42, 47, 67, 74, 87, 128, 133, 135, 138, 147, 156, 157, 160, 171, 176, 190, 208, 212, 250, 261
steroids, 120, 186, 188, 199, 206, 207, 208, 209, 211, 235, 238, 242, 246, 274
stimulation, 24, 161, 183, 219, 220, 225, 305
stimulus, 184, 194, 208
storage, 55, 57, 61, 71, 72, 95
storms, 132, 133, 134, 153
stress, 41, 116, 151, 152, 158, 159, 160, 162, 163, 174, 176, 291
stroke, 85, 153, 156, 167
structure, 24, 64, 160, 296
subcutaneous route (SCIT), 63, 249, 251, 253, 254, 259
subgroups, 185, 211, 274
sublingual route (SLIT), 63, 72, 249, 251, 252, 253, 254, 255, 258, 259, 261
supervision, 100, 228, 230
suppression, 21, 25, 174, 235, 240, 250
surveillance, 5, 102, 105
survival, 25, 60, 169, 209, 219, 270
susceptibility, 1, 2, 72, 112, 113, 134, 157, 158, 159, 170, 183, 197, 213, 286, 293, 294, 300
Sweden, 38, 41, 163, 174
syndrome, 40, 41, 66, 74, 77, 85, 90, 148, 171, 172, 174, 179, 183, 185, 195, 237, 271, 277, 281
synergistic effect, 137, 138, 241, 257
synthesis, 14, 24, 36, 121, 220, 223, 292, 293, 302

T

T cell, 18, 19, 22, 24, 25, 26, 31, 111, 120, 121, 157, 168, 196, 197, 220, 255, 275
T lymphocytes (Th2), 17, 18, 19, 22, 23, 24, 25, 26, 27, 29, 31, 36, 37, 59, 61, 97, 111, 112, 152, 158, 159, 160, 161, 164, 165, 169, 172, 174, 176, 177, 178, 196, 197, 209, 210, 255, 298
T regulatory cells, 112, 165, 255
T2 asthma, 18, 19, 27, 111, 112, 116, 117, 194, 217, 219, 224, 225, 231, 265
T2 cytokines, 18, 19, 22, 23, 28, 111, 198, 273
T2 inflammation, 18, 29, 110, 111, 112, 220, 223, 225, 233, 265
target, 21, 101, 115, 117, 186, 198, 231, 233, 265, 282
Task Force, 91, 104, 108, 187, 280
techniques, 62, 78, 84, 114, 188, 204, 256, 299
technology, 95, 176, 177, 203
temperature, 38, 63, 64, 87, 127, 128, 130, 137, 138, 139, 140, 141, 142, 143, 145, 146, 196
temporal variation, 38, 41, 50
testing, 81, 84, 85, 87, 89, 97, 99, 100, 102, 103, 186, 187, 188, 189
TGF, 25, 28, 112, 113, 121
therapeutic targets, 18, 118, 176
therapeutics, 176, 210, 285

therapy, viii, ix, 4, 24, 28, 29, 32, 63, 73, 74, 84, 85, 87, 88, 96, 107, 108, 109, 115, 116, 117, 119, 122, 127, 130, 133, 168, 169, 170, 171, 174, 176, 177, 179, 186, 187, 188, 194, 205, 206, 207, 208, 210, 211, 214, 215, 217, 218, 224, 228, 230, 231, 236, 237, 238, 240, 241, 242, 243, 244, 245, 246, 247, 262, 265, 266, 267, 269, 274, 275, 278, 279, 280, 282, 283, 285, 286, 288, 289, 291, 292, 293, 295, 296, 298, 299, 300, 301, 303, 304, 305
thunderstorm-asthma, ix, 125, 126, 128
thunderstorms, vii, ix, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 145, 148, 163
tics, 24, 118, 122, 218, 224, 225, 231, 279, 303
tissue, 18, 19, 24, 25, 28, 29, 204, 220, 224, 234, 239, 240, 246, 255
TNF-α, 25, 27, 113, 160, 162
tobacco, 60, 78, 79, 110, 150, 157, 164, 184, 225, 277
toxicity, 157, 208, 210, 211
trachea, 80, 182, 183
training, 117, 118, 123
traits, 112, 122, 185, 188, 257
transcription, 27, 158, 159, 160, 230, 236, 291, 292, 297, 298, 305
transport, 21, 23, 33, 34, 36, 60, 61, 153, 295
trees, 19, 33, 34, 35, 40, 41, 42, 44, 45, 46, 48, 49, 50, 51, 52, 116, 129, 139, 182, 205, 251
trial, 22, 72, 73, 123, 191, 210, 214, 235, 246, 247, 261, 263, 268, 269, 270, 271, 273, 274, 275, 276, 279, 280, 281, 282, 283, 298, 303
triggers, 3, 34, 78, 85, 101, 102, 108, 111, 116, 117, 135, 158, 159, 170, 184, 186, 196, 219, 266
tumor, 22, 87, 165
type-2 cytokines, 18, 19, 22, 23, 28, 111, 194, 198, 273

U

underlying mechanisms, 5, 217, 218
united airway disease (UAD), viii, ix, 181, 182, 183, 184, 185, 186, 187, 188
United States (USA), 6, 12, 14, 15, 42, 47, 67, 129, 131, 132, 133, 138, 142, 147, 148, 157, 190, 250, 261, 286, 287, 288, 289, 290
upper airways, 185, 223, 225, 227
upper respiratory tract, 182, 188, 241, 276
urban, 45, 46, 73, 135, 138, 146, 147, 150, 151, 153, 154, 156, 162, 163, 164, 233
urticaria, 99, 266, 278, 280, 305

V

vaccine, 71, 251, 257, 271
validation, 99, 213, 260
variables, 8, 138, 141, 174, 209
variations, 4, 6, 12, 14, 38, 41, 44, 48, 50, 69, 77, 147, 148, 183, 204, 251, 291, 293, 298, 303
ventilation, 62, 64, 66, 87, 102, 114, 115, 169, 170, 174, 232
viral infection, 22, 27, 78, 111, 113, 168, 170, 171, 172, 174, 176, 177, 178, 179, 224, 292
viruses, viii, 3, 30, 36, 61, 131, 167, 168, 170, 172, 174, 176, 177, 178, 179, 184, 210, 275
viscosity, 186, 198, 210, 220, 221
vision, 66, 68, 125

W

water, 85, 145, 151
weather, 69, 70, 127, 128, 133, 134, 135, 163
weeds, 19, 33, 34, 40, 46, 47, 52
wheezing, ix, 2, 6, 13, 14, 78, 79, 80, 90, 97, 110, 162, 163, 168, 169, 170, 176, 178, 193, 195, 199, 286
work absenteeism, 1, 2, 12, 109
work environment, 61, 89, 101
workers, 69, 72, 93, 95, 101, 102, 103, 105
workplace, 13, 69, 93, 94, 95, 96, 97, 98, 99, 100, 101, 103, 105
work-related asthma (WRA), 93, 94, 95, 96, 97, 99, 101, 103, 104, 105
World Health Organization (WHO), 1, 56, 73, 108, 109, 118, 119, 156, 158, 164, 174, 189, 260, 261
worldwide, 2, 4, 49, 55, 56, 109, 128, 171, 174, 195, 237, 286

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