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Asthma

From Childhood Asthma to ACOS Phenotypes

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Notice

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Meet the editor

Prof. Celso Pereira is a head-chief of Clinical Immunology Unit at Medicine Faculty of Coimbra University, and also the recently created discipline, Clinical Herbal Medicine. In addition, he is a graduated specialist in Immuno-Allergy at Coimbra's University Hospital Centre, at Portugal. He was the past president of the Immuno-Allergy Board of the Portuguese Medical Association. His main activities include clinical practice, education (pre- and post-graduate), and clinical and laboratorial research. He is a member of national committee for vaccination and a coordinator of some clinical guidelines approved and under application by Portuguese health authorities. He is also a member of a national committee for the diagnostic procedures in the field of Allergy and Clinical Immunology. His scientific interests include the participation and development of several research projects, but now he has focused his interest in the mechanisms of respiratory allergy, specific immunotherapy, and drug interactions with medicinal herbs. He has edited five scientific books, and has more than 120 scientific publications, including book chapters and scientific papers. He has published over 350 abstracts related to the developed research projects and presented his work at scientific events. He was also honored with 36 scientific awards. He was responsible for the first subcutaneous application of latex immunotherapy and for the first long-term sublingual native *Pru p3* desensitization. During his Iberian Chapter Presidency of the Latin-American Allergy, Asthma and Immunology Society, he coordinated an international multicenter study focusing aeroallergen sensitization in the Iberian Peninsula. He is an associate editor of seven scientific journals related to Allergy and Clinical Immunology. He has been participating in the organization of scientific meetings and actively collaborates with the scientific committees of several Medical Scientific Societies.

Contents

Preface

Despite the huge knowledge of the pathophysiological mechanisms of asthma, the current concerns for the clinics are the precise identification and characterization of the different phenotypes, to optimize the suitable therapy and a customized plan as much as possible. In fact, the asthma medications that we have available, and also many of those that are actually subject to clinical trials, would allow an overall functional and clinical control for the majority of our patients, but unfortunately this goal cannot be achieved in all asthmatic patients.

For this purpose the clinical specificities developed in this book, particularly from those reported in the pediatric population to those reported in complex shapes at ACOS patients, emphasize the importance of identifying not only biomarkers but also critical aspects regarding the variability in pharmacogenomics responsible for the individual response to the different drugs on the therapeutic plan.

The contribution of several well-known specialists with their profound knowledge inherent to this issue into different age groups and socio-geographical contexts has resulted in this interesting book with relevant key contents in asthma.

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Chapter 1

Allergen Control in Asthma

Ayfer Ekim

Additional information is available at the end of the chapter

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Abstract

Asthma is one of the most prevalent chronic diseases especially among children so that it continues to be a public health problem. Even though genetics is an important factor for asthma, dramatic increase of the asthma recently is related with environmental triggers and lifestyle factors. Understanding of the interaction of multiple factors causing asthma is absolutely necessary for the planning interventions strategies. Exposure to allergens is a key factor for asthma morbidity. Environmental exposure leads allergen sensitization for genetically predisposed individuals and persisting of exposure is a risk element for asthma and other allergic diseases as well. Evidences suggest that environmental triggers avoidance and control interventions preclude asthma attacks, decrease the frequency of symptoms, and the need for drugs. Thus, environmental control should be focused in the management of asthma. Identifying and controlling of indoor and outdoor environmental triggers is the cornerstone for a successful asthma management. Figuring out the reasoning factors and developing primary preventive precautions are necessary to decrease asthma development frequency throughout the world.

Keywords: Allergen, asthma, environment, sensitization, trigger

1. Introduction

Asthma and asthma-related allergic diseases are most prevalent chronic diseases in developed countries. In recent years, a dynamic increase has been observed in prevalence of allergic diseases [1–3]. Even though the exact reason for such an increase is not know, home environment design and quality of indoor allergens are thought to be the factors for those diseases [4, 5]. Allergen induced diseases like asthma is an important public health problem and a burden for health resources [6]. Asthma and allergic reactions are life threaten conditions. Additionally, such diseases have negative influence on children and adults [1, 2, 4, 7]. Although increase

in asthma for children and adults has been discussed widely in recent years, it is believed that the leading factor for this increase is environmental factors. Genetic heterogeneity and ethnicity variety, lifestyles and changes in the type of accommodation are the key factors for the development of asthma [8, 9]. Since avoidance from allergens is an important part for the management of allergic diseases, taking required precautions to prevent exposing to the allergens is the first step of symptom control. A successful identification and avoidance from environmental triggers might prevent inflammation of diseases. Avoidance from allergens in allergic diseases like asthma is more effective than treatment itself [7, 10]. So international asthma guides recommend environmental control implementations as a part of systematic approach to asthma [2]. This chapter focuses on the importance of allergens and control of allergens for the management of allergic asthma.

In the asthma patogenesis, the role of environment is getting more obvious. Asthma develops through the interaction of genetic agents with environmental exposure [1, 11, 12]. Individual allergen sensitivity is the most important feature and exposing to allergens play the key role to trigger asthma symptoms and inflammation. Asthma management might be controlled by multifaceted interventions since the reasons for asthma are multifactorial. Studies have suggested that combination of different interventions would be much more effective than a single intervention. Thus, it is essential that a successful asthma management should include multiple strategies covering pharmacological and nonpharmacological methods [1].

In allergen control, the risk of exposure should be identified for sensitive individuals, and some interventions should be planned to decrease or to remove the risk, accordingly [4, 6, 13]. In recent years, large number of strategies aiming at reducing of asthma based morbidity and mortality rates have been developed and tested [14, 17]. Within the scope of those strategies, it has been stated in many studies that the education provided for allergen control is not an effective intervention on its own to change environmental control behaviours [18]. To sum up, individual interventions are usually ineffective. Yet, effective allergen avoidance requires a detailed approach.

2. Indoor Allergens

Asthma is characterized by chronic airway inflammation caused by genetic-environment interaction [2, 3, 19]. Even though seasons are accepted as triggers for allergens, exposure to indoor allergens and their presence throughout the year means a greater risk for asthma. Many environmental factors might trigger asthma, but the most important ones are indoor allergens [20]. Indoor environment is a source of risk for health, and exposure to indoor allergens occur in indoor areas [11, 21]. Indoor environment means not only the house but also schools, offices, restaurant, and cars [22, 23]. A strong relation between pathogenesis of allergic diseases like asthma and exposure to indoor allergens has been emphasised in many epidemiological cohort studies and population surveys as well. Biological functions of allergens enhance IgE response and cause allergic inflammation directly. Environmental exposure to allergens and atopic predisposition affect the development of IgE and Th2 responses [8].

Modern life conditions and long hours spent indoors leads to high level allergen exposure, enhancing sensitization, and asthma symptoms [24, 25]. Major indoor allergens, which has been proved as triggers of allergy are dust mite, cockroach, mouse, pet dander, and mold [11, 26]. Although major allergens are present in all of the inner city houses, their presence differ in geographic regions due to climatic variety of geographic regions. Controlling of indoor allergens need much more effort compared to seasonal allergens since the presence of indoor allergies continue throughout the year [27]. In studies, it has been suggested that sensitization prevalence of children to indoor allergens is higher and those children with indoor allergen sensitization are also sensitive to outdoor allergens [28, 29].

Poor housing condition is more often the reason for exposure to the triggers and allergen sensitization. Exposure to indoor allergens may produce symptoms for asthmatic individuals [23]. It has been commonly believed that cats and dogs provoke the symptoms. However, high levels dust mites allergens in the house are the majör risk factor for sensitized individuals. Excessive moisture, inappropriate or bad heating/cooling systems, overcrowding, cockroaches, structural problems are the triggers for asthma [26]. Decreasing indoor allergen exposure causes healing in the asthma symptoms, and lessens drug use [22, 26, 27].

2.1. Dust mites

Although people have lived with dust mites for centuries, dust mites allergy has increased dramatically in recent times. Such an increase might be explained by the suitable conditions for dust mites due to the modernization of dwellings and long hours being spent in indoor areas. [16]. Dust mites are the first discovered indoor allergens. Two of the most common house dust mites are Dermatophagoides pteronyssinus (Dp), and Dermatophagoides farinae [27, 30, 31]. High levels of mites might be found in from the dust of mattresses, pillows, carpets, upholstered furniture, bed covers, clothes, and soft toys. Ninety percent of children with asthma have IgE sensitization to home dust, this might be attributed to dust mite allergens that are carried by bigger substances and that stays on the air hanging a short while. These mites occupy clothes and they are immobile. Distribution of allergens inside the house differ between rooms, more commonly in the bedroom particularly on the bed [20, 23]. Dust mites can be found in hot and humid places. Dust mites exposure is a risk factor for sensitization, and a trigger for asthma attacks [8, 16, 28, 32]. Asthma development, severity, and morbidity are strongly linked with dust mite allergy [15, 24, 31].

Numerous studies have been carried out to test the effect of dust mites removal on asthma symptoms. The most effective method to remove dust mites on the bed is to cover the bed and pillows with dust resistant clothes [30]. In a clinical study, it has been suggested that seven dust mites allergens are removed due to dust resistant cloth used to cover bed and pillows [18]. However,bed-focused interventions to remove dust mites is not effective enough to decrease the exposure. Instead, interventions focusing on patient's total exposure in a day are much more effective [30, 32]. If possible, carpets should be removed and replaced with *polietilen* coverings. Halken et al. [15] have stated in their study that semipermeable *poliuretan* bed and pillow coverings are important for children and has clinically long-term significant effects. In

addition, toys might be a source of mites and constitutes as a serious risk for asthma symptoms. Toys should be cleaned in hot water or kept in deep-frozen once in a week [15].

Acaricides is recommended as a temporary solution to remove dust mites in soft furnishings. However, some studies have suggested that it might be an effective method if combined with allergen-impermeable mattress to remove dust mites [17, 33]. Vacuum cleaning is another effective method for the removal of dust mites. Vacuum cleaners should be equipped with a special bag or high efficiency particulate air (HEPA) filters in order to diminish mites levels in the air. Additionally, wet vacuum cleaning or steam cleaning are beneficial to remove mites. Bedding staff and other items should be wasted in hot water with detergent [11, 17]. Water temperature should be more than 55^0C because colder water doesn't kill mites. Dry cleaning might be effective to kill the mites [30].

2.2. Cockroaches

Cockroach allergens are the second agent of indoor allergic sensitization after dust mites [34, 35]. Urban environment, low socio economic status, old buildings, and multifamily homes are all risk factors for cockroach occupation and high level cockroach allergens [23]. In some geographic regions, sensitization to cockroach allergens are rather common especially for children. In those regions, climate is a determinant factor and spending long hours inside the home during winter months means higher exposure to cockroach allergen. Cockroach allergens might be present in all body including shit or split [27, 34]. The most common cockroach allergens are produced by *Blattella Germanica* and *Periplaneta Americana* [20, 34].

Removing cockroach allergens in closed environments is a long term process. Although it has been considered as the best way to exterminate cockroaches, allergic material stays in the closed environment long after its extermination [28]. Thus, following the extermination of the cockroaches, the material including active allergen proteins should be cleaned with an intense vacuum cleaner. The National Cooperative Inner-City Asthma Study (NCICAS) has recently carried out a phase II study emphasising that a complete extermination of cockroach allergens in closed environments is not an easy task [2]. Education, cleaning and extermination interventions ensure a short term decrease, yet, the quantity of allergens is even higher in long term period. For the extermination of cockroaches allergen reservoirs, all the rooms should be cleaned. Studies have stated that pesticides used by professional pest control teams are highly effective to exterminate cockroach allergens. More effective interventions or chemical agents need to be tested to exterminate cockroach allergens but those agents shouldn't harm human health [10, 34].

2.3. Animals

Pet sensitization is an important risk element for asthma and allergic rhinitis. Some studies report that 50–70% of the children with bronchial asthma have sensitivity to pets [34, 36, 37]. Such high rates are the results of the trend of feeding pets at home that leads to high level exposure sensitization [38]. Among pets, cat allergy is the most common type of animal allergy. Sensitized individuals may expose to allergens directly or indirectly. Cats and dogs allergens

stay in the form of little particulates and they are very adherent to floor or clothes. People might be exposed to allergens directly by living with them or they might be exposed indirectly in animal-free areas such as schools, hospitals, or transportation vehicles. Preventing exposure to pet allergens completely is not possible. Lower dose indirect exposure to pet allergens might initiate breathing symptoms for sensitized individuals [34, 39].

Ventilation, humidity and regular house cleaning have been shown as the most effective strategies to remove pet allergens. [25]. Utilizing air-filter tools is not useful to remove mites and cockroaches allergens, however, they may constitute as an important means to reduce the quantity of pet allergens on the air – approximately 2–4 times. In the studies conducted on that issue, it has been reported that HEPA filters and vacuum cleaning lead to short term decrease in the cat/dog allergen levels, however, they do not change the levels of dust allergen concentrations [11, 14, 40]. Results of studies have suggested that the removal of pets from house has provided optimal pharmacotherapy reduced airway responsiveness significantly to methacholine, but no significant differences has occurred in the change of FEV1 [37, 38, 41]. Furthermore, it has also been stated in this study that the removal of pets from the house has led to a reduce in the doses of inhaled corticosteroids and in the frequency of follow-up visits. Alternatively, air cleaners and pet washing are other measures when the pets are not removed from the house [31]. Washing cats is aneffective short time intervention. Allergen level of the cat increases dramatically within a week after washing. Past studies have shown that repeated washing progressively decreases the amount of cat allergen, and also reduces the amount of airborne allergens originating from the animal. Animal washing procedure is important to reduce allergens, washing with tap water for at least three minutes and pet shampoo is recommended. Washing is an effective method of reducing allergens, but it is not an effective for a long-term time [14, 31]. Avner et al. [14] indicated that the amount of allergen is significantly reduced after washing, but this reduction could not be maintained for a week. As a results, removal of the pets reduce airway responsiveness in patients with pet allergic asthma more than optimal drug use [37]. Even if the pet is excluded from home environment, allergic material keeps living for several months in the same environment. Thus, removal of carpets, covering of bed and mattresses are necessary for the exclusion of allergens for highly sensitive individuals [11, 42]. For pet allergies, use of HEPA filters , mattress covers, and exclusion of cats from the bedroom leads to airway hyperresponsiveness development, and decrease in peak flow variation [28]. For highly sensitive individuals, removal of carpets and upholstery, and encasement of mattresses might be essential to diminish cat allergen levels to a reduce in allergic symptoms. High proportion of sensitized people are not willing to remove pets from their houses because they accept the pets as a member of their family. In this case, environmental control precautions should be taken seriously, such as keeping the pet outside of the bedroom, room air filtration, washing the pet once a week, keeping it in a separate area inside the home, and using HEPA filters. Nevertheless, presence of pets inside the houses always poses a risk for sensitized people [38].

Even though a consensus is available on the role pet allergens for asthma development, it has been tested by some studies that on early interaction with pets can prevent allergy and asthma development for children [27, 43, 45]. The issue whether pet exposure is a risk factor or a

protective factor for allergic symptoms and allergic sensitization has still been under discussion. Studies have focused on the possibility that pet exposure might be beneficial as they can obstacle the development of atopic diseases particularly on the early years of life [43, 46]. Ownby et al. [44] suggested in his study that living with cats and dogs relates to a lower risk of developing atopy during childhood and young adulthood. Collin et al. [36] emphasises in their study that there is no relation between having a pet and bronchial response to methacholine for an 8 year old child. The relation between exposure to allergens and allergy sensitization is a matter of discussion. The results of recent studies confirms hypothesis that keeping pets at home might lead to development of tolerance in a certain degree [46, 47].

2.4. Environmental tobacco smoke

Tobacco smoke is a public health issue and 25-35% of the people with asthma are regular smokers. Approximately 30% of the children has a difficulty to control asthma exposure to environmental tobacco smoke [19]. Environmental tobacco smoke affects individuals at all ages. Negatively, the effect of it on children is stronger, and they can not prevent themselves appropriately [13]. Passive smoking is the most important source of indoor air pollution [48]. Children are exposed to smoke not only at their homes but also in car, other public areas, or restaurants. However, home is the most common place for exposure [49].

The results of exposing to tobacco smoke at pre or postnatal stages are different. Exposure to maternal smoking at prenatal period has long-term effects on the respiratory health of children [50, 51]. Chemical substances available in smoke, transmits to the placenta and affects the health of fetal growth negatively [19]. Prenatal smoke exposure of the baby is associated with deficiency in both functional residual capacity and index of tidal respiratory flow. Furthermore, maternal smoking is associated with increased serum IgE levels, and prevalence of skin-prick test responses in children. Additionally, many studies have suggested that maternal smoking increase the risk of snarling respiration for children under 6–years old [52]. Smoking exposure size at prenatal period and lung irritation are the factors leading allergy development [28, 48, 50].

Children whose parents smoke, experience more severe asthma symptoms and have exacerbations more often [23]. It has been stated in Lang et al.'s [19] study that children exposed in indoor environmental tobacco smoke had more respiratory infections, and significantly worse asthma related quality of life. In addition to its prenatal relation with reduced airway size and its postnatal behavior as a proinflammatory lung irritant, some have proposed that environmental tobacco smoke might also affect allergy development [28].

Tobacco smoke, affecting respiratory and circulatory systems, it the primary reason for many diseases and death, thus, it is a critical issue for public health throughout the world [19]. Although there are several interventions aiming at educating and raising consciousness in people, they are not common enough as desired. Unfortunately, such a public health issue is still waiting for a solution, although lots of strategies has been developed so for. The sole way to prevent children from tobacco smoke is to giving it up inside the house and to inform parents and family members about the mandatory tobacco education programmes helds at school. This is one of the recommended strategies to raise the awareness of environmental tobacco

smoke. In addition, strong national and international policies are required for solution of this issue [2, 19, 28, 49, 52].

3. Outdoor Allergens

People expose to outdoor allergens directly or indirectly is their life-span [53]. Controlling of outdoor allergens is more difficult than controlling of indoor allergens [1]. The most common sources of outdoor allergens are pollens, fungal spores, and air pollutions. Pollens are important risk factor for allergic rhinitis. Furthermore, they cause asthma through their particules penetrating lower respiratory ways. Some evidences suggest that exposure to pollens and airborne allergens increase asthma exacerbations. In order to reduce the pollen based respiratory symptoms, sensitized people should stay at home with closed windows at certain periods of the day that is an effective method to reduce allergen inhalation. Furthermore, wearing masks in outdoor areas might be useful to reduce exposure [53].

Urbanization is an important contributor for asthma since it increases air pollution. Air pollution is a crucial risk factor for health and quality of life in urban life [54]. Children are more sensitive to air pollution and meteorological factors because their lungs have not completed development and developing immune and respiratory systems make them vulnerable to pathogens [55]. There is a consensus on the idea that air pollution might trigger asthma symptoms but the role of air pollution in the development of asthma is a matter of discussion. Air pollution is associated with asthma inflammation, increasing bronchial sensitivity, admitting to emergency departments and increasing rates in the use of drugs. The effects of air pollutants on lung functions depend on type of pollutants, their environmental concentration and duration of exposure to the pollutants. Airway mucosal induced by air pollutants and ruined mucociliary clearance facilitate transmitting and penetrating of allergens to immune system [56]. Concentration and nature of air pollutants changes among regions while the most common ones are ozone (O3), nitrogen dioxide (NO2), particulate matter (PM), sulfur dioxide (SO2), and carbon monoxide (CO) [53].

Modern lifestyle, emission gases of vehicles and air pollution are important risk factors for respiratory allergy for urban life. Controlling those allergens identified as outdoor is rather difficult however reducing exposure to those allergens might be an ideal approach [3, 38]. Individual efforts to control outdoor allergens might be insufficient since their sources are outdoor environment. Primarily, in order to reduce air-pollution based asthma inflammation high level ozone and PM2*5 levels warning should be done, traffic should be reduced in urban areas, emission rates of vehicles should be controlled by local authorities [54]. On certain days when air-quality is poor patients should avoid outdoor activities and indoor activities should be included in asthma management plan. Air pollution is a global problem and the solution

requires local national and international efforts by governments, industries and private sector authorities [55, 56].

4. Sensitization

Sensitization is the production of specific IgE antibodies to allergens and immune response begins with sensitization [3]. Asthma prevalence and incidence are affected by many factors. Allergic sensitization is one of the most important factors especially for children and adolescents [9]. Allergic sensitization increases the risk of asthma 4–20 times [23]. The effect of lifestyle factors have still been a matter of debate. On the other hand allergic disease history of the family is doubtlessly an important factor for allergic sensitization [40]. The importance of living conditions and environmental factors exposed at early childhood have been emphasised in many studies [4, 9, 22, 57].

Allergens are a kind of protein and able to penetrate the nasal and respiratory mucosa [58]. Sensitization to inhaler allergens is a major risk factor for asthma however the strength of this factor has still been debated. Furthermore, dose-response relation is another issue that has still been examined. Epidemiological evidences suggest that high level exposure to inhaler allergen is an important risk factor for atopic bronchial asthma especially in the first years of life [13, 20, 57]. It has been reported in many studies that sensitization to allergens is directly related with development of asthma symptoms, exacerbations and severity of asthma symptoms. Childhood asthma, particularly is associated with allergy sensitization and allergy exposure (**Table 1**) [13, 24, 28].

Exposure to high level allergens at early childhoods means a risk for childhood asthma. For the development of sensitization, exposure to indoor allergens at early childhood is an important determinant compared to outdoor allergens. Inner city and urban population studies have indicated that more 80% of school children with asthma sensitized to at least one indoor allergen [4, 23]. Allergic sensitization is a strong determinant for the continuity of disease at later life [40].

Skin prick test, which is the most common method to determine allergic diagnosis, is the basic test procedure to prove IgE based allergic disease sensitization. In the test different allergen extracts is pricked on upper-arm with little quantities and reaction of the person to these extracts is assessed [57]. It provides confirming the type of allergy for sensitization and different allergens can be tested simultaneously as well. The test ensures confirmation of sensitization to allergens objectively [59]. National Asthma Education and Prevention Program (NAEPP) recommends to use the test to assess exposure to allergens besides the patient's history [2]. It is helpful for specific immunotherapy since it makes right interpretation of sensitization children from the birth. As for children, the test can be re-applied if changes in symptoms or new environmental allergens are available. Experienced health professionals perform the test according to international standardized allergens. In test, ≥3 mm swolen tissue is accepted as positive allergen reaction (**Figure 1**) [58].

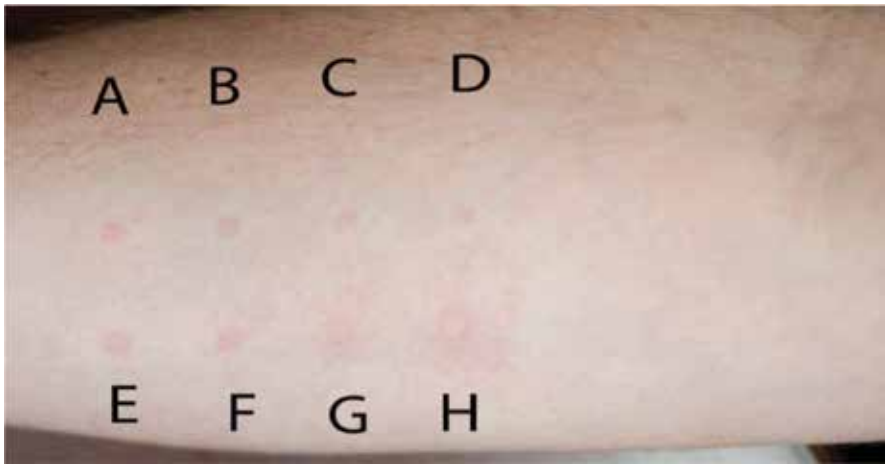

Figure 1. Skin prick test. Kwong et al. [60].

Another sensitization test is the measuring of specific IgE antibodies for a certain antigen such as Enzyme-Linked Immunosorbent Assay (ELISA) or Radioallergosorbent test (RAST). ELISA identifies IgE with a colored reaction product whereas RAST uses radioactively labelled allergen [8, 59].

Allergy history of family	Genetic influence on allergic sensitization
Gender	Underdebate
Childhood environment	Rural and farming environment
	Close interaction with furry animal at early childhood
	Air pollution
	Crowded family
	Exposure to tobacco smoke

Table 1. Factors affecting allergic sensitization.

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Chapter 2

Non-invasive Biomarkers in Asthma: Promises and Pitfalls

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Additional information is available at the end of the chapter

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Abstract

The asthma concept has evolved throughout the years: one major step in asthma management is the recognition of the chronic (airway) inflammation; another major step is further understanding of asthma heterogeneity and subsequent development of targeted therapies. While the concept of chronic inflammation, airway structural changes and their variability over time are widely accepted, their measurement and monitoring have gone through many hardships.

In this chapter, we discuss the need for applicable biomarkers in asthma management and focus on the currently available and most promising totally non-invasive samplings and detection techniques, ranging from single biomarkers to biomarker panels and composite signatures, including molecular high-throughput "omics" technologies outcomes. Limitations of these biomarkers are compared with minimal-, semi- and invasive techniques. Additionally, we discuss the benefits of an integrative systems medicine approach, considering asthma phenotypes based on cluster analysis of multidimensional biomarker datasets and its contribution to recent developments towards the promise of better understanding asthma and personalised asthma management.

Keywords: asthma, biomarkers, composite signature, phenotype, personalised medicine

1. Introduction

According to the concurrent paradigm, asthma should not be regarded as a single disease, but rather as a complex of multiple, overlapping syndromes. The heterogeneity of asthma has been

recognised already for over a century, for instance, as intrinsic and extrinsic ("allergic") asthma [1].

The introduction and subsequent validation of hypertonic saline-induced sputum analysis revealed different inflammatory asthma phenotypes: i.e. eosinophilic versus non-eosinophilic [2]. Asthma phenotypes comprise shared similar observable characteristics, produced by the interactions of an individual's genetic make-up and the environment that can be affected by several triggers and respond to treatment. However, phenotypes may vary over time and do not directly link to the underlying pathophysiology. Factor analyses involving various disease characteristics and biomarkers, including fractional exhaled nitric oxide (FeNO) levels and sputum cell differentials, helped to further define asthma (sub)phenotypes [3, 4].

In the 1990s, in analogy with animal models, asthma was thought to be a typical T-helper (Th)2- and immunoglobulin E (IgE)-driven disease, and hence, the proof of clinical effectiveness of potential asthma therapeutics was tested in the allergen challenge model. More recently, genomics and other sophisticated "omics" techniques enabled further characterisation of various inflammatory cells and other biomarkers, and helped to link asthma subphenotypes or endotypes to specific cellular and molecular pathways. For instance, gene expression profiling revealed two major subtypes: i.e. "Th2-high" and "Th2-low" asthma providing evidence for responders and non-responders to Th2-targeted therapies [5, 6]. Apart from the involvement of the adaptive immune responses, pathognomonic for parasites and allergens, more recent insight showed the major involvement of the innate system (ILC2s: innate lymphoid type 2 cells) in some asthma endotypes [7]. Interestingly, both Th2 cells and ILC2s produce type 2 cytokines (i.e. interleukin (IL)-4, IL-5 and IL-13) and these type 2 responses are mainly mediated by eosinophils. However, the underlying "upstream" mechanisms differ: while allergens mainly drive Th2-responses [8], viruses and pollutants are common triggers for ILC2-mediated type 2 responses that involve epithelial cells and IL-25, IL-33 and thymic stromal lymphopoietin [9]. Presently, it is not fully clarified how exactly both type 2 response pathways interrelate.

Apart from disease typing, the discovery of new inflammatory pathways and related biomarkers resulted into the development of endotype-specific, individualised asthma treatment.

In this review, we aim to highlight the key non-invasive and semi-invasive biomarkers currently used in the management of asthma.

2. Do we need biomarkers in asthma?

Given the heterogeneity of asthma and the evidence that standard therapy is not (fully) effective in all patients, especially in those with more severe disease and those at risk for frequent exacerbations, the need for appropriate biomarkers allowing the identification and subsequent targeted treatment of these patients has been increasingly recognised. Since asthma is multidimensional and thus presents at several different levels including clinical, physio-

logical, histological, cytological and molecular, various approaches have been developed to identify effective biomarkers (**Table 1**) [10]. In addition, given the complexity of the disease, (unbiased) biomarker clustering within different asthma populations has been performed by several research groups, which revealed different disease subphenotypes with varying disease course and/or response to treatment [3, 4].

Disease level	Parameters/biomarkers
Clinical	Age of onset Frequent exacerbators Therapy resistance Cofactors, including allergy, nasal polyps, recurrent viral infections, air pollutants including passive and/or active tobacco smoke, obesity
Physiological	Lung function (normal, reversible, fixed obstruction) Airway hyperresponsiveness
Cytological	Inflammatory cells and soluble markers in: Sputum (central airways); BAL, bronchial wash/brushings (peripheral airways)
Histological	(Trans)bronchial biopsies (inflammatory and structural cells and structures)
Exhaled air	FeNO (fractional exhaled nitric oxide) EBC (exhaled breath condensate) VOCs (volatile organic compounds: eNose) EBT (exhaled breath temperature)
Systemic biomarkers	Peripheral blood: eosinophils, CRP, IgE, periostin, cytokines
Molecular	Genomic SNP analysis (i.e. the large-scale genotyping of single nucleotide polymorphisms) Transcriptomic analysis (i.e. the measurement of all gene expression values in a cell or tissue type simultaneously) Proteomic analysis (i.e. the identification of all proteins present in a cell or tissue type) Metabolomic analysis (i.e. the identification and quantification of all metabolites present in a cell or tissue type; eNose)

BAL: bronchoalveolar lavage; CRP: C-reactive protein; eNose: electronic nose; IgE: immunoglobulin E; SNP: single nucleotide polymorphism

Table 1. Clinical and biological biomarkers in asthma.

Using a systems biology approach in large cohorts of patients, researchers within the Innovative Medicines Initiative Severe Asthma Project U-Biopred have been collecting data, including molecular analyses, tissue, exhaled air and blood samplings, as well as clinical and lung function data, and patient-reported symptoms [11]. By combining this information, the researchers aimed to generate a "handprint", i.e. a combination of clinical and biological

characteristics (biomarkers) indicative of a specific asthma subphenotype/endotype. Subsequent studies are being undertaken to test if one's "handprint" can predict the disease course and can indicate a response to (targeted) asthma treatments. This approach will provide a key step to personalised medicine [12–14].

Generally, an ideal biomarker should possess the following key characteristics: clinical relevance, adequate sensitivity and specificity for (targeted) treatment effects, repeatability, simplicity and cost-effectiveness [10].

3. Promising single non-invasive biomarkers of asthma

The concept of asthma has undergone considerable changes throughout the years, from a disease mainly manifesting by variable symptoms and bronchoconstriction to airway inflammation and remodeling. More recently, heterogeneity has gained an outstanding position in asthma definition. So far, one of the most important steps in asthma history, bringing significant reduction in morbidity and mortality, was the recognition of airway inflammation in asthma and the introduction of efficacious and safe anti-inflammatory therapy for asthma control. Despite ongoing developments, current guidelines for both diagnosis and follow-up of patients with asthma are still grounded on clinical and lung function parameters. Thus, functional biomarkers were the first objective measures coming forward into clinical practice and, in general, the promise of delivering valuable molecular, cellular or histological biomarkers to daily clinical practice has not yet been met. However, intense research in asthma has brought together scientists from academia, research institutes, the pharmaceutical industry and patient organisations, with significant progress taking place in the recent years. In this section, we discuss the currently available and more advanced non-invasive biomarkers in asthma.

Clinicians and researchers dedicated to asthma may benefit from a direct analysis of the airways, profiting the patients. In fact, non-invasive airway assessment is possible through lung function tests (LFTs) and airway sampling. Furthermore, other "more distant" to the airway biomarkers (such as blood or urinary biomarkers) can also be regarded as potentially useful, considering the systemic properties of asthma.

3.1. Functional biomarkers

LFTs are essential in routine clinical practice. They are non-invasive, well validated and reproducible. At present, LFTs provide the only generally accepted functional biomarkers to objectively aid in the diagnosis, risk assessment and monitoring of asthma. Thus, asthma definition currently implies the objective detection of variable airflow limitation, while the "best personal lung function" is a hallmark of asthma monitoring and future risk assessment.

LFTs provide relative features (phenotypes) that aid in differential diagnosis, namely in the distinction from chronic obstructive pulmonary disease (COPD), but are not diagnostic in its use. For instance, neither post-bronchodilator airway obstruction, lack of bronchodilation response or hyperinflation can be used to rule out asthma.

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Presently, LFTs patterns alone are not considered to define disease subsets that respond to particular therapies. However, lung function has been shown to be predictive of clinical outcomes and provide complementary information to subphenotype asthma. For instance, variability measures of lung function can predict the loss of asthma control and response to long-term beta2-agonist treatment [15].

Airway hyperreactivity (AHR) is a basic pathophysiological hallmark of asthma, but remains a complex component of this disease. A growing number of variable airway smooth muscle (ASM) and non-muscle factors contributing to AHR has been recognised. Besides its high negative predictive value in the diagnosis of asthma, AHR has been advocated as a surrogate biomarker related to airway inflammation to guide asthma management. It has been shown that anti-inflammatory therapy directed at reducing AHR may imply higher corticosteroid doses, but leads to improved lung function and better control [16, 17]. AHR evaluation has also been suggested useful in back titration of inhaled corticosteroids. However, the reduction in AHR with higher doses appears targeted to the persistent structural component of AHR (defined as opposed to the variable inflammation component of AHR). Emerging data support that it is the structural changes of the airway that mainly contribute to AHR (i.e. reticular layer thickness and ASM hypertrophy) [16]. This effect also depends on the type of challenge used: assessing AHR to indirect bronchoconstrictor stimuli is superior in the detection of changes associated with airway inflammation, while direct stimuli, mediated through direct interaction with ASM, better reflect the structural changes. Assessment of AHR is a useful non-invasive tool providing complementary information, though its routine feasibility in general practice can be hard to settle.

Summing up, lung function measurements may not, per se, reflect the precise underlying pathological processes responsible for different phenotypes. However, in a multidimensional approach to evaluate asthma as a complex dynamic disease, functional biomarkers and their variability must definitely be part of future composite parameters in asthma.

3.2. Exhaled air biomarkers

Exhaled breath can be sampled in a fully non-invasive manner across all age groups. However, exhaled breath analysis is not useful for analysing cellular or histological biomarkers and, in general, the search for useful molecular biomarkers has been hampered by methodologic difficulties mainly dealing with very low molecular concentrations, variability and lack of sampling and analysing methods standardisation [10].

FeNO is so far the most commonly used molecular biomarker in exhaled air. Nitric oxide (NO) is a gaseous chemical compound, which can be measured in exhaled breath either by chemiluminescence and electrochemical analysers. The American Thoracic Society and the European Respiratory Society recommendations for standardised procedures for the FeNO measurement have been published [18]. Accordingly, FeNO is measured at a flow rate of 50 mL/s, thus reflecting NO production from the central airways. Currently available devices allow accurate and highly reproducible measurements, through simple, fast and non-invasive methodology. Hand-held devices are now widely available in clinical practice and used in both adults and children (since preschool age, usually above the age of 4 years) [10].

Evidence-based guidelines for adequate interpretation of FeNO measurement have been developed [19]. This biomarker can be affected by several perturbing factors, mainly age, height and recent active or passive smoking. Other variables that have been reported to affect FeNO levels include weight, gender, race, atopic status, diet or alcohol intake [20]. Large variation of normal FeNO values exists, with wide inter-individual differences and significant overlaps between healthy/non-asthmatic and asthmatic populations. Intriguingly, the aforementioned confounding factors explain few of the substantial variations within the general population [20]. For these reasons, guideline-recommended cut-points are supported for routine interpretation of FeNO levels [19].

Presently, there is evidence to support the use of FeNO thresholds essentially for assessing the likelihood of Th2-mediated airway inflammation and responsiveness to corticosteroids [19]. Low FeNO levels do not rule out asthma [19].

Persistently high FeNO levels may be attributed to poor adherence to corticosteroid therapy, poor inhaled drug delivery or persistent/high allergen exposure [19]. This has also been suggested to reflect a highly reactive asthma phenotype [21]. Although FeNO may be indicative of loss of disease control or exacerbation, some patients remain with high FeNO despite good clinical asthma control, and clinical trials of FeNO-guided management have yielded conflicting results [22–24]. Increased knowledge on asthma pathophysiology and the source and biochemistry of FeNO may help to further understand these findings. Traditionally, FeNO is known to originate in the airway epithelium as a result of inducible nitric oxide synthase (iNOS) upregulation, which occurs with inflammation [19]. Recent data give further support to this view by showing iNOS overexpression in the airway epithelium of patients with asthma [25]. However, it is interesting to note that despite the strong association between FeNO and Th2-mediated/eosinophilic inflammation and atopy, eosinophils are not the principal cells in the airways that express iNOS and this enzyme is upregulated by Th1 cytokines [26]. Anti-IL-5 and anti-IgE therapy for asthma reduced sputum eosinophilia without affecting FeNO, contrary to IL-13 inhibition that significantly decreased FeNO [27]. Studies have shown that FeNO levels are not elevated in many patients with severe asthma, compared to mild and moderate asthma, despite evidence of airway inflammation [13, 28]. Other sources of FeNO need also to be considered. For instance, as NO is a highly reactive molecule, it can be trapped and directly regenerated by abundant free thiol-containing biomolecules [26]. One of these thiols is S-nitrosoglutathione, which has been shown to be depleted in severe asthma, possibly contributing to comparative lower FeNO levels in these patients. Another important reservoir of nitrogen species is nitrite/nitrous acid. These agents are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides. When airway pH increases, more nitrite is formed and FeNO levels fall. On the other hand, FeNO may be high with acidification [26]. Still, many questions regarding the source of FeNO and its specific role need to be explored. Another area of research that may bring additional knowledge and clinical usefulness is dedicated to partitioning of FeNO. In particular, alveolar FeNO can be obtained by measuring FeNO at multiple flow rates and has been shown to be an independent parameter that is putatively associated with increased distal lung inflammation and more severe disease [29].

In summary, the clinical importance of FeNO as a marker of Th2-mediated airway inflammation that is likely to respond to corticosteroid treatment may be "indirect," but is well established. Further analysis is needed to address the possible need to define FeNO levels cut-points in different situations, according to the presence or absence of pertinent confounders. The application of FeNO measurement to identify particular asthma phenotypes or as part of a more comprehensive panel of biomarkers including also other "Th2 type" biomarkers may allow taking better profit of this readily available biomarker [30]. Partitioning of FeNO is a promising area of research, whose clinical usefulness is yet to be established.

Other biomarkers have been studied in exhaled breath vapor namely *volatile organic compounds* (VOCs). In general, reactive oxygen species result from inflammation and promote polyunsaturated fatty acids degradation, originating volatile hydrocarbons. These VOCs are subsequently excreted in exhaled breath. Thus, exhaled VOCs may originate from systemic metabolism or from local airway inflammation. It is important to consider also that VOCs in exhaled breath may also be originated from pathogenic bacteria or from exogenous sources such as ambient air pollution [31]. Some studies have suggested that single VOCs such as pentane or ethane could be significantly higher in patients with asthma. However, VOCs profiles analyses bring significant additional value [31].

Another potential single biomarker in exhaled air is *exhaled breath temperature* (EBT), which reflects heat, a cardinal sign of inflammation. EBT has been shown to correlate with bronchial blood flow [32], which is advocated as the main mechanism to explain EBT changes in disease status.

Several studies have shown that EBT is higher in patients with asthma [32–34]. Conflicting data have been reported regarding a possible association between EBT and asthma control, with several studies supporting [34, 35], and others rejecting this relation [36, 37]. Correlation between EBT and other biomarkers, such as sputum eosinophils and FeNO, has resulted in inconsistent reports [32, 37]. Furthermore, EBT has been shown to increase after eucapnic voluntary hyperventilation, methacholine challenge test or exercise, but no difference was found between asthmatics and healthy individuals [38], suggesting this increase in EBT to be physiologic.

However, it is important to stress that different methods have been used to measure EBT. Some studies used a flow and pressure-controlled maximal slow continuous exhalation to residual volume to measure EBT, while others measured EBT in tidal volume until a temperature plateau was reached. Different variables have been analysed: plateau EBT, rate of temperature increase, time to achieve plateau EBT. These different methods preclude results comparison and, to our knowledge, no study has analysed both methods simultaneously. The recent development of improved, easier-to-use, portable devices has improved feasibility, including in children and in the elderly [34, 36, 39].

Moreover, further studies are needed when it concerns interpretation of the results. Variables such as room temperature and relative-ambient humidity may influence the results [39]. Some studies point a correlation between gender [37, 39], age [36, 39] and lung volume [36], which

needs to be addressed. No significant correlation has been documented between EBT and auricular temperature, suggesting EBT to be a distinct variable and not just another measurement of body temperature [33, 34].

Conclusively, EBT assessment may be an appealing method enabling completely non-invasive and patient-friendly evaluation and deserves further standardisation and validation as a potentially useful biomarker in asthma.

3.3. Exhaled breath condensate biomarkers

Exhaled breath has been a source for intense research in the latest years and many other biomarkers have been studied. *Exhaled breath condensate* (EBC) has the advantage of being a more stable matrix than exhaled breath vapor, including volatile and also non-volatile compounds. It is obtained by cooling exhaled air and is thought to reflect the composition of the airway lining fluid. Many molecules have been analysed in EBC, including metabolites and also proteins. Although methodological recommendations for exhaled breath sampling and analysis have been published [40], the procedures for EBC collection and biomarker detection are not fully standardised and there is significant heterogeneity between different working groups yielding (highly) variable data.

Many biomarkers analysed in EBC reflect oxidative stress. Among these, the most extensively studied include H_2O_2 and isoprostanes.

H_2O_2 is a reactive oxygen species that contributes to oxidative stress within the airways. A meta-analysis has reported that EBC H_2O_2 concentrations were significantly higher in adults with asthma, and associated with disease severity and control [41]. This has also been reported in children. Of importance, smoking increases H_2O_2 levels. EBC H_2O_2 levels were inversely correlated with lung function parameters and improved with inhaled corticosteroids [41]. Thus, EBC H_2O_2 has been suggested a promising biomarker for asthma control monitoring.

Oxidative stress can also be assessed through the determination of lipid peroxidation-derived products. *8-isoprostane* derives from arachidonic acid peroxidation. Increased levels of 8-isoprostane have been found in EBC in patients with asthma, correlating with disease severity [42]. EBC 8-isoprostane levels have been shown to be particularly useful to indicate asthma control and severity in childhood when combined with different markers [30]. Increased 8-isoprostane levels in EBC of children with exercise-induced bronchoconstriction (EIB) have been described, suggesting a role for oxidative stress in EIB [43].

Markers of inflammation have also been addressed. *Leukotrienes (LT)* are important mediators of airway inflammation in asthma, and the most extensively studied molecular biomarkers of inflammation in EBC. Increased levels of LTs have been detected in EBC of patients with asthma, correlated with disease severity and were effectively reduced by oral corticosteroids or LT receptor antagonist [44, 45]. However, the reported effect of inhaled corticosteroids on LTB4 EBC levels is controversial [46]. LTs have been suggested as markers of asthma severity [42]. Likewise, LTs have been associated with EIB severity [47].

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Various *cytokines* and other molecules have been analysed in EBC. In particular, IL-4 has been found to be higher in EBC of patients with asthma, especially in asthma associated with atopy [30, 42]. Cytokine ratios and biomarker panels in EBC including cytokines have been suggested to be useful to assess asthma control (including IL-4 and interferon-gamma) and to predict asthma exacerbations (e.g. IL-5) [30, 48].

Last but definitely not least, the measurement of *pH* is one of the simplest and most technically validated biomarkers in EBC. EBC pH reflects airway acidification [49]. Several research groups have found higher pH levels in healthy subjects, compared to patients with asthma [10]. Significant decline in EBC pH occurred during asthma exacerbations. EBC pH shows good reproducibility, having low running costs and normal data sets have been published in self-reported healthy subjects [50].

Although some biomarkers may be useful to measure in EBC, samples are highly diluted, biomarker concentrations are difficult to measure, require specialised equipment, laboratory techniques and normalisation standards are lacking. Unfortunately, EBC has been hampered by serious drawbacks in the methodology, detection techniques and result interpretation, all consistent with large intra and intersubject variability, precluding validation for most single biomarkers.

3.4. Biomarkers in non-respiratory specimens

Other non-invasive matrices have also been analysed in search for biomarkers in asthma. *Saliva* is a readily available specimen and allows metabolites, proteins and also deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) extracting (although buccal swabs perform better), including also oral microbiota assessment. *Cotinine* in saliva has been one of the most extensively studied biomarkers, with interest in asthma as a measure of tobacco exposure. Salivary *cortisol* has also been used for the evaluation of adrenal function. Morning salivary cortisol was significantly lower in patients with asthma than in healthy individuals, and poor asthma control has recently been associated with lower salivary cortisol levels [51]. Preliminary data have suggested that *inflammatory salivary markers* may also be associated with asthma control, including eosinophil-related (such as eosinophil cationic protein) and myeloid/innate mediators [52]. Additionally, a significant decrease in salivary antioxidant enzyme-peroxidase activity was observed in children during asthma exacerbations [53]. A salivary pH decline has also been associated with asthma and AHR [54]. Another area of research includes the analysis of oral microbiota, which may change in asthma, either through disease status or its pharmacotherapy. The interest in saliva studies in relation to asthma is still preliminary and the role of many possible confounders needs to be considered.

Although *urine* does not directly reflect the airways, samples are easily obtained across the full age spectrum. Several urine molecular biomarkers have been described to be associated with asthma. Here, we focus on four molecules which have been studied in more detail.

Of the potent lipid inflammatory mediators comprising the cysteinyl *LTs*, only LTE4 is stable, making this molecule the dominant LT detectable in biological fluids. Urinary levels of this end product of LT metabolism have been shown to be elevated in asthma, both in children and

adults, and in patients with aspirin-exacerbated respiratory disease [55, 56]. It has been associated with the degree of airflow limitation and acute exacerbations [55, 57]. Although inhaled corticosteroids are the most effective treatment for asthma, they do not alter LTE4 excretion. Urinary LTE4 levels have been suggested as potential predictors of better response to anti-LT therapy compared to other therapeutic approaches, though further studies are needed, including other biomarkers, to predict individual responses.

As LTs, *prostaglandins* (PG) are the end products of arachidonic metabolism. PGD2 results from cyclooxygenase pathway and is excreted in urine after being metabolised to 9α,11β-PGF2. Increased urinary excretion occurs in patients with asthma and after challenge tests, and a negative association has been found with lung function [58].

Bromotyrosine is another molecule with possible interest in asthma. It is generated from protein oxidation by eosinophils. The oxidised amino acid is stable and excreted in urine. Urinary bromotyrosine levels are higher in patients with asthma and have been associated with asthma control and lung function, predicting exacerbations [59]. Its levels have been shown to reduce during inhaled corticosteroid therapy. High urinary bromotyrosine levels could predict a favorable clinical response to inhaled corticosteroid therapy, especially in combination with high FeNO values [59]. These results warrant further developments.

Though urinary biomarkers may become useful tools, many require specialised equipment and their measurement is not fully validated or standardised. There is a current need for normalisation standards and assessment of intra and inter-individual variation to select the potentially useful biomarkers. It is also important to address urine dilution when reporting quantitative absolute results.

3.5. Airway imaging biomarkers

Airway imaging biomarkers are also emerging, offering the potential of adding complementary information, namely on small airways function and remodeling. High-resolution computed tomography (HRCT) images are used to measure airway narrowing, wall thickening, air trapping and ventilation inhomogeneity [27]. The first two measures have been correlated with lung function and asthma severity. Increased parenchymal lucency has also been associated with severe asthma exacerbations, lung function and neutrophilic inflammation. HRCT is easily performed though it requires that lungs are scanned at a standard volume for validity and reproducibility. The risk of exposure to significant ionising radiation needs to be considered and normal ranges have not been established.

4. Composite biomarkers in non-invasive sampling: what is known and what could be useful in the future?

The complexity and dynamics of asthma drive the need to establish distinct disease phenotypes and endotypes. There are several different triggers in asthma, with various pathophysiological pathways in parallel resulting in clinical expression that may be rather similar. Therefore,

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repeated multiscale, multidimensional measurements may be needed to capture this complexity, which may yield more useful information than single or even panels of combined biomarkers [60]. In this view, molecular composite signatures may be obtained by high-throughput "omics" technologies, which are increasingly standardised. Several large-scale studies of the genome, transcriptome, proteome or metabolome have produced an enormous amount of data and it is pivotal to follow the available guidelines in order to avoid false discoveries [60]. The composite high-dimensional signatures or fingerprints are based on pattern recognition underlying complex non-linear biology systems. Some evidence that this approach may be successful in asthma has already emerged concerning differential diagnosis [61]. Regarding non-invasive, direct assessment of exhaled breath, it is interesting to note that while many problems arise in specific molecular biomarkers validation, recent studies have shown encouraging results with the application of metabolomics strategies to study exhaled biomarkers [60, 62, 63].

Among "omics" systems biology, metabolomics is considered the one that comes closest to phenotype expression. It involves the identification and quantification of small molecular weight metabolites. Real-time metabolomics measurements are already feasible for several clinical applications with electronic noses (eNoses). These handheld, portable devices can capture various combinations of VOCs in exhaled breath, with nanosensors arrays. The nanosensors are based on conducting polymers, metal oxide, metal oxide field effect transistors, surface or bulk acoustic waves, optical sensors, colorimetric sensors, ion mobility spectrometry, infrared spectroscopy, gold nanoparticles and gas-chromatography (GC) coupled with mass spectrometry (MS) or flame ionisation detection [60, 64]. The pattern recognition algorithms using various eNose sensor systems indicate fingerprints of exhaled VOCs, called breathprints, which have shown to discriminate patients with asthma from healthy subjects and COPD with accuracies between 80% and 100% [65]. Breathprints have also been studied to phenotype asthma. Recent studies indicate that eosinophilic and non-eosinophilic asthma can be distinguished when using a composite eNose platform. Breath analysis by eNose could also predict the response to corticosteroids with greater accuracy than sputum eosinophils or FeNO [66]. These data suggest that composite signatures of breath analysis could be used for assessment and monitoring of airway inflammation. Important methodologic issues of technique optimisation and standardisation deserve deeper analysis, from breath sampling, to modulating factors including comorbidities and incompatibility between eNoses. These should enable external validation to determine possible disease-specific breathprints with clinical applicability.

Besides the analysis of exhaled breath vapour with GC-MS and eNoses, the novel metabolomics approach has also been applied to EBC. It has been shown to enable characterisation of metabolic compounds in even small EBC volumes, using high-resolution proton nuclear magnetic resonance (NMR) or MS. This has proved capable of discriminating healthy individuals from those with asthma [62, 63, 67, 68]. It could also discriminate between severe and non-severe asthma [63], supporting the hypothesis that severe asthma has specific metabolic features.

Interestingly, the metabolomics analysis of urine also discriminated healthy individuals from those with asthma [69], and could distinguish patients with stable asthma from those with acute exacerbations based on profiles [69, 70]. Metabolomics analysis of urine samples has also been recently suggested as a useful clinical tool to differentiate asthma from COPD [71].

Pinkerton et al. [72] demonstrated for the first time that differences between healthy controls and asthma patients could be detected via micro-RNA (miRNA) expression in EBC, and suggest that different types of inflammation may have unique miRNA signatures. These small non-coding RNAs are known to be important in the post-transcriptional regulation of inflammation, thus opening a new research field using non-invasive direct air sampling.

Proteomics has also recently been applied to EBC. Liquid chromatography (LC)-MS has been used to separate and detect proteolytic peptides present in EBC with differentiating profiles based on asthma status [73]. However, this preliminary study faced several problems such as insufficient sample volume, possible salivary contamination and difficulties in peptides identification due to their low concentration.

Besides allowing an overview of molecular signatures, the "omics" approach may potentially lead to new knowledge regarding asthma pathophysiology, due to its untargeted, hypothesis-generating approach. All biomedical researchers are facing not only the opportunities but also the challenges in accessing, managing, analysing and integrating diverse data sets that are larger, more diverse and more complex than ever before, and that exceed the abilities of current management and analysis approaches [60, 74]. Composite biomarkers research such as that coming from molecular profiling assays including various "omics" is a live example that needs to be critically interpreted and cautiously validated to yield truly significant advances in personalised medicine.

5. Non-invasive biomarkers limitations: can more invasive sampling do better?

Asthma syndromes are characterised for being dynamic, with varying changes in symptoms pattern, lung function, inflammation and remodelling throughout time. In this setting, non-invasive direct airway sampling, such as exhaled breath analysis, seems especially appealing, allowing easy and repeatable measures over time. However, low molecular concentrations and variable sample dilution lead to difficulties in methods sensitivity and validation, with consequent issues in replication of biomarker findings (**Table 2**). In comparison, bronchoscopy allows direct visual examination of the airways and direct collection of fluid (bronchoalveolar lavage, bronchial washing) and tissue (brushing, biopsy). These techniques are mostly impractical because they are invasive, require specialised equipment and qualified personnel, have contraindications and carry potential risks / complications. Therefore, ethical issues preclude bronchoscopic sampling broad use in asthma, even less when repeated samplings are needed, thus being mainly reserved for selected severe patients and for research purposes. Apart from practical issues, standard bronchoscopy techniques hold several other limitations, including lack of reproducibility and sample dilution effect, despite recently proposed

http://dx.doi.org/10.5772/62790

improvements (Table 2) [75]. In between invasive and non-invasive airway samplings, semi-invasive induced-sputum analysis may also reflect the airways and is easier to perform. Moreover, although indirect, blood sampling is minimally invasive and is a known relevant biomarker source in asthma.

Biomarker source	**Pros**	**Cons**
Exhaled breath	Totally non-invasive Validated for FeNO measurement Portable (FeNO, eNose, EBT, EBC) Direct results (FeNO, eNose, EBT) Multiple molecular biomarkers May be collected across all ages May be collected in severe patients Allows serial measurements	Validation not complete (except FeNO) Many perturbing factors Upper airways/salivary possible contamination Require expertise, expensive and time-consuming specialised lab assays (EBC) Soluble markers subject to dilution
Induced-sputum	Semi-invasive Validated tool Molecular and cellular biomarkers Useful to guide treatments (sputum-eosinophils)	Impossible in young children Contraindicated in severe bronchoconstriction / active cardiovascular disorders Rescue medication / procedures needed Non-repeatable over short time-period (<12 to 18 h) Procedure itself may induce changes in airways/lab results Upper airways/salivary possible contamination Require expertise, expensive and time-consuming specialised lab assays Soluble markers subject to dilution
Bronchoscopy	Direct airway assessment	Invasive Several medical contraindications Rescue medication/procedures needed Non-repeatable in many patients Expertise and experience required for procedure Require expertise, expensive and time-consuming specialised lab assays BAL markers subject to dilution Procedure itself may induce changes in airways/lab results (BAL)
Blood	Minimally invasive Some biomarkers routinely available (e.g. eosinophil counts) Useful to guide treatments (e.g. eosinophils counts) Molecular and cellular biomarkers May be collected across all ages May be collected in severe patients Allows serial measurements	Not directly reflecting the airways Not patient-friendly in all subjects (e.g. children) Require expertise, expensive and time-consuming specialised lab assays (some biomarkers)
Urine	Totally non-invasive May be collected across all ages May be collected in severe patients Allows serial measurements	Not directly reflecting the airways Require expertise, expensive and time-consuming specialised lab assays

BAL: bronchoalveolar lavage; EBC: exhaled breath condensate; EBT: exhaled breath temperature; eNose: electronic nose; FeNO: fractional exhaled nitric oxide.

Table 2. Pros and cons of main biomarker sample sources in asthma.

In this section, we will discuss these sampling methods and related current main biomarkers for asthma management.

5.1. Sputum biomarkers

Induced sputum is a validated sampling method of the more central airways. Sputum is collected after inhalations of hypertonic saline. Although relatively safe, induced-sputum requires specialised training, equipment and laboratory processing. Monitoring lung function during the induction procedure reduces the risk of excessive bronchoconstriction. Patient's active cooperation is needed for collection, making this technique unsuitable for some patients, especially for children below the age of 7 years [76].

Induced-sputum provides a rich source of soluble and cellular biomarkers and has exceptionally allowed a successful single biomarker-based clinical management approach in asthma. This is the case with sputum eosinophil percentage, which identifies patients who have eosinophilic and non-eosinophilic asthma phenotypes and can be predictive of poor asthma outcome and targeted treatment response, with demonstrated treatment-guided superior efficacy in reducing asthma exacerbations in adults [2, 27, 77, 78]. Thus, sputum eosinophil percentage acts as a key marker and correlates with severe exacerbations and AHR. It has also been useful in a panel of biomarkers to select patients who may benefit from IL-5 targeted therapies, including mepolizumab (anti-IL-5), reslizumab (anti-IL-5) and benralizumab (anti-IL-5R). In contrast with adults [77, 78], eosinophil sputum-guided therapy was not associated with decreased asthma exacerbations or improved asthma control in school-aged children and adolescents [79]. Sputum inflammatory phenotype was shown to be unstable in children with asthma, and this was not related to treatment or disease control [80].

Besides eosinophils, other sputum biomarkers are currently in research. Sputum neutrophils are often related to severe non-eosinophilic asthma with fixed airway obstruction. Soluble sputum biomarkers have been associated with asthma severity (e.g. eosinophilic cationic protein, LT, IL-4, IL-5, IL-13, IL-6, IL-12, tumour necrosis factor-α, granulocyte-macrophage colony-stimulating factor), exacerbations (e.g. IL-8, neurokinin A) or remodelling (procollagen synthesis peptides, tissue inhibitors of metalloproteinase or transforming growth factor-β) [10]. Many biomarkers can be measured, but most require highly sensitive detection methods and results may be affected by sputum processing or variable dilutions. These factors need to be taken into account to select and validate useful biomarkers in sputum.

Induced sputum may also be an interesting source for composite biomarkers. Unsupervised clustering of induced-sputum gene expression profiles identified three transcriptional asthma phenotypes that related to clinical and inflammatory parameters (resembling eosinophilic, neutrophilic and paucigranulocytic asthma) [81]. Differentially expressed genes were related

http://dx.doi.org/10.5772/62790

to immune and inflammatory responses, proving a framework to investigate asthma endotypes.

In summary, logistic and practical difficulties have precluded the wide use of induced sputum in clinical practice, but sputum eosinophil percentage is recommended as a supplemental measure in future asthma clinical research studies to identify specific cellular profiles and to predict or to monitor a treatment response in adult patients [27]. It is important to highlight that sputum eosinophils and FeNO are not duplicative outcome measures, even though low sputum eosinophil and low FeNO are strongly linked [27].

5.2. Blood biomarkers

Peripheral blood can be collected across all age groups, with minimal risk. Some biomarkers are routinely standardised in medical institutions and therefore readily available, such as eosinophils, total serum IgE and allergen-specific IgE. The latter are used to define atopy, which can be accurately, easily and more readily detected by skin prick test. Atopy modestly increases the probability of asthma, but is not essential for diagnosis. Though it is useful to characterise patients, atopy itself is recognised to be heterogeneous, including both "Th2-high" and "Th2-low" phenotypes [5]. Specific sensitisations are useful in clinical practice to suggest clinically relevant allergen avoidance and consider allergen-specific immunotherapy. However, total IgE or allergen-specific IgE quantification cannot predict the response to treatment and are otherwise weak biomarkers in asthma.

Blood eosinophil absolute count has long been associated with asthma and remains a recommended supplemental asthma biomarker [27]. Although it may not reflect the airways and be unspecific, blood eosinophilia supports asthma diagnosis and is an independent risk factor for exacerbations and fixed airflow limitation. Blood eosinophil counts are useful to subphenotype asthma and to monitor systemic biologic effects of pharmacologic interventions in patients with asthma, including (inhaled) corticosteroids, anti-IgE, LT antagonists and 5-lipoxygenase inhibitors [27]. Furthermore, blood eosinophil counts emerged as predictive biomarkers of clinical benefit from IL-5- and IL-13-targeted therapies, being associated with a "Th2 bronchial signature" [82].

Another promising "Th2-high" serum biomarker is the extracellular matrix protein periostin. The expression of periostin is upregulated by IL-13 in bronchial epithelial cells and, unlike IL-13, is abundant and readily detectable in peripheral blood [82]. Interestingly, a multi-centre study collecting matched sputum, bronchoscopy and peripheral blood samples from patients with asthma showed that serum periostin was the best single predictor of airway eosinophilia, with a further advantage of lower intrasubject variability over time than FeNO or blood eosinophilia [82]. However, conflicting results have recently been reported [83, 84]. Nevertheless, periostin levels have been associated with asthma severity and its levels have also been shown to be important to predict lebrikizumab (anti-IL-13) clinical benefit, with greater reduction in severe exacerbations and greater improvement in lung function in the "periostin-high" patients [85]. A greater decrease in exacerbations with anti-IgE therapy has also been reported in "periostin-high" patients. Healthy subjects and lebrikizumab-treated patients still

have measurable levels of serum periostin, thus other systemic sources of periostin than IL-13 need to be explored [82].

Overall, blood eosinophils, serum periostin and FeNO reflect "type 2" airway inflammation in different ways and are only weakly correlated; therefore, combinations of these biomarkers obtained with minimally or non-invasive samplings may further enable optimisation of treatment benefit [82, 86, 87].

Recently, application of "omics" technologies to peripheral blood and invasive sampling with unsupervised clustering are yielding crucial data to capture the complexity of various asthma phenotypes and add new insights on asthma endotypes and treatment response. Given its maturity, transcriptomics analysis using microarrays is the current state-of-the-art method for asthma signature discovery [60]. For instance, gene expression profiling of bronchial epithelium identified distinct subtypes of patients with asthma with "Th2-high" or "Th2-low" phenotype [5], supported the involvement of endotoxin and macrophage activation in corticosteroid resistance, and suggested that corticosteroids also exert their beneficial effects through activity on bronchial smooth muscle [60]. "Omics" technologies developments, with data comparison and validation, will lead to the integration of composite signature biomarkers in phenotyping asthma and improvements in our understanding of asthma. Ultimately, breakthroughs in asthma treatment may be reached through the development of innovative targeted therapies [12, 60].

Non-invasive procedures for biomarker analysis form the backbone for day-to-day clinical asthma management. However, invasive tests may provide important information to phenotype and direct therapy in patients with severe refractory asthma [88]. These techniques bring significant additional knowledge in asthma research that needs to be integrated with non-invasive procedures outcomes to allow truly innovative steps in biomarker discovery for asthma management.

6. Asthma phenotypes based on cluster analyses

In general, milder asthma phenotypes respond well to standard therapy with corticosteroids (with or without long-acting beta2-agonists), while those with more severe disease urged the development of new therapeutic modalities. To enable the development of effective (targeted) therapies, it is crucial to understand the pathophysiological mechanisms driving these subsets of asthmatic patients. Haldar et al. performed a cluster analysis on baseline data of 184 patients with mild to moderate asthma coming from different general practitioners (GP) and baseline data of 187 patients with refractory disease from specialist settings [3]. Additionally, a third dataset comprised baseline and longitudinal data of 68 patients with refractory disease followed for 12 months. Hierarchical cluster analysis revealed five different clusters, with some overlapping features between patients from GP and specialist origins. Most importantly, patients with concordant symptoms and (eosinophilic) inflammation (based on sputum analysis) were mostly coming from GP and were characterised by overall milder, often atopic, well-controlled disease, with a benign disease course. Alternatively, patients with uncontrol-

led disease, characterised by either discordant symptoms (i.e. many symptoms, little airway eosinophilia or non-eosinophilic inflammation) or discordant inflammation (few symptoms, prominent airway eosinophilia) mostly originated from the specialist settings. Commonly found confounders consisted of obesity and non-compliance. Overall, these findings supported a symptom-guided management for mild-moderate "concordant"-type asthma, while "discordant"-type refractory asthmatics might benefit from inflammation-guided therapy [78].

Using unsupervised hierarchical cluster analysis in a group of 726 patients from the Severe Asthma Research Program (SARP) revealed five distinct clinical subphenotypes within this population [4], showing some overlap with the findings by Haldar et al. [3]. The results of both cluster analysis studies underscore disease heterogeneity, even in subsets of patients with similar clinical characteristics, with potentially different pathophysiological and immunological mechanisms, requiring different therapeutic approaches.

Further analysis into the molecular mechanisms underlying different asthma phenotypes revealed at least two distinct subsets with a "Th2-high" and a "Th2-low" profile, respectively [5], based on the expression of IL-13 inducible airway epithelial genes (POSTN (periostin), CLCA1 (chloride channel regulator 1) and SERPINB2 (serpin peptidase inhibitor clade B, member2)) as previously described by this research group. Not unexpectedly, patients with Th2-driven asthma responded well to inhaled corticosteroids while those with a "Th2-low" profile did not. Hence, there is an urgent need for effective therapeutic options for "Th2-low" asthmatic patients that appeared to comprise approximately 50% of the study population, and hence, in reality may be larger than originally thought.

Additionally, these findings urged phenotyping of patients (i.e. including an adequate target population) and/or using an appropriate disease model [8], for adequate interpretation of effectiveness data in targeted intervention studies. So far, several applicable (surrogate) biomarkers have been validated to phenotype potential responders and to monitor the effects of currently available (or under development) targeted therapies, i.e. anti-IgE, and Th2-pathway targeted therapies (anti-IL-5, anti-IL-4 and anti-IL-13) [86]. Presently, biomarkers including blood eosinophils, FeNO and serum periostin thus moved the first steps to personalised medicine [87]. Further insight into the heterogeneity of Th2-driven/type 2 asthma, "Th2-low" subsets, as well as further refinement of sensitive (composite) biomarkers should be considered the next steps in this promising direction to optimise and personalise asthma management.

7. Conclusions

The complex heterogeneity and dynamics of asthma with varying response to standard treatment is driving the search for distinct asthma phenotypes and endotypes. While inhaled corticosteroids can effectively control asthma, therapeutic responses are individualised (though clinical manifestations may match), can be incomplete in a significant number of patients and no curative treatment exists.

In this setting, biomarkers are needed to innovate asthma management. As indicators of pathophysiologic processes or pharmacologic responses, biomarkers can be useful for asthma diagnosis and phenotyping, prediction of future risk or treatment selection or evaluation of response. Non-invasive sampling has the advantage of being patient-friendly and allowing repeatable measurements across all age and severity groups. More direct airway or distant assessment non-invasive sampling and analysis are currently possible, yielding molecular, cellular, functional and imaging potentially clinically useful biomarkers.

For the promise of delivering valuable new biomarkers to the clinic to come forward, it is mandatory that standard optimised procedures are set for sample collection and analysis, and that resulting data are critically processed, explored and cut-off values are well-defined. This will allow comparison of results and replication, with external validation in different population settings.

Though relevant single biomarkers have been found in asthma, increasing evidence shows that biomarker panels do better and composite signatures may indeed soon be integrated in phenotyping/endotyping of asthma. Multiscale, high-dimensional biological, together with standard clinical measures are adding new relevant knowledge. This systems medicine approach is helping to generate new hypotheses and (re)discover pathways and related biomarkers, linking phenotypes to endotypes and ultimately leading to truly innovative treatments for patients with asthma syndromes.

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Chapter 3

Matrix Metalloproteinases in Asthma-Associated Airway Remodeling – Dr. Jekyll or Mr. Hyde ?

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Additional information is available at the end of the chapter

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Abstract

Matrix metalloproteinases (MMPs) are Zn^{2+}-dependent endoproteases, which digest extracellular matrix (ECM) components and various non-ECM molecules. Main physiological role of MMPs concerns regulation of tissue remodeling and regeneration. The production and activity of MMPs are tightly supervised by multistage control mechanisms. These mechanisms include regulation of gene expression, and various post-transcriptional/post-translational modifications. However, without proper control MMPs reveal dual nature, similarly to character from the novella by R.L. Stevenson, "Strange Case of Dr Jekyll and Mr Hyde". They become dangerous molecules, involved in cancer metastasis, or cardiovascular diseases.

Recent studies revealed that MMPs are also engaged in asthma. Despite extensive research, exact role of MMPs in this process remains unclear and there is no agreement among scientists, regarding two opposite concepts. The followers of "destructive hypothesis" postulate detrimental effect of MMPs on mucosa. Accordingly, MMPs-mediated damage stimulates chaotic regeneration, and progressive remodeling. Oppositely, enthusiasts of "protective hypothesis" postulate that MMPs actually do not allow formation of excessive collagen deposits, and thus they protect from tissue fibrosis.

The better understanding of "MMPs — Jekyll or Hyde ?" story may be clinically relevant, especially while considering therapies focused on modulation of MMPs activity. Therefore, this issue requires instant elucidation.

Keywords: airway remodeling, asthma, extracellular matrix, matrix metalloproteinase, destructive hypothesis and protective hypothesis

1. Introduction

Matrix metalloproteinases (MMPs) represent group of 25 endoproteases, which require a presence of zinc ions to reveal their proteolytic activity. According to worldwide accepted nomenclature, MMPs have assigned numbers from 1 to 28. However, till now no respective molecules have been ascribed for numbers 4, 5 and 6, whereas MMP-18 was identified only in *Xenopus* frogs. [1, 2] Apart from regulation of extracellular matrix (ECM) turnover, MMPs are also involved in controlling of numerous non-ECM molecules, including cytokines and growth factors. Thus, MMPs are key molecules in embryo- and organogenesis, angiogenesis and tissue regeneration. However, they are also main destructive factors, responsible for cancer progression, aortic aneurysm rupture or delayed healing of chronic wounds. [3, 4] Recently, their involvement was postulated also in some inflammatory diseases affecting respiratory tract, among them chronic obstructive pulmonary disease and asthma. [5] In this chapter authors will focus especially on possible role of MMPs in asthma and asthma-associated alterations in architecture and function of respiratory tract mucosa, which are better known as airway remodeling.

2. MMPs — portrait of the family

2.1. MMP structure

Based on molecular structure, substrate specificity and mechanism of activation, MMPs are classified into four groups: gelatinases, matrilysins, archetypal MMPs and furin-activated MMPs (Fig. 1.). Formerly, MMPs were divided into six types – collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others. However, nowadays this classification possesses rather historical meaning. The overall structure of MMPs reveals some common features, which are similar in all members of the family. [2, 3] One of these features is the presence of signaling peptide, located on the N-terminus of newly synthesized proteins. This leader sequence is necessary for the insertion of maturating MMP molecule into cistern of endoplasmic reticulum, and then it is removed. Unlike to other family members, in MMP-23 the N-terminal signaling sequence is substituted by a type II transmembrane domain, which allows anchorage of these molecules in cell membrane.

The next part common in MMP structure is approximately 80 amino acid-long prodomain. It contains conserved “cysteine switch” motif, responsible for maintaining the latent form of enzyme by the blockade of its catalytic site. The main constant segment present in all family members is their catalytic domain. This sphere-like domain is composed of 160-170 amino acids. It contains shallow slot with two zinc ions inside, which constitutes an active site of MMP molecule. Exclusively, catalytic domains in both gelatinases, MMP-2 and -9, contain unique fibronectin II-like inserts. Most MMPs (except for MMP-7, -23 and -26) have short hinge segment of approximately 10-30 amino acids, which connects the catalytic domain with hemopexin-like domain. Exceptionally, MMP-9 molecule has the longest hinge region, composed of 64 strongly O-glycosylated amino acids. The C-terminal hemopexin-like domain,

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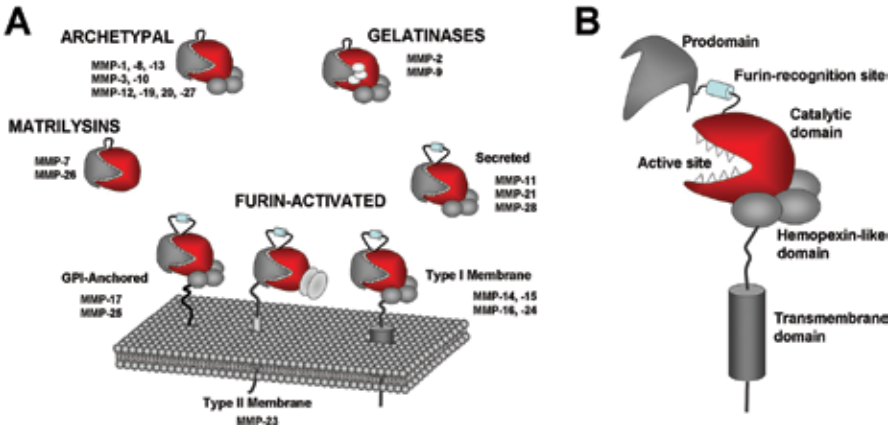

Fig. 1. Schematic representation of MMPs family. (A). Main groups of the family and their structure. (B). An example of schematic structure of MMP. Detailed description in text.

not present in MMP-7, -23 and -26, is composed of approximately 200 amino acids and is considered as docking spot for tissue inhibitors of MMPs (TIMPs). In MMP-23 molecule the hemopexin-like domain was replaced by a cysteine-rich immunoglobulin-like domain.

The representatives of membrane-type (MT) subgroup of MMPs (except of already mentioned MMP-23) have hemopexin-like domain connected to a type I transmembrane domain with a short intracellular tail (MMP-14, -15, -16 and -24, also known as MT1, -2, -3 and -5-MMP, respectively), or a cell membrane-anchoring glycosylphosphatidylinositol (GPI) moiety (MMP-17 and -25, known as MT4- and MT6-MMP) (Fig. 1).

Finally, three of secreted MMPs (MMP-11, -21 and -28), all the membrane-type MMPs and MMP-23 have a specific sequence between their prodomain and the catalytic domain, which is recognized by furin. This subtilisin-like serine proteinase from trans-Golgi apparatus and the endoplasmic reticulum removes the prodomain from the catalytic domain and thus may lead to intracellular activation of MMP molecule. [1–6]

2.2. Substrate specificity

MMPs are able to digest main components of extracellular matrix (ECM), including high molecular weight polymers of native and denatured collagens and elastin, as well as small ECM molecules, like fibronectin, laminin and aggrecan. Moreover, MMPs may process numerous non-ECM molecules, among them various adhesion molecules, dystroglycan, syndecans, growth factors, pro-cytokines, and their receptors. MMPs were shown to activate via proteolysis pro-forms of interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF), Fas ligand, transforming growth factor (TGF)-β, but also other members of MMPs family (Table 1). [1, 3, 7–9] Noteworthy, some of these cytokines, including vascular endothelial growth factor (VEGF) and TGF-β, may be further entrapped in three-dimensional net of extracellular matrix

components or by their binding proteins. Therefore, MMPs may be necessary to reveal biological activity of these factors, through their enzymatic discharge from ECM.

Group	Representatives	Main ECM substrates	Non-ECM substrates
ARCHETYPAL MMPs	**Collagenases** MMP-1, -8- 13	collagens, gelatin, fibronectin, aggrecan…	pro-IL-1β, pro-IL-8, pro-TNF, other MMPs, PAI, IGFBM
	Stromelysins MMP-3, -10	collagens, gelatin, elastin, fibronectin, laminin, aggrecan	pro-IL-1β, other MMPs, IGFBP, MMP/TIMP complex, fibrinogen, plasminogen, antitrombin III
	Others MMP-12, -19, -20, -27	collagen IV, gelatin, elastin, fibronectin, laminin	fibrin, plasminogen, myelin basic protein
MATRILYSINS MMP-7, -26		collagen IV, gelatin, elastin, fibronectin, laminin, integrins…	other MMPs, MMP/TIMP complex, fibrinogen, plasminogen
GELATINASES MMP-2, -9		collagens, gelatin, elastin, fibronectin…	pro-IL-1β, plasminogen, other MMPs
FURIN-ACTIVATED MMPs	**Secreted** MMP-11,-21,-28	collagen IV, gelatin, laminin, fibronectin	casein, IGFBP
	Type 1 transmembrane MMP-14,-15,-16,-24	collagens, gelatin, elastin, laminin, vitronectin	other MMPs
	GPI-anchored MMP-17,-25	UNK	
	Type II transmembrane MMP-23A,-23B	UNK	

Table. 1. Representatives of MMPs family with their main substrates (detailed description in text). ECM – extracellular matrix, Non-ECM- other substrates, PAI – plasminogen activator inhibitor, IGFBP – insulin-like growth factor-binding protein, TIMP – tissue inhibitor of MMPs, UNK – unknown.

2.3. MMP expression

Due to a high proteolytic activity and broad substrate specificity, MMPs are recognized as key molecules, engaged in cell proliferation and migration, tissue growth, remodeling, and regeneration. For this reason their expression and activation has to be maintained under precise multistage control. These controlling mechanisms include regulation of gene expression, post-transcriptional and post-translational modifications, but also several ways of pro-enzyme activation or inhibition of active MMP. [3, 4] Nevertheless, if these mechanisms fail, similarly to the well known character from the famous novella by R.L. Stevenson, "Strange Case of Dr. Jekyll and Mr. Hyde", MMPs may also reveal their dual nature. Without sufficient

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supervision, these endoproteases may become highly dangerous effector molecules, engaged in various pathologies. These conditions include cancer metastasis, formation and rupture of aortic aneurysm, delayed healing of chronic wounds and many others. [1, 3, 10, 11] Recent studies have provided evidence that MMPs may also be involved in pathogenesis of asthma, mainly asthma-associated airway remodeling. [5]

Among all MMPs, only MMP-2 and MMP-9 are produced constitutively, whereas the expression of majority of MMP genes requires some trigger, e.g. tissue damage, or inflammatory reaction. It was found that the promoter region of genes encoding for MMPs comprises sequences recognized by two main specific transcription factors, AP-1 and NF-κB. Both transcription factors merge expression of many inflammatory response-engaged molecules, including MMPs with several intracellular signaling pathways, induced by cytokines and growth factors. Indeed, it was proved that MMPs expression may be controlled by variety of growth factors, including TGF-β, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNF, etc.). Moreover, the promoter activity of MMPs may also be supervised by family of Ets transcription factors. Since their conserved binding site is located close to target sequence for AP-1, they may interact each other and thus modulate promoter response to various stimuli. [3, 4, 10, 12–14]

The rigid control of MMP genes expression may also be granted by their epigenetic modification. This mechanism is based on alteration in chromatin conformation, which is mediated by differential acetylation-deacetylation of nucleosomal units, due to activity of an enzyme – histone deacetylase (HDAC). Noteworthy, it has been shown that such regulation may result in various responses of particular MMP genes. *In vitro* stimulation with TNF or IL-1β, with simultaneous suppression of HDAC activity resulted in decreased expression of MMP-1 and MMP-9, but increased production of MMP-3. [14, 15] Finally, the expression of MMPs may also be modified on the post-transcriptional level, by the influence on stability or degradation of their transcripts. Recently, it has been proven that the expression of several MMPs may be negatively regulated by the small molecules of non-coding RNA, known as microRNAs (miRs), in mechanism of RNA interference. It has been demonstrated that miR-9, miR-24 and miR-133a may bind to the 3′-UTR of mRNA for MMP-14 (MT1-MMP) and they directly block its translation. On the other hand, down-regulation of miR-199a-5p in murine model induced MMP-1, possibly via Ets-1 derepression. [16–19]

2.4. MMP activity

All members of MMPs family are expressed as inactive pro-enzymes. This condition is assured by previously mentioned "cysteine switch", a specific interaction between zinc cations from the active site of the catalytic domain, and a cysteine thiol group from the prodomain. The renouncement of inhibitory influence of the prodomain on the catalytic domain is critical for activation of pro-enzyme and may take place in two concurrent ways (Fig. 2). [3, 14, 20]

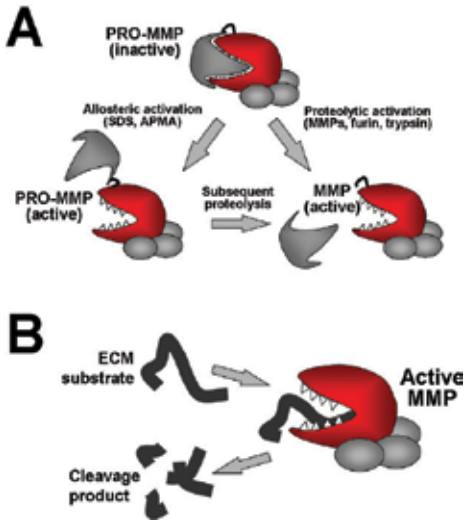

Fig. 2. MMPs activation. (A) Two pathways of MMPs activation. (B) Schematic representation of substrate cleavage. Detailed description in text.

2.4.1. Activation

The first pathway of MMPs activation is based on direct cleavage of their prodomain. It may be carried by several extracellular proteolytic enzymes, including other MMPs, as well as cysteine, serine and aspartate proteases. This pathway also involves already mentioned intracellular processing and activation by furin. Due to removal of prodomain, molecular weight of pro-enzyme activated in this pathway is significantly reduced, as compared to its initial size. Thus, in zymograms of substrate-specific zymography with SDS-polyacrylamide gel electrophoresis (PAGE) the activated MMP appears as the lower band, below that, corresponding to latent form of enzyme. [21–23]

The second pathway depends on interaction of cysteine thiol groups from prodomain with various compounds, including free radical, disulfides, some detergents with sodium dodecyl sulphate (SDS), alkylating agents, heavy metal ions and organomercurials, with 4-aminophenylmercuric acetate (APMA). This interaction may induce allosteric conversion in MMP structure, which leads to an exposure of the active site in the catalytic domain. Therefore, although the prodomain still remains attached to the entire molecule, such MMP may reveal its proteolytic activity. On the other hand, that MMP, despite being activated, has the same molecular weight, as its inactive pro-form. That explains, why such full length-MMP may be visualized in zymograms on the same level as latent pro-MMP. Noteworthy, the prodomain may be further removed by auto-cleavage, that results in decrease of MMP molecular size and, similarly to the first pathway, an appearance of the lower band in zymograms. [3]

Results of recent studies suggest that *in vitro* proteolysis requires only a substrate and respective MMP, whereas *in vivo* systems usually involve some additional component. These accessory factors may include membrane-, or ECM-associated peptides and glycosaminogly-

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cans, which may determine specificity, as possibly, catalytic rate of MMPs. Accordingly, such accessory molecules may work as a kind of adapters, which bind a substrate and MMP and thus enable their close interaction with an effective concentration. [21–23]

2.4.2. Inhibition

As was already noticed, the precise control of MMPs expression and activity is essential for homeostasis of the entire body. Therefore, to counterbalance the mentioned stimulators and activators of MMPs, some agents revealing inhibitory properties are also required. Apart from best known family of specific tissue inhibitors of metalloproteinases (TIMPs), there are also less specific endogenous inhibitors, among them α2-macroglobulin, family of serine proteinase inhibitors (serpins), thrombospondin-1 (TSP-1), tissue factor-pathway inhibitor (TFPI)-2, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), etc. [3, 10, 21, 24]

Members of TIMPs family (numbered from 1 to 4) are the best identified specific endogenous inhibitors of MMPs. They are expressed and released by various cell populations, including macrophages, platelets, smooth muscle cells, etc. The mechanism of their action depends on reversible chelating of Zn^{2+} cations from active center of MMP's catalytic domain and, thus, abolishes its proteolytic properties. Since MMP – TIMP interaction occurs in a stoichiometric ratio 1:1, the MMP/TIMP ratio seems to better reflect presumable biological impact of both agents, instead of absolute amount of each of both proteins. Moreover, it is noteworthy that studies concerning *in vivo* interactions between MMPs and TIMPs are also interfered by the highly effective serum antiprotease – α2-macroglobulin. [3, 6, 14]

Although all TIMPs may interact with various MMPs, they differ in their specificity, e.g. TIMP-1 preferentially binds to membrane type-MMPs, whereas TIMP-2 is considered as important regulator of MMP-2 activity. Interestingly, the latter regulation actually involves TIMP-2-dependent activation of MMP-2. In this unique mechanism TIMP-2 works as bridging molecule between hemopexin domain of MMP-2 and MT1-MMP (MMP-14), which mediates cleavage of prodomain in "immobilized" MMP-2.

Apart from mentioned above endogenous MMPs inhibitors, there is also an increasing number of exogenous compounds, which reveal direct and/or indirect modulatory properties towards the activity of MMPs. [3, 4] Since they have potential clinical relevance in a treatment of asthma and asthma-associated remodeling, they will be further described in next paragraph (see 2.7).

2.5. Methods of MMP measurement

Increasing interest in the role of MMPs in asthma and, especially, asthma-associated remodeling encouraged scientists to develop more specific and sensitive methods to detect MMPs in analyzed samples. However, main obstacle in MMPs research is that most commonly used methods, i.e. enzyme-linked immunosorbent assay (ELISA) and zymography, do not allow simultaneous assessment of amount and activity of MMPs. [3]

2.5.1. ELISA

Standard ELISA is a routine laboratory technique, which allows a quantitative detection of minute amounts of MMPs in solution (picograms per ml) using specific antibodies, usually conjugated with peroxidase-based detection system. Noteworthy, standard method provides data concerning specific protein concentration, without any information regarding actual activity of MMPs. Nevertheelss, such activity could be roughly estimated using specially designed ELISA sets, which enable differentiation between truncated forms of activated MMPs and prodomain-containing latent MMPs. However, as mentioned above, allosteric activation not necessarily leads to prodomain removal. Therefore, data provided by ELISA alone are not fully conclusive, and should be verified by some activity assay. [3]

2.5.2. Zymography

The substrate-specific zymography is the most commonly used method to evaluate MMPs activity in tested samples. This assay is based on initial separation of samples using electrophoresis in modified polyacrylamide gel, followed by its incubation in reaction buffer and subsequent staining. The key component of such modified gel is substrate, specific for enzyme being analyzed (e.g. collagen for MMP-1 and -13, gelatin for MMP-2 and -9, casein for MMP-1, -3, -7, -10, -12, -13), which is homogenously distributed in whole gel volume. Since polyacrylamide gel contains sodium dodecyl sulphate (SDS), the speed of migration in electrophoresis is determined by molecular weight of separated proteins, resulting in shifted local condensation of full length pro-enzyme and truncated forms of MMPs. During incubation in calcium- and zinc-rich reaction buffer, MMP molecules become reactivated and digest own specific substrate only in place of their condensation. After wash in the staining solution, e.g. Coomassie Brilliant Blue, the entire gel becomes stained, with except of unstained area corresponding to digested substrate. Noteworthy, when compared to respective molecular weight standard, the localization of unstained area enables better identification of analyzed MMP, whereas the size of digested / unstained bands well correlates with amounts of detected enzyme. This amounts may be further determined by comparison to reference sample, e.g. known amounts of recombinant MMP. [3, 25]

Although substrate-specific zymography is sensitive (picograms per sample), and relatively cheap method, it has some weak points. The first issue is long and time-consuming protocol. The next, more important concern, is uncontrolled allosteric activation of MMP mediated by SDS. Since it may strongly affect results of assessment, in current research standard zymography is often replaced by other, faster and more reliable activity assays.

2.5.3. Fluorescent activity assay

The unintended interaction with SDS may be avoided, when instead of polyacrylamide gel, MMPs activity is assessed in SDS-free reaction mixture. The measurement of substrate proteolysis in solution implements technology of fluorescence resonance energy transfer (FRET) using substrate (e.g. casein or gelatin) tagged with fluorochrome and quencher. Until labeled substrate stays untouched, the entire energy from fluorochrome is absorbed by

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quencher, with no fluorescence detectable. When the substrate is cleaved, the fluorochrome-quencher interaction becomes disrupted, that is associated with increased emission of fluorescence under UV light. Since the increase of fluorescence is proportional to enzyme activity, with known quantities of MMP as reference, and with fluorescence reader, this method allows very fast (within few minutes) measurement of proteolytic activity revealed by small amounts (nanograms per ml) of MMP in tested samples. [3, 26]

Noteworthy, in contrast to standard zymography, the fluorescence assay enables studies on proteolytic activity of MMPs, and analysis of modulation of this activity by various agents, e.g. natural and synthetic inhibitors. However, the method is very sensitive to reaction conditions, which may vary depending on protocol of sample preparation. The key factors are concentration of non-ionic detergents, and presence of exogenous protease inhibitors (frequently used to prevent proteolysis in biological material) or metal ion chelators (e.g. EDTA). On the other hand, the tissue sample preparation itself may lead to artificial activation of MMPs or release of their natural inhibitors. [3]

The main disadvantage of the basic variant of mentioned fluorescent method is its non-specificity. Therefore, when analyzing biological samples, to determine, which MMP contributes to the degradation of labeled substrate, it is necessary to use a panel of MMP-specific antibodies, to inhibit proteolytic activity of selected enzyme. Although a such approach enables precise identification of all contributors of observed proteolytic activity, it also significantly increases the cost of that analysis.

2.5.4. Immunozymography assay

Recently, a modification of mentioned above fluorescent method was introduced into market. The method combines specificity of standard ELISA and functionality of fluorescent activity assay. In a first step the sample is applied onto test plate, coated by antibody specific for MMP of interest. Then MMP molecules, which are captured by antibody, convert a latent detection reagent into its active form. The activated detection reagent catalyzes enzymatic conversion of colorless substrate into color product. Since the amount of product directly correlates with number of active MMPs, the use of standard calibration curve allows precise measurement of active MMP molecules concentration in tested samples. Furthermore, when using organomercurials (e.g. APMA) to activate pro-MMPs in tested material, it also allows an assessment of latent form of MMPs. Thus, the assay incorporates advantages of standard zymography (the assessment of MMP activity and discrimination between pro- and active forms of these enzymes), specificity of ELISA and exceptional sensitivity, reaching 0.1 pg/ml. Therefore, it may be the best choice for research concerning MMPs activity in samples, where the minute amounts of MMPs are expected, e.g. condensates of exhaled air. [27]

2.5.5. In situ zymography

The distribution of MMPs in tissue specimens may be studied using immunohistochemistry. However, to assess the local activity of these enzymes, directly on the place of their production, the *in situ* zymography may be used. Similarly to mentioned above fluorescent activity assay,

this method also utilizes FRET technology. The tested specimen is incubated with substrate labeled with fluorochrome-quencher complex and then analyzed using fluorescent microscope, or confocal laser scanning microscope. Similarly to fluorescent activity assay, *in situ* zymography does not identify particular MMPs, unless used with specific neutralizing antibodies. Furthermore, it does not provide information concerning quantities of active MMP. Nevertheless, it is still valuable supplement to other methods in MMPs research. [3]

2.5.6. Reverse zymography

As previously mentioned, various factors which affect MMPs activity, may be analyzed using fluorescent activity assay, western blot or respective ELISA. However, to detect natural tissue inhibitors of MMP (TIMPs) some functional assay, better known as reverse zymography, has been developed. The method is based on specific interaction between TIMPs from analyzed sample and MMP of interest. Similarly to standard zymography, samples are separated in polyacrylamide gel, which is supplemented with homogenously distributed substrate (e.g. gelatin), but also selected MMP. After electrophoresis the gel is incubated in reaction buffer. Since both, MMP and substrate, are present in the entire gel volume, MMP cleaves the whole substrate, except of places corresponding to the TIMPs condensation after electrophoresis. In these places TIMPs protect substrate from digestion, therefore, after Coomassie staining they appear as blue bands, whereas the remaining gel volume stays unstained. [3]

2.6. MMPs in patients with asthma

2.6.1. Mr. Hyde…?

Extensive studies, focused on asthma and asthma-associated airway remodeling, have revealed clear involvement of MMPs in pathogenesis of that disease. [5] However, the exact role of MMPs in this process remains vague. The postulated link between metalloproteinases and asthma was based mainly on observations concerning increased amounts and/or activities of various MMPs in samples collected in patients with asthma. The samples were obtained using various methods of collection and/or various material, among them serum or plasma, mucosal biopsies, induced sputum, broncho-alveolar lavage (BAL) fluid and, most recently, exhaled breath condensates (EBC). [27–31] Majority of studies concerned MMP-9, however, other MMPs, including MMP-1, -2, -3, or -12 were also studied. It is noteworthy that substrate specificity of mentioned MMPs entirely enables their self-sufficient work with full repertoire of ECM components. Collagen IV, and laminin, two main components of basement membranes, are cleaved by MMP-9 and MMP-12. Native molecules of collagen I, main fibrillar ECM component of mucosal connective tissue, are initially digested by MMP-1, whereas their further degradation may be continued by all mentioned MMPs (MMP-2, -3, -9, and -12). Elastin molecules are degraded mainly by MMP-12, but also MMP-2 and -9. [3] Although nominal values of MMPs (especially MMP-9) concentrations differed between various studies, in vast majority of mentioned reports similar regularity was observed. MMPs levels and /or activity in individuals with asthma were several fold higher than in control subjects. [28, 29, 32] The number of MMPs-positive cells in sputum or BAL inversely correlated with values of forced

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expiratory volume in 1 second (FEV1), whereas MMPs amounts in sputum, BAL and EBC positively correlated with severity of disease. They were significantly higher in severe asthma or in asthma exacerbation, and lower in mild asthma or in remission. [31, 33] Also bronchial smooth myocytes/myofibroblasts (BSM) from mucosal biopsies of patients with fatal asthma produced increased levels of MMP-9 and -12, whereas BSM from non-asthmatics expressed only small quantities of MMP-2,-3 and -9. [34]

These observations could support concept of "destructive hypothesis", which emphasizes detrimental effect of metalloproteinases on disease progression. In this scenario, similarly to Mr. Hyde from previously mentioned novella by R.L. Stevenson, MMPs reveal their dark nature. The overexpression and hyperactivation of these enzymes may result in progressive damage of epithelium, basement membrane and subepithelial connective tissue. These events may endorse local inflammatory reaction, and thus further increase the damage zone. [5, 35] On the other hand, they may induce excessive and poorly controlled tissue repair, with increased deposition of ECM components, proliferation and hypertrophy of myofibroblasts, as well as goblet cells hyperplasia with mucus hypersecretion. These changes result in structural and functional changes in bronchial tree mucosa, which is known as airway remodeling (Fig. 3). [5, 34] In fact, in animal model of asthma it was found that an increase in MMP-9 activity in the airway mucosa was associated with epithelial damage, alteration of subepithelial basement membrane, but also increased levels of TGF-β and subepithelial collagen deposition. [36, 37] In patients with asthma Vignola and coauthors have observed positive correlation between sputum levels of MMP-9 and the intensity of functional and structural abnormalities, which may be easily visualized using air flow measurement and high resolution computed tomography, respectively. [38, 39]

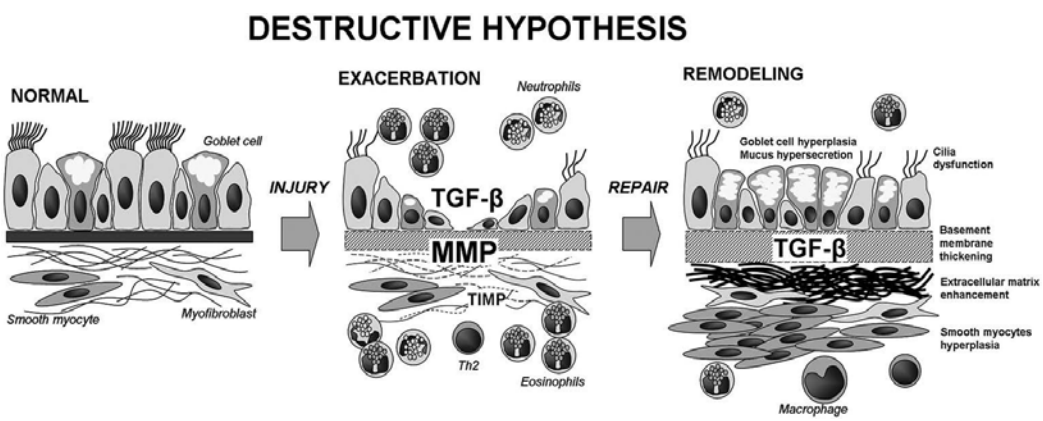

Fig. 3. Schematic representation of the "destructive hypothesis". Detailed description in text.

Interestingly, some authors did not confirm direct correlation of MMPs levels with symptoms severity, especially when using serum or plasma samples for the analysis. [30] The last finding may suggest that a main source of MMPs overproduction in asthma is located in airway system, with limited systemic influence. This assumption may be supported by association

between MMP-9 level measured in breath condensates and predominant population of inflammatory cells in induced sputum or BAL. Barbaro and coauthors have shown that patients with neutrophilic airway inflammation revealed MMP-9 concentrations significantly higher than individuals with severe eosinophilic asthma. [40] Thus, one could conclude that, essentially, neutrophils and, to a lesser extent, eosinophils would be main sources of MMPs in mild and severe asthma. Nevertheless, there is strong evidence that functional and structural changes in bronchial wall are also contributed by other producers of MMPs – epithelial cells, bronchial smooth myocytes, fibroblasts and mast cells. [5, 27] In fact, recent studies have shown that epithelium- and myocytes-derived metalloproteinases may be involved in pathogenesis of asthma and asthma-associated remodeling much earlier, even before clinical manifestation of first symptoms. That hypothesis emerged as an ancillary result of embryological studies, focused on development of bronchial tree. It has been proposed that pouches of epithelium, submerged in mesenchyma, work together during organogenesis as an functional entity, which was named the epithelial-mesenchymal trophic unit (EMTU). [41] Apart from large variety of cytokines and growth factors, produced by EMTU during embryogenesis, the important role in regulation of bronchial growth play metalloproteinases. Their list is still expanding and includes several soluble MMPs, mainly MMP-3, -9 and -12 [34, 42], as well as membrane-type 1 MMP (MT1-MMP/MMP-14). [43] The latter is involved in regulation of cell proliferation, migration and differentiation, since all these events are in some extent associated with pericellular proteolytic activity of this membrane-bound MMP.

Recently, another membrane-bound metallopeptidase, member of distinct class of disintegrin and metalloproteinases (ADAM), denoted as ADAM33, has been added to this list. [44] Similarly to MT-MMPs, function of these metalloproteinases relies on degradation of ECM components located in the close proximity to the cell, that enables further growth and branching of respiratory tree. However, this involvement also comprises processing of cytokines and their receptors. Therefore, although they remain under strict control, including methylation-dependent epigenetic regulation of promoter activity [45], even small abnormality in that system may be responsible for aberrant function of EMTU. This may lead to enhanced response to some stimuli, e.g. oxidative stress or viral infection. [46] Such triggers may result in reactivation of EMTU in adulthood, excessive stimulation of epithelial cells and bronchial myofibroblasts. They start again to express large quantities of metalloproteinases and cytokines, among them TGF-β. Both mentioned enzymes, ADAM33 and MT1-MMP/MMP-14, are supposed to trigger TGF-β-dependent stimulation of BSM and subepithelial fibroblasts, which, in response, start with excessive production and deposition of ECM components. Hereby EMTU reactivation may be associated with an increased risk of airway malfunction. Interestingly, in some individuals the initial ultrastructural changes in basement membrane, a characteristic feature of asthma-associated remodeling, were observed long before the onset of clinical symptoms of disease. [47, 48] Accordingly, one could expect, that EMTU hypothesis should be supported by some genetic background. Indeed, analysis of genome-wide association has indicated the possible role of nucleotide polymorphisms of metalloproteinases in increased susceptibility to asthma. [49] Among them, a significant association with early onset of bronchial hyperresponsiveness and asthma was noted in case of several polymorphic variants of ADAM33. [50] However, due to ethnic variability, the

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significance level of this association differs between analyzed populations. Noteworthy, among analyzed polymorphisms of ADAM33, only T1, V4, T+1 and F+1 variants were found to correlate with asthma in the general population [51], whereas other, including ST+7 or haplotype H4, were characteristic solely for certain populations. [52, 53]

Furthermore, several groups have suggested correlation between increased risk of asthma development and occurrence of some nucleotide polymorphisms in genes encoding for "classic" matrix metalloproteinases, mainly MMP-9. The postulated associations concerned single nucleotide polymorphisms (SNPs) of MMP-9 gene, located in promoter region (-1562C/T), the substrate binding site in catalytic domain (279Q/R) and TIMP docking region of hemopexin domain (574P/R and 668R/Q). [5, 54] However, majority of mentioned studies were conducted in rather small groups, with various ethnic origins. Therefore, results of these studies, although relevant, should be considered with some caution. Till now, based on studies involving group of 4,000 children, only allele R of 279 SNP was confirmed as being associated with significant increase of asthma risk. [54] On the other hand, some SNPs in MMP genes may also be associated with eventual benefit for a patient. Recently, the mutant T allele of MMP-2 promoter (-1306C/T) SNP, supposed to decrease MMP-2 production, has been described as conferring significant protection against asthma in North Indian population. [55]

2.6.2. … or Dr. Jekyll ?

As already mentioned, research involving asthma patients, animal models and *in vitro* studies provide strong support for "destructive hypothesis". [56] MMPs are abundantly produced and activated during acute and chronic asthma, and their level negatively correlates with lung function. However, those observations should be interpreted with some caution, especially since recent studies yielded some contradicting data. [57, 58] Based on these data an opposite, or "protective hypothesis" has been formulated. According to this concept, MMPs are responsible for cleavage of excessive amounts of ECM components, which are secreted in response to inflammation-mediated damage of mucosa. [59] Therefore, MMPs are supposed to protect mucosa from uncontrolled fibrosis, whereas their natural inhibitors – TIMPs (especially TIMP-1), since they prevent cleavage of abnormal ECM deposits, would, paradoxically, appear as key detrimental molecules in that system (Fig. 4). In fact, increased TIMP-1 concentrations in BAL were related to persistent wheezing in preschool children. [31] However, an attribution of altered ECM turnover solely to MMPs activity or TIMPs concentration seems to be unfounded simplification. Presumably, enhanced accumulation of matrix components results rather from imbalance between proteolytic activity of MMPs and anti-proteolytic properties of TIMPs. Consequently, instead of absolute levels of MMPs or TIMPs, their relative amounts, expressed as respective MMP/TIMP ratios, may be more relevant for course of disease. Indeed, in several studies decreased MMP-9/TIMP-1 ratio was observed in sputum, BAL and mucosal biopsies of adults and children with asthma. [60, 61] Moreover, it was associated with the airflow aggravation and reduced airway lumen, observed in computed tomography of asthmatic patients. [62] The low MMP-9/TIMP-1 ratio was also reported in asthmatic smokers with persisted airflow obstruction and thickening of bronchial mucosa. [63]

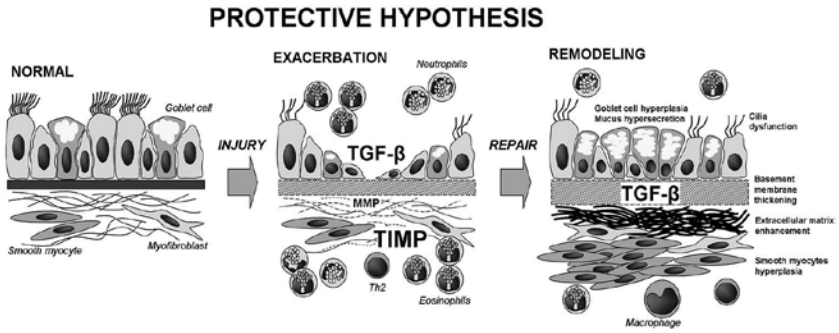

Fig. 4. Schematic representation of the "protective hypothesis". Detailed description in text.

When considering protective role of MMPs in asthma and asthma-associated remodeling, their main function concerns normalization of ECM turnover. However, one has to mention MMP-mediated regulation of cell-to-cell and cell-to-ECM interactions, as well as their involvement in cytokines/growth factors network. [56] As previously described, MMPs have been shown to process some cytokines, including TGF-β and VEGF, but also cleave several cell surface receptors, among them fibroblast growth factor (FGF) receptor 1 (FGFR1), CD44, or alpha subunits of receptors for IL-2, -5 and -13. [56, 64–67] Possibly, MMPs could also reveal their protective effect on asthma progression by interference with trafficking of immune cells and/or shedding key receptors engaged in Th2 signaling, thus attenuating allergic inflammation. This hypothesis was confirmed in murine models with MMP-deficient animals. Indeed, in mice lacking MMP-2, -8, -9 or -19 an allergen challenge resulted in increased allergic inflammation and airway hyperresponsiveness with augmented release of Th2 cytokines – IL-4, -5 and -13. [65, 68–74] Interestingly, MMPs deficiency was also associated with delayed clearance of immune cells from the airway. [68, 69, 71] This finding could be explained by involvement of MMPs in conditioning of leukocytes. [71] The possible mechanism of that phenomenon may exploit MMP-9-mediated cleavage of IL-2Rα subunit on the surface of T lymphocytes, which results in down-regulation of their proliferative capacity and subsequent apoptosis. [75] Apart from mentioned Th2 cytokines, MMPs may modulate inflammatory and immune response via processing of CC and CXC chemokines. Metalloproteinases have been shown to cleave macrophage inflammatory protein (MIP)-2 and monocyte chemoattractant proteins (MCPs) – MCP-2, -3, and -4. [76, 77]

The data mentioned above imply, that allergic inflammation and airway remodeling seem to be intricately related to MMPs activity, since MMPs may represent key mediators, or rather modulators, involved in vigorous crosstalk between airway constituent cells, invading inflammatory cells, and the extracellular matrix. [78] From that point of view the idea concerning protective role of MMPs in asthma and asthma-associated remodeling seems to be convincing. However, the issue becomes more complicated, when analyzing involvement of MMPs in processing of ECM components and their direct input in airway destruction and remodeling. Noteworthy, both concepts, "destructive" and "protective", may be supported by some clinical data. In fact, there is still reasonable doubt, whether MMPs reveal some

http://dx.doi.org/10.5772/62440

similarity to Dr. Jekyll, or they are recognized rather unfriendly, like Mr. Hyde. However, in addition to some philosophical background, this issue has also an outstanding practical meaning, especially in context of possible pharmacological interventions in asthma-associated remodeling, which may be addressed to modulate MMPs activity. Obviously, when favoring "protective" role of MMPs, they would require some support to increase MMP/TIMP ratio. In contrast, if considering the "destructive hypothesis" as more likely, an opposite action should be undertaken. According to that concept, actually the inhibition of MMPs should provide some benefit for patient. Therefore, univocal clarification of that issue is of great clinical relevance.

2.7. MMP modulation — pharmacological interventions

Apart from previously mentioned (see chapter 2.4.2) endogenous or "physiological" inhibitors, several exogenous MMP modulators are also available. Noteworthy, in addition to few agents originally designed as MMP inhibitors (e.g. batimastat or marimatsat), nowadays in clinical practice are used many drugs, originally not intended to modulate MMPs activity. [3, 4, 10] The list of these agents is still expanding and includes tetracyclines, inhibitors of angiotensin converting enzyme (ACE), inhibitors of cholesterol synthesis (better known as statins), corticosteroids, etc. Some of them display direct inhibitory influence on MMPs activity (tetracyclines, ACE inhibitors), whereas in others mechanism of their action is indirect and more complex. Moreover, modulation of MMPs expression has been related to the use of clarithromycin, imatinib, inhibitors of Rho-kinase, antagonists of VEGF receptors, as well as inhibitors of some signaling pathways, including NF-κB, MAPK, and others (Fig. 5). [79–81]

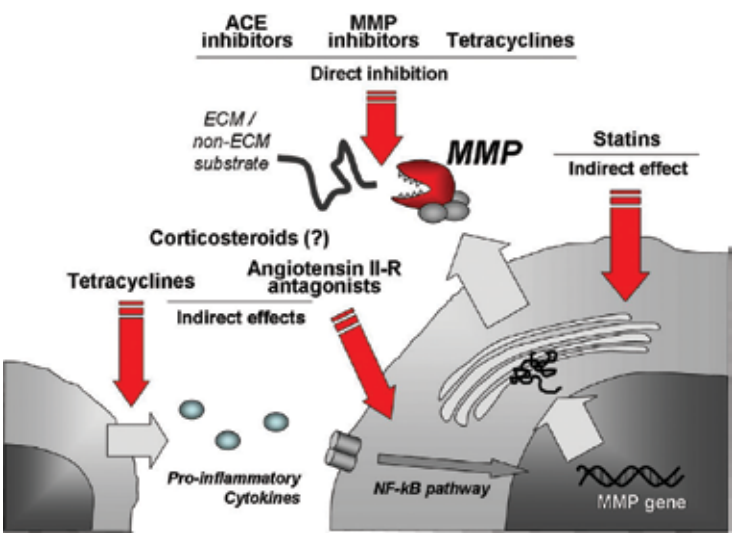

Fig. 5. Schematic representation of main strategies for MMPs modulation. Detailed description in text.

2.7.1. MMP inhibitors

The first group of broad-spectrum MMP inhibitors (batimastat, marimastat, ilomastat, etc.) is based on hydroxamate derivatives. These small zinc ion chelators were originally developed as anti-cancer drugs, and were expected to protect from cancer metastasis and tumor-related angiogenesis. [82] In murine asthma model a treatment with these compounds (marimastat, neovastat, GM6001, and others) was associated with reduced development of allergic inflammation, whereas in patients with atopic asthma these agents decreased bronchial hyperresponsiveness after allergen challenge. [68, 83, 84] However, due to low specificity, nearly all first generation synthetic MMP inhibitors may reveal inhibitory activity against various zinc-containing metalloproteins, including numerous non-MMP enzymes and transcription factors. Therefore, due to reported severe adverse effects, including so-called musculo-skeletal syndrome, mentioned compounds are currently withdrawn from the clinical practice. Regrettably, also novel MMP inhibitors, designed to specifically target particular MMPs, although encouraging in animal studies, did not ensure better safety and, most importantly, satisfactory clinical efficacy in humans. [85–90] The possible explanation of unexpectedly low clinical effectiveness of specific MMP inhibitors concerned compensatory induction of other MMPs after specific down-regulation of the target one.

Noteworthy, some other strategies of MMPs inhibition include use of monoclonal antibodies or anti-sense technologies. [3, 91] However, relatively high cost due to sophisticated technological process, and parenteral route for administration are enumerated as main limitations for development and broad use of these solutions.

2.7.2. Tetracyclines

Tetracyclines are natural antibiotics discovered in *Streptomyces*, which, apart from well defined anti-microbial properties, may also reveal some other, non-antibiotic activities. Tetracyclines may stabilize ECM turnover, presumably by direct inhibition of catalytic site of MMPs, but also indirectly, by suppression of inflammatory cascade and modulation of MMPs expression. [92] Indeed, anti-MMP properties of semi-synthetic tetracycline — doxycycline have been reported in various clinical conditions, including adult periodontitis, abdominal aortic aneurysm, atherosclerosis, autoimmune diseases and in cancer research. [93–96] Thus, doxycycline has received a Food and Drug Administration approval as potent MMP inhibitor, nevertheless its effect on asthma and asthma-associated remodeling still requires extensive studies. Data available from animal studies are promising in terms of attenuated airway hyperresponsiveness after allergen challenge and decreased airway inflammation. [97–99] Moreover, it was observed that long-term administration of doxycycline together with standard therapy was associated with significant improvement in lung function parameters and possible reversal of remodeling in patients with chronic asthma. [100]

2.7.3. Statins

The inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, better know as statins, are potent inhibitors of cholesterol biosynthesis. Due to their mechanism of

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action statins are currently used as standard constituent of primary and secondary prevention of atherosclerosis and arterial insufficiency. In addition to decreasing serum cholesterol levels, statins are know to exert various pleiotropic, cholesterol-independent effects. [3, 101] The latter are mediated by inhibition of isoprenoids, which modulate the function of intracellular signaling molecules. Thus, statins, among them lovastatin, cerivastatin, simvastatin, rosuvastatin, or pitavastatin, may reveal some anti-inflammatory activities, including inhibition of MMPs. Therefore, they have been extensively studied mainly in cancer and cardiovascular diseases. [102, 103] Recently, in a randomized controlled study atorvastatin has been shown to significantly reduce sputum concentrations of acute inflammatory mediators, including MMP-8 and -9, in smoking asthmatic patients. [104] Thus, statins, especially when combined with standard therapy, may offer some protection from exacerbation and, possibly, airway remodeling. Noteworthy, simvastatin was found to modulate TGF-β-induced mesenchymal-epithelial transition of alveolar epithelial cells *in vitro*. Interestingly, simvastatin sufficiently suppressed TGF-β to induce expression of connective tissue growth factor (CTGF) and MMP-2 and -9, but it failed to reverse TGF-β-induced morphological changes in epithelial cells. [105] Therefore, although that observation could be a rationale behind the use of statins in prevention of subepithelial fibrosis and airway remodeling, this issue still requires further research.

2.7.4. Renin-Angiotensin System modulators

Another group of potential MMP modulators comprises several compounds designed to control the function of the renin-angiotensin system (RAS). These agents, although originally designed to manage arterial hypertension through the inhibition of angiotensin-converting enzyme, appeared to be effective also against MMPs. The mechanism of their action is based on dose-dependent, direct blockage of active site in catalytic domain of the enzyme. [3] Interestingly, also antagonists of angiotensin II receptor were found to modulate MMPs expression, possibly by inhibition of NF-κB pathway. [106] Since early clinical experience with modulators of RAS may suggest their great potential in novel therapy of respiratory diseases, these compounds are recently in focus of interest of several groups. [5, 107, 108]

2.7.5. Inhaled corticosteroids

Inhaled corticosteroids (ICS) are currently used as a standard treatment to control asthma symptoms. However, it has to be determined, whether they can block or even reverse epithelial–mesenchymal transition and subepithelial fibrosis in the respiratory tract of asthmatic patients. Noteworthy, decreased amounts of MMP-9 in reticular lamina of basement membrane have been recently shown to contribute to the beneficial effect of ICSs on epithelial–mesenchymal transition in chronic obstructive pulmonary disease. [109] Unexpectedly, data from clinical studies in asthmatic patients are limited and disappointing. [27, 110] In particular, no significant decrease in levels and/or activity of MMP-9 was observed after prolonged therapy with ICS in patients with asthma. [27, 111] This finding may suggest that inhaled corticosteroids alone could not be as effective in preventing asthma-associated airway remodeling, as postulated previously. [27, 110]

In contrast to ICS alone, the improved control of asthma severity, possibly due to better modulation of MMPs system, may be achieved by introducing a combination therapy, which comprises ICS and long acting β-agonists (LABA). Although both, *in vitro* and *in vivo* studies, confirmed superiority of combination therapy over ICS or LABA alone in this regard, they did not determine exactly, how this combination may affect MMP levels. [112–114] Possibly, the augmented effect of ICS-LABA combination can be, at least partially, explained by LABA ability to modulate NF-κB signaling pathway. Thus, inhibition of NF-κB will result in decreased expression of MMP-9 gene, as has been recently shown for ultra-LABA – indacaterol. [115] Indeed, combination of ICS with LABA as both, maintenance and reliever therapy, significantly reduced MMP-9 levels in induced sputum of asthmatic patients. [116, 117] Remarkably, MMP-9 levels, observed in patients with asthma before and after combined treatment with ICS and LABA, seem to reflect the intensity of airways remodeling, as they revealed good correlation with bronchial wall thickening, visualized using high-resolution computed tomography. [116]

2.7.6. Leukotriene-receptor antagonists

Leukotriene-receptor antagonists (LTRA) may be considered as an alternative to ICS as "first-line asthma-controller therapy" or as "add-on therapy" in patients already receiving ICS. Montelukast, most commonly used LTRA, was found to decrease the expression of MMP-9 in activated eosinophils *in vitro*. [118] In children with asthma a treatment with LTRA resulted in clinical improvement –reduction of symptoms and increase of peak expiratory flow, which were associated with significant decrease of MMP-9 levels in plasma. [119] In experimental asthma model in mice LTRA treatment was shown to reverse airway remodeling and decreased airway hyperresponsiveness after allergen challenge. Again, mentioned improvement correlated with decrease of MMP-2 and -9 levels in BAL fluid. [120]

3. Conclusions

Despite extensive studies focused on role of MMPs in asthma and asthma-associated remodeling, our knowledge regarding this issue is still far from a satisfactory level. Since there is no agreement among scientists regarding superiority of "destructive" or "protective" concept, the clarification of "Dr. Jekyll or Mr. Hyde ?" issue seems to have outstanding clinical relevance, especially when considering possible pharmacological interventions. Regrettably, the interpretation of results concerning exact place of MMPs in asthma pathogenesis may be impeded by different methodology and various populations analyzed in these studies. Such differences may certainly affect result of MMPs assessment across the studies. [57, 121] On the other hand, these discrepancies can be ascribed to real differences in MMPs amount and/or activity, depending on sample type and disease severity. Furthermore, local expression and activity of individual MMPs may vary in different airway compartments, thus adding complexity to the network of allergic inflammatory response. [61, 78, 122] Accordingly, the distribution of MMP-2, MMP-9 and MMP-12 in bronchial wall was shown to differ between

large and small airways. Moreover, it varied between healthy controls and patients with asthma, and further changed depending on severity of disease. [61, 78]

Noteworthy, mentioned compartmental differences may be easily averaged for the entire bronchial tree, especially when using site-unspecific samples, like induced sputum, BAL, breath condensate and, obviously, serum or plasma. On the other hand, due to such averaging, small local changes, although clinically relevant, may be disregarded. Therefore, further research should focus on more precise assessment of distribution and activity of MMPs, the balance between MMPs and their natural inhibitors, as well as association of those findings with alterations in architecture and function of respiratory tract mucosa.

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Chapter 4

Asthma in Preschool Children

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Additional information is available at the end of the chapter

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Abstract

A proper diagnostic and therapeutic approach in children under 5 years who have symptoms of respiratory distress, of varying intensity, more or less continuously or in acute and repeated episodes must observed. In many cases, the dominant symptom is cough, which has been linked to the existence of asthma ('equivalent asthmatic coughing'). As respiratory symptoms are common to many processes that affect this system, an appropriate differential diagnosis is required before starting treatment, which is often not appropriate.—Concept. Epidemiology—Predisposing factors, risk factors and triggers—Respiratory symptoms addressed from a pathogenic point of view, in order to better understand the possibilities of these symptoms to appear: Pathogenesis of dyspnea, cough, secretion and bronchial breath sounds.—The inflammatory reaction is the pathogenetic basis of asthma, and hence, anti-inflammatories are the most appropriate treatment. But there is no evidence that inflammation is a permanent fact from the start of the disease or that it exists in other respiratory processes. The appropriate methods to assess inflammation in children under 5 years and the evaluation of results in published studies will be presented. The conclusion is that it has been shown that in mild to moderate and sporadic cases, inflammation persists.—Atopy and asthma: onset and evolution—Clinical and allergologic diagnosis—Diagnostic evaluation of the dominant symptoms, relating directly to their pathogenesis.—Exploration of respiratory function, according to age: younger and older than 2 years.—Differential diagnosis based on the dominant symptoms.—Treatment. (a) Etiologic: immunotherapy in <5 years: standards. (b) Pathogenic: anti-inflammatory (corticosteroids). Indications of pre-inflammatory: chromones and anti-leukotriene: montelukast. (c) Treatment regimens: treatment of seizures.

Keywords: preschool children, diagnosis, treatment, immunotherapy, genetic and trigger factors

1. Introduction

Episodes of breathlessness or wheezing are common in preschool children primarily in the first 2 years, being estimated that 50% of infants have episodes of this nature, and their causes are needed to be known. The insufficient development of the immune system opens up to frequent viral infections, but other factors can contribute, such as the home environment, particularly if the mother is a smoker. Given the importance of genetic predisposition, family history of allergic diseases and asthma in particular is always is to be known, taking into account the famous expression 'all that wheezes is not asthma'.

2. Epidemiology

Some anatomical and physiological characteristics of the infant and young child's airways predispose to the development of processes that lead to the narrowing or bronchial obstruction, manifested by common symptoms, such as coughing, dyspnoea and noisy or wheezing breathing.

The *narrower airway calibre* is a basic fact, which contributes to the obstruction due to the inflammation of the mucosa, the smooth muscle constriction or the increased secretion of tracheobronchial mucus glands.

Infant *physiological increase of vagal tone*, which continues during the first years of life, as Montgomery and Tepper [18] demonstrated by methacholine inhalation, is known. Pathologically, bronchial hyperresponsiveness (BHR) is a key element in the pathogenesis of asthma. Having certain anomalies in the protein chain of the beta-receptors of the smooth muscle, such as the substitution of glycine for arginine at position 16 and glutamate for glutamine at position 27, is a characteristic of individuals with atopic predisposition. But in non-predisposed subjects, BHR is usually secondary to the inflammatory reaction that occurs in various circumstances in the bronchial mucosa.

Regarding bronchopulmonary infectious pathology, it is the well-known *immaturity of the immune system*, which in some children continues for several years (infant transient immunodeficiency), facilitating the development of bronchial inflammation processes.

It is not always easy to establish the true diagnosis of asthma in early life, as the evolution of symptoms over the years will confirm the diagnosis by excluding other possible causes of dyspnoea or wheezing, supported by immuno-allergological and respiratory function studies.

In line with these concepts, Martinez et al. [16] distinguish the different phenotypes of the bronchospastic pathology in preschool children, identifying asthma and transient bronchitis (wheezy bronchitis) that encompass various processes suffered by a group of children who, after preschool age, do not show no broncholability, all a consequence of the predisposing factors cited above. However, it is not always easy to determine the phenotype of a particular patient, and in the course of time, as they evolve, the criteria may even have to be modified. Hence, the need to pay attention to the characteristics of the symptoms and their evolution, in addition to a number of circumstances, such as the suffering from other allergic processes by

the same child (eczema, allergies to milk proteins), a precocious start of symptoms, or the existence of similar pathologies among siblings, parents or other close relatives, or environmental pollutants, climate, etc. The lack of a family history in approximately 20% of cases adds another obstacle to the diagnosis.

In most children, asthma begins in the first 5 years of life. A study in Spain in 1982 showed that in 76% of asthmatic children, the process had started before 4 years of age. Several studies indicate that between 15 and 35% of preschool children have had an episode of respiratory distress, with or without wheezing or other breathing noises; however, 60–65% of these children would not suffer crises after the third year. The high diagnostic confusion in this group of children with 'transient bronchitis' comes not only from symptomatic similarity but also from the terminology that has been used to label the process. The appearance of these temporary symptoms can be due to various causes, such as viral infections (respiratory syncytial virus (RSV), parainfluenza, influenza), causing bronchiolitis, which may recur, even more in immunodeficient children. Other possible causes can also be household pollutants (tobacco smoke, cleaning chemicals or industrial products) or weather changes (sudden cooling), among other.

3. Predisposing factors, risk factors and triggers

Prevention of diseases is one of the challenges that medicine faces, therefore knowing the risk factors for each of the processes we aim to prevent is essential to carry out effective measures to achieve the proposed objectives. The best known risk factors and triggers that lead to allergic diseases, especially asthma and related bronchopaties, are as follows.

3.1. Atopy: genetic bases

The hereditary component in allergic diseases is well known, being asthma the most important, so that in approximately 70–80% of patients, close family members with the same or other allergy-related pathologies are identified. Allergic (atopic) predisposition is polygenic, that is there are several genes that support polymorphisms, which prompt the abnormal response of the organism to allergens.

In infants and pre-schooler children, the diagnosis of asthma is based on demonstrating the causality of allergic symptoms, and BHR, which is an essential condition for the diagnosis of the disease at any age. Polymorphisms in genes that lead to variants of the β_2-adrenergic receptor in the bronchial smooth muscle are the basis for congenital BHR (primary BHR), which may be also due to the harmful influence of certain exogenous factors such as when smoker women do not drop the habit during pregnancy, thereby harming the normal development of the lung. The existence of congenital BHR is the first precursor of asthma in young children. Moreover, also in these early years, BHR may worsen or appear by the inhalation of pollutants present at home or in the exterior, being this cause of BHR (acquired BHR) the most prominent at later ages.

In children with wheezy bronchitis (transient), such family circumstances are not given, but the causes are different, such as pulmonary immaturity, viral infections or environmental pollution (micro-habitat).

3.2. Foetal immunity and atopy: intrauterine sensitization

The influence of the mother in the transmission of atopic predisposition is greater than the father's, possibly because of the intimate connection between the former and the foetus. Pregnancy maintenance requires an immune environment with predominance of activity of Th2 lymphocytes, which prevents the rejection of the foetus. Thus, the foetus develops in an environment in which cytokines from that lymphocyte subclass, especially interleukin (IL)-4, IL-10 and IL-5, are predominant, increased if the pregnant woman is atopic, suffering or not from any allergic disease. Although IL-13 is a cytokine of the Th2 group, it has been observed that it is produced to a lesser extent in foetuses and neonates at risk of atopic disease, which could be related to the immaturity of the development of the activity of T cells [27]. Along the same lines, low production of IFN-γ by mononuclear cells could be related, thereby reducing the suppression of Th2 activity, defect that is maintained for at least the first 2 years of life [24].

Sensitization to food allergens or pneumoallergens may occur already during pregnancy, as the foetus can produce IgE from the 11th week of gestation, even IgE specific to antigens, as has been shown to occur against parasites (helminths, filaria) in some countries.

What is yet to be clarified is the route by which the allergens that have passed to the mother by inhalation or digestion are brought into contact with the foetus for the immune stimulation that promotes IgE production to occur.

What it is also to be elucidated is the relationship that may exist between the degree of maternal exposure to allergens and the possibility that the foetus becomes sensitized. It does not seem that an environment rich in pneumoallergens cause a greater number of foetuses affected or that the diet of pregnant woman devoid of the most allergenic foods reduce the number of sensitivities.

3.3. Risk factors

Lung development

Regardless of atopic predisposition, various circumstances constitute an added risk for the early onset of asthma, which can also promote the development of wheezing transient bronchitis. Some of these facts can act on the lung during foetal life affecting both the development and maturation of the immune system, but also after birth, especially in the first year of life.

Lung development can be affected by certain circumstances. Prolonged stress of the mother can affect the imbalance of Th1/Th2 activity, due to the excessive production of cortisone that occurs in this emotional condition, since the hormone exerts its immunomodulatory activity in favour of Th2 activity [11]. Similarly, some incidents during pregnancy can affect the

development of the lung, such as uterine bleeding, placental insufficiency or other processes that alter the physiology of the uterus favouring premature birth and a consequent low birth weight. Also, prolonged malnutrition of the pregnant mother is detrimental to the overall development of the foetus and particularly of the lung, increasing the risk for prematurity in which many of these pregnancies end.

Smoking during pregnancy and lactation

Among the risk factors that affect lung development in both its structure and mechanisms of immune defence, the greatest interest is focused on the smoking habits of the mother during pregnancy and the first year of life, including also other smokers in the vicinity of the newborn and infant.

There are no doubts about the relationship between smoking and respiratory disease of the small child, with an increase in the prevalence among children of school age. Nevertheless, it is difficult to prove whether the functional changes are already initiated in the foetus or if this occurs later during lactation. Gilliland et al. [9] found decreased respiratory function ($FEF_{25–75}$) in children whose mothers smoked during pregnancy, but not after birth, suggesting that the injuries already started in the foetus.

Modern methods to assess respiratory function allow the study of BHR already in the first months of life, having shown a slight but real increase in the children of smoking mothers. Regarding immunity, several studies in cord blood appear to show that tobacco produces some changes in the immune response of the foetus, having found higher levels of IgE and a significant production of Th2 cytokines (IL-13, IL-5mRNA, IL-6), after *in vitro* stimulation of lymphocytes with allergens (ovalbumin, mite).

Environmental chemical pollutants

Various common contaminants at home may act as irritants that can cause or increase bronchial reactivity or produce an inflammatory condition of the airways. This may precipitate both sensitization to environmental allergens and be the cause of respiratory symptoms of varying intensity, from irritative cough to wheezing bronchitis. The most common environmental pollutants have a variable influence, in logical dependency on the concentration of chemicals, ventilation, housing characteristics and location (urban, suburban, rural).

It seems less likely that the outside environmental irritants may cause the same problems in young children, perhaps because the degree of exposure is lower than in later ages. However, in big cities or in the vicinity of industrial areas, pollution can be a serious risk, from which only small infants that spend little time outdoors will be protected.

3.4. Trigger factors

Early sensitization

As already mentioned, sensitization to allergens seems possible already during foetal life, although the onset of symptoms of asthma may take some time, depending on the degree of

exposure and environmental conditions in addition to the genetic load. But it is after birth when the child is more exposed to common domestic allergens, being mites the most common (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), but sensitization and subsequent development of allergic symptoms in the respiratory tract are associated with atopic predisposition, concentration of allergens and allergenic potency of each of them, and their ability to release the antigens when the particles are deposited on the wet surface of the mucosa, as well as the involvement of adjuvants, as are the aforementioned pollutants or the intercurrent respiratory infections.

Viral infections

Respiratory tract infections caused by viruses are responsible for a high percentage of bronchospasm in young children, for which their characteristic tendency for allergic symptoms in their in upper respiratory tract or even crises of wheezing and/or dyspnoea had already been revealed. But also in non-atopic children, RSV mainly can cause lesions of the bronchial mucosa that facilitate contact with allergens, causing sensitization to them, with a simultaneous increase in broncholability. Also in not-predisposed children, RSV infections and other viruses trigger wheezing crisis or broncho-obstruction, as a pathogenic base for transient or wheezy bronchitis. The cause of these disorders lies in diverse mechanisms, dependent some on the injury of the mucosal epithelium and others on the activation of the cells involved in the immune–allergic response (**Table 1**).

RSV belongs to the *Pneumovirus* genus of the *Paramyxoviridae* family. Through a protein (G), the virus attaches to the surface of target cells, resulting in the fusion of the lipids of the cell membrane by another protein (F), which facilitates the insertion of RNA into the invaded cell. The G protein promotes a Th2 response, whereas the F protein stimulates the production cytokines in Th1 lymphocytes. In connection with the predominance of one or other protein, it has been established the existence of two types of RSV, A and B, whose variety may depend on the different clinical manifestations [13].

The metapneumovirus is responsible for 10–15% of the respiratory infections in children, with the same clinical impact than the VRS, with which shares a certain genotypic and epidemiological similarity. They also belong to the *Paramyxoviridae* family and are genetically similar to the pneumovirus of the avian flu.

The influenza virus A and B, and parainfluenza 1, 2 and 3 are less aggressive but also cause obstructive crisis in atopic and non-atopic children, because they cause desquamation of trachea–larynx–bronchial epithelial cells and bronchial submucosal oedema with infiltration of neutrophils and mononuclear cells, lesions that are reversible within 6 weeks [3].

Moreover, it is possible that in healthy children, RSV infection may be the cause of asthma to be developed later, but is more likely to result in the wheezing bronchitis process that will persist for some time. It is shown that, after severe infection, deterioration of the lung function occurs with increased bronchial reactivity to methacholine or histamine and to physical exercise, as well as the existence of specific IgE antibodies against the virus, which decreases over time, which could be related to the transience of broncho-obstructive crises.

More recently, rhinovirus, with its three subgroups A, B and C, has been identified as the main cause of respiratory infections which will later trigger half of asthma attacks in early childhood. The *in vitro* rhinovirus contact with blood mononuclear cell from adult asthmatics, compared to healthy subjects, demonstrates the production of IL-4 only in patients and less IFNy in these than in controls, with a IFNy(gamma interferon)/IL-4 ratio over three times lower in asthmatics, which shows that rhinoviruses directly induce the activity of Th2 cells, which does not rule out a greater responsibility of rhinoviruses in the pathogenesis of asthma.

Bacterial infections

Unlike viruses, bacteria do not exercise such a harmful function. Some bacterial antigens have an immunomodulatory capacity and repeated infections are what promote the predominance of the activity of Th1 versus Th2 lymphocytes, preventing sensitization to allergens.

Although the protective action against sensitization to pneumoallergens and food in children under 2 years of age seems confirmed, there are studies that nuance the findings, as is the potential coadjuvant effect of endotoxins with sensitization to mites and perhaps other pneumoallergens.

— Epithelial lesions
Increased neuronal sensitivity
Increased penetration of allergens
Decreased ciliary clearance
Neural endopeptidase reduction and increased tachykinins
— Increase in mediators from mast cells
Histamine
Arachidonic acid metabolism: prostaglandins, leukotrienes
— Increase of cellular mechanisms involved in inflammation
Increased production of cytokines
Stimulation of chemotaxis: eosinophils, neutrophils
— Regulation of β_2 receptors
— Production of specific IgE against viruses
Allergic sensitization
Mediators release

Table 1. Mechanisms responsible for bronchobstructive crises caused by viruses.

Moreover, at very low concentrations (10 ng/ml), endotoxins are able to activate alveolar macrophages and promote bronchial inflammation, increasing bronchoreactivity both in asthmatic and healthy people; therefore, it is possible that in the severity of asthma, environ-

mental endotoxin levels may have an outstanding role not only in adults but also from school age, causing especially wheezy bronchitis or non-atopic asthma (6,10).

Conversely, if the asthmatic has a bacterial infection with respiratory location, it is most likely that obstructive symptoms will be intensified by the action of bacterial enterotoxins that act as superantigens, which promote the mechanisms by which the inflammation of the respiratory mucosa is established.

4. Pathogenesis of common symptoms in obstructive bronchopathies

The mechanisms by which the symptoms of asthma occur in preschool children do not differ from those that cause the process at any age. The permeability of the bronchial lumen is maintained by a neuro-chemical mechanism, but airway calibre may be affected either by an imbalance in this mechanism or by the intervention of certain cells and their biochemical mediators involved in the inflammatory reaction, usually triggered in childhood by an allergic reaction or an unfavourable home environment. In the obstruction of the wider bronchi, the dominant mechanism is the constrictor one, of neuro-chemical cause, whereas in the peripheral bronchi (small airways), inflammation is the greatest cause, through cellular mediators that are also constrictors.

BHR and inflammation are the pathogenetic basis of asthma. BHR is usually present already in the newborns of atopic families, although it is true that certain exogenous harmful agents can increase it. Similarly, the regular or intense exposure to these pollutants and also viral infections can cause broncholability in non-predisposed children. This increased broncholability occurs as the result of inflammation that such elements, including allergens, produce in the bronchial mucous membrane. These facts are demonstrated in adults and children from school age, but in preschool children under five, the immediate influence of these exogenous factors is less certain. The injured bronchial epithelium is restored after the aggression that leads to crises of dyspnoea or wheezing, and the permanence of the injury can depend on the intensity of the aggression and the repetition of the same, leading to more or less severe and repeated symptoms. Therefore, there is doubt whether the inflammatory reaction is established from the onset of the first symptoms or whether that permanence occurs after the recurrence of the crisis or after the most serious crises, on which the therapeutic approach may depend. Various conventional methods are used to study bronchial inflammatory reactions, although in younger children, it is not always easy to conduct them. The most common are the following:

Direct methods: (1) Cell study from sputum obtained by bronchoalveolar lavage (BAL), induced sputum, forced cough or aspiration. (2) Bronchial biopsy.

Indirect, non-invasive methods: (1) Exhaled nitric oxide measurement. (2) Exhaled breath condensate: evaluation of several measurements of the inflammation. (3) Blood eosinophilia assessment and eosinophil cationic protein (ECP) in serum levels.

The inflammatory reaction is a key event in the pathogenesis of asthma, as it is evident in adults and children from school age. It seems certain that inflammation is present even in the first

episodes of dyspnoea in children who later go on to develop allergic asthma, but there is no unanimity in the recognition of this fact. In the work of Maclennan et al. [14] in preschool children with episodes of severe dyspnoea repeated between 2 and 12 times in the previous year (it is known that from the third episode, asthma can be diagnosed in young children), it is found that a percentage of them had an increased serum IgE levels although it is not significant in relation to a control group.

It is needed to know whether the persistence of inflammation and its intensity corresponds with the frequency and severity of the crisis, that is, if the mucosal lesions are restored after mild episodes that occur at long intervals of time, with implications in the therapeutic approach aimed at preventing recurrences.

5. Initiation and evolution

The precocity of the onset of episodes of respiratory distress may be related to the severity of the process and its evolution over time. Although in some children, the first crisis occurs in the first months of life, the process has a markedly evolutionary nature, not presenting the first crisis of dyspnoea but after a period of time, which varies from child to child.

5.1. Asthma crisis: early onset

For the suspected allergic asthma to be well-founded, it is necessary that the crisis is repeated for at least three times, as it is known that a large number of infants suffer an episode of dyspnoea, possibly of infectious cause, estimating that this happens in between 15 and 32% of children under 5 years, who cannot be labelled as asthmatics until the atopic cause is confirmed, related to the progressive evolution of the process.

The first episodes of dyspnoea are usually caused by viral infections and occur most frequently in the cold months, and it is not uncommon that they are accompanied by light fever. Associated symptoms are similar to asthma: rhinitis, dry cough, shortness of breath, wheezing, dyspnoea, intercostal retractions.

Depending on the intensity and the phase of the crisis, auscultation will show from wheezing to silent areas as well as fine or coarse crackles, indicative of bronchoalveolar involvement.

In any case, it is necessary to make sure there is a bronchiolitis, by RSV, of which prognosis and treatment can differ from that of a simple catarrhal process.

After the first year, it is likely that crises are not triggered by viral infections, but that other environmental factors are responsible, not ruling out weather conditions changes, even in atopic children.

The repetition of three episodes of dyspnoea should alert of the possibility that those are the first manifestations of asthma, which will continue in the following years. It is therefore necessary to assess in each case the various predisposing and boosting elements of atopy and

asthma, such as the incidence of allergic disease in parents or close relatives, the coincidence in the child of other allergic processes or the environment in which the patient lives.

5.2. Atopic processes precursors of asthma

In chronological order, but not occurring in all cases, the first manifestation of atopy might be the sensitization to foods, mainly cow's milk when breastfeeding is absent, with symptoms in the first weeks of life. Eczema associated with sensitization to foods, from the third month, and rhinitis, as the first manifestation of respiratory allergy, that usually precedes asthma, both identities related with the persistence and increased intensity of the other two previous allergic processes.

The correlation between early sensitization to foods and later development of asthma is difficult to determine, but in these cases, given the repeated respiratory crisis by possible viral infection, the allergy study must be expanded.

Atopic eczema

Although atopic eczema may be the only manifestation of atopy in many children, there is no question about the relationship between eczema that start of in childhood and asthma, of which the first symptoms sometimes coincide with a cutaneous process, although in most cases, respiratory symptoms appear months or years later, even after skin lesions have disappeared or been attenuated, as it happens in many children before their third year.

To predict the risk of respiratory disease in children with eczema, an early allergologic study is advised. An elevated total serum IgE will be the early sign that will alert of the atopic nature of the process, but skin tests and assessment of serum IgE specific to foods and to the most common aeroallergens at home (mites, animal epithelium), which will alert about the risk of respiratory disease, which will start later.

Besides eczema, it is frequent that allergy to cow milk protein is revealed by digestive (vomiting, diarrhoea) or anaphylactic symptoms, which are also indicative of atopic predisposition and might be a precedent of asthma.

Rhinitis, rhinoconjunctivitis, rhinosinusitis

Not surprisingly, allergic sensitization is initially produced in the respiratory mucosa, as it is directly accessible to airborne allergens present in the air we breathe; and therefore, very often, respiratory pathology of allergic cause starts with symptoms of rhinitis. The frequency and persistence of nasal symptoms in early childhood are well-known fact, and their causes are diverse, from adenoiditis to allergic rhinitis.

Because of the proximity of the conjunctival mucosa, with similar characteristics to the respiratory one, it is not uncommon the concurrence with conjunctivitis (rhinoconjunctivitis). From the second or third year, it is neither uncommon the simultaneous conditioning of maxillary sinusitis, which may be limited to the inflammatory reaction of the mucosa, but that is often complicated by superinfection. Greater doubts arise in the relationship of rhinitis with

otitis media present in some children, although it seems that nasal provocation with pollen can cause a dysfunction at the inner ear level.

6. Clinical and allergologic diagnosis

The diagnosis of asthma in the first years of life is based on a thorough questioning (anamnesis) and in the allergologic study. Given the difficulties and controversies surrounding the concept of the disease at a such early age, the issue is to obtain data that, with the smallest possible doubt, may allow on one hand to establish the syndromic diagnosis, that is, the existence of intermittent episodes of bronchial obstruction and, on the other, allergic causality, in most cases, or the responsibility of other exogenous factors able to increase bronchial reactivity, which usually occurs at later ages. When the allergologic study is negative, the questioning may provided valid information to guide diagnosis to some other of the process in which coughing or signs of bronchial obstruction often dominate the case history, having to complete the study in accordance with the suspected diagnosis.

Physical examination is essential, since even if the child is asymptomatic while being examined, important data, such as a chest deformity, the presence of paradoxical breathing with depressed abdomen on inspiration, or the pathologic auscultation when the child breathes deeply, among other information, can be obtained. Moreover, functional exploration will also provide data that can be decisive for the definitive diagnosis.

6.1. Anamnesis

A good interrogation often provides critical data to guide the diagnosis. It should include information about family precedents and medical history, diseases of probable allergic cause cited above, symptomatology, chronology of symptoms, medication use and its effectiveness.

6.2. Other medical history

Primary immunodeficiencies also occur in repeated bronchopaties, by viral or bacterial infections, with symptoms that may remind those of asthma; hence, the need for information on whether there have been relatives with any of these diseases (Wiskott–Aldrich, Hypo or agammaglobulinaemia, Di George, etc.), processes in which predisposition is transmitted by recessive inheritance.

6.3. Symptomatology: chronology

It is not enough to know that the child has episodes of cough or dyspnoea, but it must be also known if the cough is dry or soft, if breathing difficulty improves or worsens after a coughing spell, if there is expectoration, or if it is predominant at night, among other features. It is also necessary to know the intensity of respiratory distress, if it is accompanied with nasal flaring, or if it disrupts sleep. The existence of fever is also a point of great interest because an infectious trigger can be assumed.

The chronology of the succession of symptoms in each episode is another fact of interest, as it is whether dyspnoea appeared abruptly or was preceded by nasal or pharyngeal symptoms, as well as at what time it began, for example, if it was at night or after eating some food. The biggest interest is in the chronology of the process, taking very much into account the age at which symptoms started and whether they were intense from the beginning (mucoviscidosis could be suspected of).

6.4. Medication: use and effectiveness

Usually, when a child presents some of the symptoms that characterize bronchial obstruction, some medication is usually given, and their effectiveness must be critically evaluated, in order to better guide the diagnosis. It is not always possible to reduce or eliminate breathing difficulty with a bronchodilator, when mucous secretion is predominant, or preventing relapse with an inhaled corticoid, when there is no inflammation because the airway obstruction is of another nature.

6.5. Allergologic study

Skin tests

They may already be positive even in the second month of life, to cow's milk protein (casein, β-lactoglobulin). At 4 months, it is possible to show sensitization to other foods, especially egg proteins, increasing the percentage in the coming months, as new foods are introduced. Sensitization to pneumoallergens comes later. It is estimated that approximately 40% of atopic children under 3 years are sensitized to dust mites, reaching 70% in those over 4 years. Figures for animal epithelia range from 3 to 5% in the younger, being 6–8% at around 4–5 years of age. Sensitization to pollens also depends on the place of residence, in relation to the time and intensity of exposure.

The *prick test* is the least traumatic and totally reliable and reproducible technique that has replaced intradermoreaction. It is virtually painless, very well accepted by young children and easily performed with the same technique at any age.

Usually, the study is limited to dust mites, the most common being *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, but others can be added, such as *B. tropicalis, Acaro siro* or others that are common in the geographical area of residence: animal epithelia, cat and dog (the most frequent, even if they are not present at home) or other animals with which they may have contact. Fungi can be found anywhere at home. The most important, that allow conducting immunotherapy, are *Alternaria tenuis* and *alternata*, and *Cladoporium* ssp. At homes with high humidity, other fungi must be tested, such as *Aspergillus, Penicillium, Fusarium, Mucor,* etc. Earlier than at 3–4 years of age, sensitization to pollens is unlikely, although there is no objection to include in the list of allergens a mixture of pollen from wild grasses (*Poa pratensis, Festuca, Dactylis glomerata, Lolium perenne, Phleum pratense*) that are most often sensitizing. Other pollens depend on the geographic area in which they reside.

Total serum and specific IgE

From birth, the serum non-specific IgE level increases to reach figures close to those of the adult into adolescence (**Table 2**). IgE has some physiological functions, such as defence against helminth parasites, although it is supposed to participate in other defence mechanisms. In the atopic individual, the regulator mechanism of IgE production is genetically altered by the prevalence of the activity of Th2 lymphocytes already mentioned. Therefore, it is common in these patients to find an elevated serum IgE level, not having yet been specifically produced against any allergen. Hence, the finding of an elevated total serum IgE may be linked to symptoms suggestive of allergic disease, and in consequence, this finding has considerable guidance significance. However, in any case, it must be taken into account that the possibility of elevated total serum IgE, especially if the figure is very high, might be related to other processes such as parasites or certain immunodeficiencies (**Table 3**).

The assessment of total and specific to allergens IgE is based on radioimmunoassay, fluorometric or enzyme techniques. As a first step, in a suspected allergic disease, a method that includes a mixture of allergens that are suspected to be frequent in childhood (food and airborne allergens: Phadiatop® infant, Immuno-CAP, 3gAllergy) can be used, the positivity of which may indicate specific sensitization to any of the antigens included. Subsequently, the allergen that led to the positive test must be identified in order to establish the preventive and specific treatment as appropriate.

Comparing the diagnostic value of skin tests and the specific IgE, it can be said that both are equivalent; however, it is always advisable to check the diagnosis with both tests. To complement the immunoallergologic study, it is required to assess other serum immunoglobulins, IgG, IgM and IgA, as it is not uncommon that the deficit of some of them may favour respiratory infections. It is not unusual that this happens when there is selective deficiency of IgA, a common immunodeficiency (1/700 in the general population and 1/200 people with allergies) that sometimes goes unnoticed by a low clinical expression in most cases.

Eosinophilia: cationic protein

In peripheral blood, increased eosinophils above 500 cells/mm^3 may indicate allergic reaction in any organ system (lung, skin, digestive tract) where the process is in an acute phase or immediate to clinical reaction. The presence of eosinophils in the bronchial exudate or tissue obtained by biopsy reveals the characteristic inflammation of an allergic reaction. When not in these cases, eosinophilia may be normal, and very high levels are usually due to many processes, parasitosis most of the time.

More valuable is the elevation of serum level of the enzymes from eosinophils, mainly ECP, easy to determine. It is necessary to be cautious when assessing the increased serum ECP as a marker of a certain bronchial inflammation, given the variability of the figures that can be found. According to Koller et al. [12], it could be a good marker even after the first episode of dyspnoea, finding a higher ECP in infants which a year later were diagnosed with asthma than in those who did not developed the disease, estimating that figures higher than 20 μg/l

could have a strong prognostic value. However, Pohunek et al. [23] found no differences in ECP levels in children under 3, between both diagnoses when children were asymptomatic, as opposite to when they were suffering a crisis. From his work, it can be deducted the interest to assess the enzyme during a bronchobstructive crisis, in yet not diagnosed children, especially during the first episode, for the possibility of establishing prophylactic measures to prevent or delay the establishment of asthma.

Age	**Geometric mean kU/l**	**+1 SD**	**+2 SD**
Umbilical cord	0.3	–	–
6 weeks	0.7	2.1	6.1
3 months	0.8	1.8	3.8
6 months	2.7	6.6	16.3
9 months	2.4	4.2	7.3
12 months	7	21	58
2 years	11	26	61
3 years	11	21	40
4 years	20	37	70
7 years	26	75	221

Table 2. Normal serum IgE levels (Kjellman and Johanson, 1976).

Parasitic infections
Ascaris lumbricoides
Toxocara canis
Entamoeba haemolitica
Echinococcus
Trichinella spiralis
Filaria
Others
Immunodeficiency disorders
Wiskott-Aldrich S.
Di George S.
Hiper IgE S.
Selective IgA deficiency

Table 3. Non-allergic process more frequents in small children that occur with high IgE.

Since allergic rhinitis often precedes the onset of bronchial symptoms, eosinophil count and ECP in nasal mucus can be a good indicator of allergic predisposition and the possible progression of the disease to the lower airways. Presence of allergy will be suspected if the percentage of eosinophils of the total cells of the smear exceeds 10%. In the same sample, ECP and even total and specific IgE can be assessed, in which variation after immunotherapy can be a good indicator of the effectiveness of the treatment [15].

7. Respiratory function exploration

7.1. Children under 2 years of age

In the diagnosis of asthma or of wheezy bronchitis, exploration in the infant has a relative utility. However, the greatest value of early exploration is focussed on distinguishing the processes that occur with breathing difficulties of various kinds, some of bronchopulmonary origin (cystic fibrosis, bronchopulmonary dysplasia, primary ciliary dyskinesia, bronchitis obliterans, malformations) or extrapulmonary (vascular rings, ductus persistence, other heart disease), which can raise the differential diagnosis with severe asthma or resistant to habitual treatment. Exploration in infants should be restricted due to the risk involved in the need to sedate the child, requiring hospital environment, which should be restricted to the following indications, in order to diagnose other possible diseases with apparent symptoms of asthma, although symptoms can have a different origin:

1. Presence of tachypnea, hypoxia, cough or dyspnoea of unexplained causes that cannot be studied with other techniques.
2. Serious and chronic obstructive diseases that do not yield to the usual treatment of corticoids and bronchodilators, both inhaled.
3. Non-characteristic respiratory symptoms, with the likelihood of an extrabronchial origin, in order to assess the origin and severity of the symptoms (e.g. vascular rings).
4. Research, necessary in all diagnostic or therapeutic procedures, but risk must always be assessed and therefore have the corresponding parental consent and approval by ethics committees.

Methodology

The most common method is the rapid thoracoabdominal compression at ordinary volume. As the child breathes normally and at the end of an inspiration, an insufflation of the jacket is provoked to induce maximal forced expiration, thus obtaining the parameter of the maximum flow at functional residual capacity (VmaxFRC). Both the figure obtained and the appreciation of the layout of the flow-volume curve will provide information on the permeability of the airways and the degree of obstruction, if any.

Less suitable is the one based on thoracoabdominal compression provided by a pneumatic jacket, a kind of inflatable vest connected to an air compressor through a non-distensible thick

tube. The exhaled airflow is measured with a pneumotachograph of appropriate size that adapts to the mouth through a mask. The biggest drawback is the need to sedate the child with chloral hydrate orally, which is not without risks.

As forced expiration is provoked during a normal respiratory movement, that is ordinary volume, this technique does not provide the value of the forced vital capacity (FVC) obtained with a spirometry, and therefore, no FEV_1 or the FEV_1/FVC ratio and FEV_1% can be obtained. To acquire these values, we must perform the technique known as rapid thoracoabdominal compression with previous insufflation, which is artificially forcing a deep breath, for which, by a compressor connected to an oral–nasal mask, air is blown into child up to the maximum vital capacity and requiring a pressure of 2–3 kPa (20–30 cm H_2O). Afterwards, a rapid inflation of the jacket is performed to provoke the forced expiration. This way, valuable data comparable to those provided by spirometry, can be obtained.

7.2. Children over 2 years of age

Although the following techniques are used in older children who do not cooperate during the exploration, some of them are also used in infants with devices adapted to their size (plethysmograph), requiring sedation of the child. With them, precise information on airway resistance (AWR) and compliance can be obtained.

The AWR is defined by the difference of pressure between both ends of the airways, that is the mouth and alveoli when an air flow of 1 l/s occurs. The circumstantial narrowing of the bronchi increases resistance, deducing the intensity of the obstruction by the degree of the increase in the value of the AWR.

Compliance refers to the intensity of the rigidity of the respiratory system, defined by the increase of the volume produced divided by the increase of the pressure unit, so that the less pressure necessary to produce an equal increase in volume, the higher the compliance.

The resistance can be measured through well-known techniques, such as interruption of flow (Rint), forced oscillation and its oscillometric impulse variation and plethysmography, a technique which also allows the compliance to be known.

Flow interruption (Rint)

The interruption of the airflow during normal breathing causes rapid and complete occlusion of the airways, enabling the measurement of the pressure in the oral cavity immediately before the interruption and during the normal respiratory cycle using a pneumotachograph. The measurement of the alveolar pressure is deduced from the pressure value established in the mouth when the momentary interruption of flow occurs. If this is suddenly interrupted, for a split second, an immediate rise in pressure occurs in the mouth. The pressure measured this way is very similar to that found in the alveoli, allowing the measurement of *resistance*, comparing this pressure with that prevailing in the mouth just before shutting off the flow. The Unit used to measure the resistance is Pascal in litres per second. In short, Rint is the most simple and affordable method to learn the AWR in young children, with the only requirement

being that they breathe normally through the tube that is fitted into the mouth, preventing nasal breathing by pinching the nose.

Forced oscillation: impulse oscillometry

Forced oscillometry is based on the application of oscillatory pressure changes and therefore airflow, measuring the resistance from the relationship between the two. Easy to perform, it only requires that the child breathe calmly through a tube—not longer than 1 m—attached to the mouth and a volume of 100 ml, having inserted a pneumotachograph in between. The patient's air flow is superimposed with a oscillanting flow of 2 ml at a frequency of 10 Hz coming from a sinusoidal generator, thereby leading to pressure variations which are not perceived by the child and are proportional to the 'total' patient breathing resistance.

Best results are obtained with a newer variation of this process, the *impulse oscillometry* (IOS). A controlled deviation of the membrane of a loudspeaker, which is adapted to a nozzle, leads to the excitation of the airflow generating pressure impulses, from which ratio, the value of the central airways resistance and the lung elasticity is obtained; and therefore, the values result from the relationship between the exerted pressure and the airflow [4].

Plethysmography

A plethysmograph measures the changes in volume of the thorax. Its principle is the law of Boyle and Mariotte, whereby the volume occupied by a mass of gas, at constant temperature, is inversely proportional to the pressure. By plethysmography, intrathoracic gas volume (TGV) through the volumetric variations of the thorax is measured simultaneously with the AWR, and from these values, conductance can be calculated.

Bronchodynamic tests

The above techniques report on the current state of the permeability of the lower airways but do not provide information on the level of BHR, the increase of which is significant in the diagnosis of asthma. Hence, the need to carry out tests to make it evident, that is bronchoconstrictor (methacholine, histamine) and bronchodilator (β-mimetic) tests. The three technical variations mentioned can be used for this purpose

8. Differential diagnosis based on the dominant symptoms

Many processes that start at preschool age manifest similar symptoms to those of bronchitis or asthma. The too early start of these symptoms, sometimes shortly after birth or in the first months of life, is a matter of warning about the possibility of a process less frequent than those, but even if the process has a late start, the other possible causes of cough or dyspnoea should never be forgotten, avoiding the comfortable position of starting a routine treatment, which may not always be beneficial and could even delay the establishment of appropriate therapeutic measures.

The assessment of cough as a symptom, that in the case of asthma is often maintained between the crises of dyspnoea or wheezing, has some characteristics that can guide to others diagnosis not uncommon at preschool age, with symptoms that may be common to those of asthma, and therefore is mandatory to take them into account in order to conduct a correct differential diagnosis (**Table 4**).

Dominant symptom: cough	Pseudoasthmatic bronchial symptoms	LESS common processes
Maxillary sinusitis	Immunodeficiencies	Congenital anomalies
Adenoids	Bronchiolitis	—Laringo and tracheomalacias
Rhinopharyngitis	Bronchitis obliterans	—Vascular rings
Whooping cough (pertussis)	Wheezy bronchitis	Alpha-1-antitrypsin deficiency
Primary ciliary dyskinesia	Tracheobronchial foreign body	Hypersensitivity pneumonitis
Eosinophilic bronchitis	Mucoviscidosis (cystic pancreatic fibrosis)	Pulmonary hemosiderosis
	Gastroesophageal reflux	Alveolar proteinosis
	Tumour or mediastinal adenopathy	Eosinophilic lung

Table 4. Most significant processes in the differential diagnosis.

9. Practical summary for diagnostic approach

1. Medical history

- **a.** Family history: allergic pathology, respiratory or genetic pathology.
- **b.** Own background: allergic comorbidity (eczema, allergies to milk proteins, etc.), recurrent infections
- **c.** Symptomatology: for each one of the symptoms, it is necessary to specify the characteristics, age of onset, duration, severity, recurrence, association between them.
- **d.** Environment: smoking, irritating substances, pets, rural area

2. Clinical examination

- **a.** In a symptomatic stage or during an interval
- **b.** General inspection: development, nutrition, colour of skin and mucous membranes, acrocyanosis. If there is coughing: characteristics. Nasal or mouth breathing.
- **c.** Thoracic inspection: circulation, retractions, respiratory rate, dyspnoea. Auscultation: wheezing, rhonchus, crackles, localized or generalized, changing or not present when coughing. Localized hypophonia. Percussion: localized dullness.
- **d.** Nasopharyngeal: nasal flaring, anterior or posterior rhinorrhea, appearance of mucus. Size and appearance of the tonsils: visible adenoids?

3. Initial Investigations in all cases

   a. Complete blood count. ESR
   b. Immunoglobulin G, M, A and E
   c. Paranasal sinuses X-ray. Adenoid RX if hypertrophy is suspected

4. If all the above rules out the existence of a pathology different of asthma or wheezy bronchitis, continue the study to confirm it.

5. If the above tests fail to lead to that conclusion, if the symptoms are important or if after an initial diagnosis of asthma or bronchitis no improvement is achieved with standard therapy (inhaled bronchodilators and corticoids) the following tests should be made:
   a. Sweat test. Also required in the initial study, if symptoms are of an early onset, severe, with general deterioration or a family history of cystic fibrosis
   b. Esophageal pH monitoring
   c. Thoracis X-ray, front and profile: assessing abnormal masses, adenopathy, condensation, etc. Mediastinal swaying following the suspicion of a foreign body aspiration, especially in children under 3 years
   d. Other imaging techniques based on radiological findings or presumed diagnosis: computed tomography, magnetic resonance, gammagraphy.
   e. Serum antibodies against RSV, influenza, parainfluenza and other

6. If the above tests do not lead to a diagnosis in accordance with the clinical suspicion or in case of uncertainty, it is recommended that, successively:
   a. Lung function study
   b. Serum precipitin against actinomycetes
   c. BAL: hemosiderophages (also in gastric contents), PAS+ protein, eosinophil
   d. Alpha-1-antitrypsin serume
   e. Bronchoscopy
   f. Lung biopsy

10. Treatment

Although asthma treatment at any age is based on the same principles, the characteristics of the disease and the pathophysiology in pre-scholar children require adjusting each of the measures available to the peculiarities of the age.

Overall, these measures aim primarily to combat the cause of the disease, that is *etiological* treatment based, in one part on environmental measures (reduction of household allergens and reducing environmental pollutants) and on the other in desensitization by immunother-

apy. The *pathogenic* treatment is based on fighting the bronchial inflammation, trying to prevent or eliminate it, being chromones, anti-leukotrienes and corticoids the appropriate medications, and finally, the *symptomatic* treatment with short-acting bronchodilators, mainly β2-agonists.

10.1. Etiological: specific immunotherapy

Immunotherapy is the only therapeutic procedure, for its widely proven effectiveness, which decreases the sensitivity (desensitization or hyposensitization) to the responsible allergens, in which its mechanisms of action are essential to prevent further sensitization, as is well known (in short, the Th1/Th2 balance).

WHO supports the term 'allergy vaccine' and considers this as the only treatment that can alter the natural course of allergic diseases and also prevent the development of asthma in patients with allergic rhinitis.

Although the age to initiate immunotherapy has been questioned, multiple studies and the own experience show the need for an early start, from the third year of age, provided that the requirements set out in **Table 5** are met.

Recent and extensive literature reviews conclude that it is a serious need to consider the start of immunotherapy in children under 5 years of age that fulfil the required conditions ([7, 8])

— **Correct clinical diagnosis**
— In case of asthma:
— mild to moderate intensity
— respiratory function within normal limits: parameters not <70% of those foreseen
— Accurate allergologic diagnosis, skin tests and serum specific IgE are sufficient
Exceptionally provocation with allergen will be used
— Right choice of allergen/s to be included
— Extract quality: purity, standardization, coadjutants
— Correct therapeutic regimen monitoring: dose, intervals
— Periodical clinical controls
— Minimum period of 3 years, and at least 1 year without onset of symptoms
— Prescription and control by a allergy and paediatrics expert
—Early onset

Table 5. Premises for immunotherapy for optimal results in paediatrics.

Some of the few problems that can cause subcutaneous immunotherapy could be avoided if administered by sublingual channels, which is virtually risk-free. A problem in young children is the difficulty of successfully administering the pharmaceutical preparation, keeping it under

their tongue for the right time, which is likely to be simplified with the most recent preparation in tablets or in atomiser (spray) (WAO).

10.2. Pathogenic: antiinflammatories

Chromones

Under this description, cromolyn sodium and nedocromil are included. Both act by blocking the chloride channels of the membrane in the cells involved in the allergic reaction, especially mast cells and eosinophils, but also epithelial and nerve cells. On the activation of chloride channels depends the crossing of Ca ions into the cell, required for the activation thereof to occur. When these cells are stimulated (allergens, non-specific agents), there is a release of mediators (mast cells) and the commencement of the elements involved in the allergic reaction. When idle, chloride channels are closed and this is what is achieved with chromones, the blocking of the channels, thereby preventing activation of the cells that would lead to the allergic reaction. For this reason, we must consider that chromones act more like preventive agents of inflammation than anti-inflammatories.

Although currently they are less used as inflammation preventives, cromolyn could be indicated in preschool age, before asthma reaches a significant degree of severity, that is in mild intermittent or persistent asthma and in moderate asthma, in which case an inhaled corticoid or salbutamol could be added.

Anti-leukotrienes

These are medications that block the action of cysteinyl leukotrienes that might prevent the onset of one of the most important mechanisms involved in the production of the inflammatory reaction of the bronchial mucosa. They specifically avoid the activity of those on specific receptors localized in bronchial smooth muscle and in bronchoalveolar blood vessels. This is intended to prevent the development of inflammation in the initial period of obstructive bronchial disorders and also contribute to avoid the swelling to persist or increase, once present when asthma has been clinically established.

Knowledge of the receptors of the leukotrienes is a key for the intended purpose. Pharmacological studies have determined that cysteinyl leukotrienes activate at least two types of receptors, named CysLT1 and CysLT2, being the first found in the bronchial muscle and the second in the pulmonary venous system. Depending on the consequences of the activation of these receptors by the corresponding leukotriene, the antagonists used therapeutically will produce diverse positive effects, even more prominent in children than in adults. On one hand, they achieved a discrete bronchodilation resulting as a consequence of counteracting the prolonged constrictor effect of leukotrienes.

In short, anti-leukotrienes behave more like inflammation preventives than as anti-inflammatories, an activity that is better performed by inhaled corticoids, and therefore, both treatments can be used simultaneously.

Of the preparations available (pranlukast, zafirlukast and montelukast), only the latter is indicated in children under 5 years of age, available in chewable tablets and granules. Since the permanence of the inflammatory reaction in all probability depends on the recurrence of episodes of dyspnoea and their intensity, montelukast may prevent the progression of the process in milder cases without resorting to inhaled corticoids. The advantage of oral administration and the single daily dose make this drug of easy acceptance and secure compliance. In asthma of moderate or severe intensity, it will be necessary to combine the treatment with inhaled corticoids.

Several studies confirm the efficacy of the treatment with montelukast in children between 2 and 5 years of age even reducing the number of episodes caused by viral infections (5). Respiratory function improves from the early days of treatment, even in children under 2 years of age as a result of the reduction of the inflammation.

Corticoids

Physiologically, corticoids activate the cytoplasmic receptors that most cells have; the same which corticoids administered therapeutically act upon, with a power that depends on the affinity of each product with these receptors, entering into competition with the natural hormone. They are known to inhibit various cytokines, especially ILs (IL-1, IL-2, IL-3, IL-4, IL5, IL-6 and IL-13), tumour necrosis factor alpha (TNF-α) and the colony stimulating factor in granulocytes and macrophages (GM-CSF), while increasing the IFN-γ and IL-12, that is they reduce the action of Th2 leukocytes, and consequently, the number of basophils, eosinophils and mast cells in bronchial epithelium is reduced. The risk of any side effects resides on the fact that only between 10 and 40% of the drug administered by inhalation reach the bronchi, depending on the inhalation system. The rest of the substance remains in the mouth and is swallowed, getting into the general circulation via the gastrointestinal tract. They are mostly metabolized in the liver, being removed, but another part remains in the bloodstream, reaching the lung, where they produce the same effect as corticoids administered by other means. At the same time, part of the fraction that reached the lung by inhalation passes into the bloodstream, following the same path as the ingested portion. Some of these drawbacks are avoided with ciclesonide, with little oral bioavailability (only 10% of the active molecule is generated in the oropharynx), so that the pharmacotherapy is mainly dependent on deposit and pulmonary absorption.

The most prominent undesirable effects are the hypothalamic–pituitary–adrenal (HPA) axis suppression and growth retardation that, although it seems to be compensated in years not affecting final adult height, it must be considered in order not to fall into the usual trend of increasing doses when the desired effects are not achieved. Prolonged treatments or higher doses may result from severe hypoglycemia to cushingoid features. In children, it is rare that osteoporosis occurs. Depression of immunity caused by corticoids may increase the risk of infections. Other less important effects are of cutaneous nature, such as oral thrush or skin atrophy, less common in children.

A weighting between dose/guideline/efficacy/duration of therapy/adverse effects should be established. Effective maximum doses of inhaled corticoids in children have been established,

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which is 400 μg/day for beclomethasone dipropionate and budesonide, and 200 μg/day for fluticasone propionate. Despite the better tolerance with ciclesonide, only studies from 4 years of age have been published, with a maximum dose of 160 μg/day. Higher doses of any of them do not improve results and increase the risk of side effects. The daily dose is usually divided into two intakes, but can be simplified without losing efficiency, administering once the total daily dose in the morning, as the physiological hormone production would be reduced if taken at night. In mild-moderate asthma, there is an increased permeability of the airways, and therefore, the drug penetrates with greater ease making the most of the administered dose, hence the recommendation to use lower doses in milder cases.

The oral or intravenous administration of corticoids is reserved for asthma crises of medium or severe intensity, either at home or in hospital. The dose of 1–2 mg/kg/day of prednisone, prednisolone or methylprednisolone should not be prolonged for more than 5 days, but if more time is required, it should be progressively reduced.

Although inhaled corticoids may show a symptomatic improvement, in the long term, the course of the disease does not seem to change; therefore, the duration of the treatment will depend on the results and even a diagnostic review should be considered.

10.3. Symptomatic: bronchodilators

Beta 2-agonists

The indication of short-acting β_2-agonists (salbutamol, terbutaline) is the bronchospasm crisis, whatever its intensity. The most appropriate route of administration is inhalation, achieving improvement in a few minutes, in the case of acute asthma attacks. Continued inhalation is indicated for the inpatient treatment of severe crisis. Other routes of administration are subcutaneous and, above all, continuous intravenous to which we must turn in severe crisis. The oral route may be useful in mild cases or after achieving a significant improvement with the inhaler.

In preschool children, the most common is the metered-dose inhaler (MDI) with a spacer or with a nebulizer when it is necessary to continuously administer the drug. Otherwise, the efficacy appears to be similar with both inhalation systems. Powdered formula, for which there are different systems (turbuhaler, diskhaler, accuhaler), is less useful at this age, because a greater degree of collaboration is required.

Anticholinergics

Ipratropium bromide, is a derivative of atropine, which does not produce the side effects of atropine. Because of its low lipid solubility, it hardly passes biological membranes and produces an effect almost exclusively in the bronchial tree, when administered by inhalation. The recommended dose for all ages is 0.04 mg three or four times daily. The greatest benefits are achieved in asthma attacks triggered by non-specific agents, which may act via vagal.

The bronchodilator action is more deferred with this product, but it remains longer than with the β_2-mimetic, and in some cases might be administered simultaneously, for which there are

even preparations with formoterol or salbutamol and ipratropium bromide, although it is preferable to dispense them separately.

11. Therapeutic schemes

11.1. Treatment of crises

The therapeutic approach in episodes of respiratory difficulty should be preceded by a reflection on the different diagnostic possibilities, since various acute processes may manifest common and sometimes confusing symptoms, needing different treatments, care and control. The severity of the case will be visible by observation and by assessing dyspnoea, intercostal, subcostal or supraclavicular retractions, colouring and sensory. The assessment of breath sounds that can be discernible by auscultation is essential for diagnosis as it can indicate the location of the process (trachea, central or peripheral bronchi, alveoli), if bronchospasm, condensation or atelectasis are present, which can be deduced from the presence or prevalence of wheezing, stridor, crackles or coarse crackles, decreased murmur, among other respiratory noises. Other causes of severe respiratory distress that may resemble an asthma attack, should also be considered, especially the frequent aspiration of a foreign body, which requires radiological confirmation, and bronchiolitis, both of a sudden onset and which can also occur in children that already suffer from asthma. Once it is concluded that it is a bronchospasm crisis, its severity will be assessed and of this assessment will depend the therapeutic activities:

1. Mild: β-mimetic inhaled: according to severity: 2 doses × 20 min, maximum 3 doses, or every 4–6–8 h depending on severity; maximum 24–36 h.
2. Moderate: add oral corticosteroids: 1–2 mg/kg/day: 3–4 days
3. In both situations, after the improvement, treatment with β-mimetic continue orally (±1 week) or mucolytic and expectorant, if required.
4. Grave whose intensity, more complex processing conditions outlined in **Table 6**.

Immediate:
- Oxygen inhalation by flow of a β-mimetic:
  - Salbutamol: 2.5–5 mg
  - Terbutaline: 5–10 mg
    - (Half dose in children under 1 year)
  - Intravenous hydrocortisone 100 mg
- In more severe cases, adding:
  - Aminophylline e.v.: 5 mg/kg in micro-drip (20 min) followed
    - continuous infusion of 1 mg/kg/h
      - (Investigate whether there were received a dose above)
    - Inhaled ipratropium bromide 0.25 mg (0.125 in infants)

Controls:
- Symptomatology
- Oximetry: to reach SaO_2 > 92%

Monitoring
- If no improvement in 15–30 min:
  - Salbutamol: Continuous inhalation or every 30 min until

to get better. After follow the evolution every 1–4–6 h

Aminophylline: continuous infusion (monitor serum)
Ipratropium bromide: every 6 h
Hydrocortisone repeated every 6 h, or
Methylprednisolone ev: 1–2 mg/kg/day (spread in 4 doses)

If there is no improvement or worse:
Admission to Intensive Care Unit:
Protocol status asthmaticus

Leaving the hospital:
Salbutamol or terbutaline inhaled or oral, to a week
Methylprednisolone oral, decreasing doses, depending on the dose and previously administered treatment days

Medical surveillance: 2–4days

Table 6. Treatment of severe asthma attacks in children under 5 years of age (Abridged outline various guides).

11.2. Maintenance treatment

Pharmacological treatment mainly depends on the frequency and intensity of the symptoms, the severity of which is deduced for each single case (**Table 7**).

When symptoms occur sporadically (occasional episodic) unless there is a crisis, a treatment is not usually needed. When the frequency is higher (frequent episodic), it is recommended to start with montelukast, which will act as an inflammation preventive and if there is a failure to control the symptoms, a low-dose inhaled corticoid will be added.

If symptoms occur more frequently, with intermittent crises (moderate persistent), apart from montelukast, inhaled corticoids without exceeding the maximum recommended dose will be added. If control is not reached, cromolyn sodium nebulizer three times a day will often achieve satisfactory results. Once symptoms are controlled, continue with montelukast and inhaled corticoids.

From the third year of age, when the allergic causality of the process is certain and the responsible allergen has been identified, immunotherapy is the fundamental etiological treatment, provided that the above criteria are met, following WHO recommendations.

Occasional episodic	**Infrequent episodes: 1 every 4–6 weeks or less**
	Asymptomatic intercrisis
Frequent episodic	Over 1 episode in 4–6 weeks
	Intercrisis-isolated symptoms, which do not affect the child's activities or sleep
Moderate persistent	Very frequent exacerbations
	Frequent intercrisis symptoms that interfere with daily activities and sleep
Severe persistent	Daily or nearly daily symptoms, with frequent episodes of respiratory distress
	Disrupted daily activity and sleep

Table 7. Classification of asthma in children under 6 years of age.

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Chapter 5

Monitoring Asthma in Childhood: Still a Challenge

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Additional information is available at the end of the chapter

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Abstract

Asthma monitoring should be focused on patient outcomes and goals. Using clinical practice tools allows the clinicians to detect problems such as bad adherence to maintenance therapy, comorbidities, or other external reason for a poorly controlled asthma. To succeed in the process of asthma control, doctors need the participation of the family. Because such educational task requires good agreement between patient environment and doctor, it might be difficult to achieve. However, it is worth to implement because the benefit is a life without symptoms of asthma with a minimum medication.

Keywords: Noncontrolled asthma, management, adherence, children, monitoring

1. Introduction

Asthma is the most common chronic disease in childhood. It is clinically characterized by episodes of wheezing, dyspnea, cough, and chest tightness with different grades of severity. Most patients are free of symptoms between these episodes or "attacks," either because asthma is well controlled or because it is the natural course of the disease [1, 2]. Although this episodic nature can make patients, parents, and health care professionals interpret asthma as an acute or intermittent disease when episodes are infrequent, asthma is in fact a chronic disease characterized by ongoing inflammation of the airway mucosa, even when the patient is asymptomatic. Successful long-term management of the disease therefore requires careful

follow-up and monitoring. However, guidelines on asthma do not provide recommendations that are unanimous [3].

An overwhelming number of 334 million people suffer from asthma worldwide. The most recent global survey calculates that 14% of children experience asthma symptoms [4]. It is difficult to quantify the global economic burden of asthma, but estimates are high enough to encourage active interventions. The indirect costs for children, which are not insignificant, include school Absenteeism; whereas the direct costs are even larger, and include costs from hospitalization, emergency department (ED) visits, unscheduled doctor or nurse visits, and medication. Controlled asthma imposes far less of an economic burden. Strategies towards improving access and adherence to evidence-based therapies are, therefore, likely to be effective in reducing the economic burden of asthma [3, 5]. One of the basics for this goal in developed countries, where access to care and medication is already guaranteed, would be to achieve and maintain asthma control with the least possible medication [6]. In keeping with this paradigm, the concept of problematic severe asthma has been used to describe children who have uncontrolled asthma despite being prescribed multiple controller therapies, including inhaled corticosteroids (ICS), long-acting beta-agonists (LABA), and leukotriene receptor antagonists (LTRA). However, only a minority of children with uncontrolled or problematic severe asthma have true therapy-resistant asthma [7, 8]. Most children with poorly controlled asthma can be in fact well controlled by addressing the basics of asthma management, including patient and parent education, achieving and maintaining correct inhalation technique, avoiding exposure to relevant allergens and irritants, identifying and treating comorbidities, and, perhaps most importantly, identifying poor adherence and helping patients and parents to improve it.

This chapter reviews the recommendations on how to monitor asthma during childhood, focusing on patient outcomes and goals. Using some clinical tools will allow the clinicians to detect situations, such as poor adherence to maintenance therapy, comorbidities, or other external reasons for uncontrolled asthma. To reach a high degree of success, the participation of the whole family in the process of asthma control is needed. Such educational task requires good agreement between the patient, parents, and the health care professionals, which may be difficult to achieve. Despite these difficulties, it is worthwhile to try and implement, as the benefit is a good quality of life for the patient with asthma. We will also search in this chapter for evidence on reliable direct instruments that may be helpful to achieve asthma control.

2. Pillars of asthma management

Comprehensive asthma management includes reviewing the following items: adherence to daily controller therapy, teaching and maintaining proper inhalation technique, controlling exposure to main triggers, reconfirming the diagnosis of asthma, and excluding other causes of respiratory symptoms or comorbidities [9]. Addressing these pillars of asthma diligently management will help to ensure asthma control in most cases, without the need of increasing medication [10].

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We discuss each of these pillars of asthma management throughout this chapter, but first, we discuss the components of an asthma follow-up and monitoring program.

2.1. Asthma follow-up

2.1.1. How, when, and who?

Primary care practitioners are usually the first to encounter asthma symptoms in children. Typically, they prescribe medication after a concise education session during a short visit. Parents are encouraged to use the medication at home as long as the child is symptomatic and to come back if they encounter problems in managing the child's symptoms. This results in a relatively high proportion of unscheduled visits [11]. In addition, many parents feel that they are expected to manage their child's asthma on their own [12]. This approach has been characterized as a "reactive" follow-up strategy of asthma [11] and appears to be common in primary care, even though it does not follow national and international guidelines for the management of asthma in children.

The alternative approach to asthma management can be characterized as a proactive approach, following the pillars of asthma management as outlined in international guidelines. This approach includes scheduled follow-up visits, providing repeated tailored education, agreement on treatment goals and methods, ensuring optimal inhalation technique, and addressing patients' and parents' beliefs and concerns; which has shown to help to improve asthma control [13, 14]. This model of management is more common in secondary care centers. The evidence of its effectiveness makes follow-up and monitoring key components of successful asthma management in children [15].

After establishing that a scheduled follow-up plan is more effective, other aspects of these visits, such as who will monitor these patients and how often, need to be determined. One of the proposed models implicates the asthma nurse. This specifically trained health professional is of great importance in a close and time-consuming management. The main role of the asthma nurse is to provide reinforcement of the patient's and parents' knowledge of the disease, to promote adherence to the management plan, to check the inhalation technique, and to adjust the medication according to symptoms of asthma [10]. In fact, the recent evidence suggests that adults with selected chronic diseases can be successfully managed only by nurses [16]. The outpatient management of childhood asthma by asthma nurses has been compared to the one led by pediatricians. Childhood asthma was proven to be successfully managed by an asthma nurse, in close collaboration with a pediatrician [10].

Educational asthma programs are definitely improved if an asthma nurse is included in the team. A follow-up schedule with alternate follow-up visits by asthma nurses and pediatricians implies a follow-up visit every 3 months. Additional follow-up visits can be planned individually if needed, according to the criteria of the pediatrician or of the asthma nurse [10]. Other members of the team would include nutritionists, psychologists, or physiotherapists, when comorbidities are detected.

2.1.2. What to monitor?

2.1.2.1. Impact of symptoms on life

Symptoms presented since the last visit is the first approach to define asthma control. Although most guidelines provide control scores to establish a degree of asthma control, it is difficult to turn this evaluation into a number because asthma control is a multidimensional concept [6]. The scores of questionnaires on asthma control have several limitations. They only provide information about the situation in the preceding 4 weeks. This makes asthma control scores much variable over time and show little concordance with the risk of exacerbations [17, 18], which is one of the main issues to consider during asthma monitoring. Quality-of-life instruments should help in the task of delimiting asthma control. They share some limitations with asthma scores: children with similar degrees of asthma control or lung function impairment differ considerably in their quality-of-life questionnaire scores, which is partly explained by psychological factors influencing their disease concept [19, 20]. The current consensus is that these instruments provide independent additional information on disease status, complementing other monitoring instruments [19–21].

A way of defining the risk of asthma exacerbation could be the use of reliever medication. However, this information seems to be independent from the risk of exacerbations or other data, such as lung function or inflammation [22]. In fact, the degree of airway narrowing that is perceived as dyspnea of enough severity to prompt the use of reliever medication varies considerably between individuals [23]. Furthermore, other psychological factors influencing this perception can play an important role. Thus, the use of reliever medication is not a reliable way of measuring asthma control.

A practical clinical approach is to review symptoms during follow-up and to consider other factors of the disease. Patients and parents are most concerned about the impact of the disease on daily life [6]. The three things children worry about their asthma control are the need of daily medication, having severe asthma attacks, and not being able to engage in sports and play [24–26]. Follow-up visits should take this into account, starting the clinical interview focusing on patients' outcomes (exacerbations, visits to the ED or hospital admissions; sports limitations or other daily limitations; identified or nonidentified triggers; etc.) and discussing the use of medication, not only the rescue medication but also, most importantly, the daily medication [6].

2.1.2.2. Lung function

The latest asthma guidelines do not include lung function as a main way of monitoring asthma control [27–29]. There are different ways of measuring lung function; but the usefulness of lung function measurements in the follow-up of asthma has not been firmly established [1].

The main two ways of studying lung function are measuring forced expiratory volume in one second (FEV_1) and measuring its reversibility after administration of a bronchodilator [30]. Reduced lung function is an independent risk factor for future asthma exacerbations [31]. FEV_1 levels have shown to improve considerably during treatment with ICS. Normal FEV_1

levels are being found in most children with mild-to-moderate asthma, rendering bronchodilator reversibility negative [32]. As a practical approach, most children with stable, controlled asthma and good adherence to ICS therapy have normal values of FEV_1 [32–34]. Reduced lung function in asthma is only found when they are measured at the time when asthma symptoms are present, or when adherence to ICS is not achieved [30].

Positive bronchodilator response (PBDR), even in patients with FEV_1 >80%, could be another way of monitoring asthma. Children with PBDR have been shown to suffer from poorly controlled asthma, with increased beta2 agonist use, nocturnal symptoms, and exercise limitation [35]. Furthermore, children with consistent PBDR, defined as an increase of 12% or greater in basal FEV_1 in every scheduled visit, had more unscheduled Visits, required more systemic corticosteroids, had more nocturnal awakenings, and missed more school days [36]. However, no study has assessed whether the follow-up that includes PBDR helps to better control asthma when compared to the standard follow-up.

Peak expiratory flow (PEF) values are more effort dependent than FEV_1. Neither isolated PEF measurements nor home PEF monitoring has been demonstrated to be useful in asthma monitoring, because they are not sufficiently sensitive or reliable to monitor airway obstruction [37–39].

Whether it is possible to recognize reduced lung function relying only on history and physical examination during follow-up and whether lung function measurements are able to detect asthma risk of exacerbation with enough anticipation are yet to be answered. In fact, previous studies have shown that it is possible to predict reduced lung function or increased risk of exacerbation, without requiring objective measurements [40].

Finally, one could think that lung function monitoring would help to improve patients' and parents' adherence and, therefore, to improve asthma control. However, studies testing this hypothesis have failed to support it [38, 39].

In summary, the usefulness of lung function monitoring in asthma management is limited. It may be useful during the follow-up when a diagnosis confirmation is needed or when poorly controlled asthma is suspected [6], mainly in poor perceivers.

2.1.2.3. Airway inflammation

Exhaled nitric oxide (FeNO) has been proposed as a noninvasive marker of underlying airway inflammation. FeNO values differ widely among healthy children, which make it difficult to establish reference values. Therefore, FeNO measurement does not appear to be a reliable instrument in asthma diagnosis [41]. FeNO measurements have been thoroughly studied as a monitoring instrument in asthma. Studies in children comparing a standard follow-up with a FeNO-monitored one have shown no evidence of superiority of the FeNO monitoring approach in predicting asthma exacerbation or improving asthma symptoms, while it has been related to higher daily dose of ICS [42]. Similar results are obtained when using sputum eosinophil counts to monitor asthma [6]. As they do not seem to provide further information on asthma control and could favor a step-up of ICS, airway inflammation monitoring should not be recommended in clinical practice to follow-up asthma control.

2.1.3. Patient-centered care and self-management concept

There is a wide consensus among experts that getting the basics right in asthma management helps to control the disease in most children with uncontrolled or problematic severe asthma [9]. This starts with a patient-centered follow-up. The self-management concept is probably the best expression of this patient-focused management.

Self-management means that the patient (or in the case of children, the patient and parents) has the ability to manage symptoms, recognize their possible causes and consequences, and can institute appropriate treatment, following the plan previously agreed with the health care professional. This active role from patient/parents is needed to support the pillars of well-controlled asthma: the parents and the patient should know how to use reliever medication properly, recognize and manage exacerbations, avoid or control known triggers, and agree with the decision of giving daily controller medication to their child [43].

Patients and their parents have certain perceptions of their illness and medication, which strongly determine their self-management behavior [25]. These beliefs can be modified by good asthma education [14]. A prerequisite for successful asthma education is to establish an effective patient–physician partnership through the use of appropriate communication skills. However, this is difficult to achieve, because most doctors have not been trained in communication techniques required for this patient-focused care. This consists of discussing illness and medication perceptions of the parents, shared decision making, and motivational interviewing. It has been shown that physicians trained in communication skills obtain better adherence and improve their patients' asthma control [43–46], as patients are more likely to take the steps necessary to improve their asthma control (if they are satisfied with the partnership) [47].

Compared to a doctor-centered consultation, a patient-focused follow-up interview has some differences: approaches must be based on equality, by listening to patients' concerns and preferences showing genuine interest, and offering medical advice based on patients' preferences. This interview should finally arrive to an agreed management plan [48, 49]. Nowadays, asthma guidelines strongly recommend such tailored management plans, as a way of improving asthma control [27]. During follow-up, this agreed plan needs to be reviewed and adapted when necessary. In this sense, starting the follow-up interview letting the patient or their parents talk about their concerns since the last visit, is a good way of reinforcing patient–physician partnership [43]. However, soliciting the patient's agenda (patients' worries and questions) has only limited effects on health outcomes by itself. The beneficial effects of patient-centered care are more pronounced when it includes facilitation of the patient to ask questions, to take the initiative, to provide information, and to be actively involved in controlling the consultation and in disease management [50]. Patients are more forthcoming with questions, opinions, concerns, and preferences when the physician uses partnership building, such as direct question about patient's views and open-ended questions and avoiding interruptions. The process of giving medical advice comprises discussing available options and supported deliberation. After taking in consideration both the medical evidence and the patient's perspective, the deliberation should come to certain point where one of the options appears to be the best possible strategy. Sometimes, patients and parents need time to consider this,

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discuss it at home, and then come back for a further round of deliberations. The final result of this process of negotiation is a mutually agreed solution, which the patient and parents are happy to embrace and follow [47].

In summary, what patients need for effective self-management is that the medical visit provides understanding of the disease state, the treatment options, the need for lifestyle changes, the need for daily medication, and the willingness to consider changes in the management. These strategies supported on patient concerns and preferences and shared decision making, will cover the patient needs [47]. **Figure 1** shows the process of patient-focused visit and self-management.

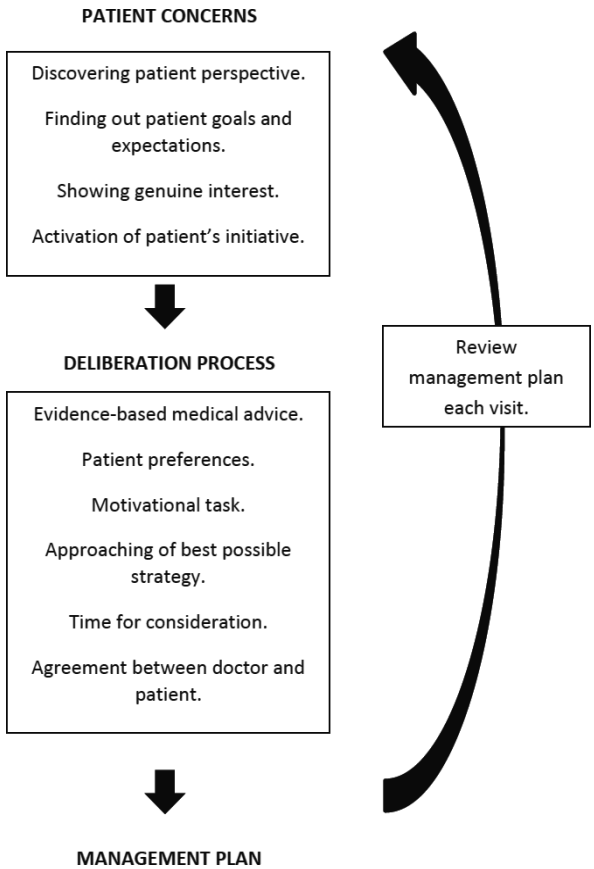

Figure 1. Process of the patient-focused visit and self-management.

2.2. Adherence to daily therapy

Adherence to daily medication is one of the pillars of successful asthma management. Studies reveal that children with asthma only take between 30% and 70% of the prescribed doses [51]. Poor adherence appears to be the main reason why the patient remains symptomatic despite

treatment with ICS [9, 10]. Adherence to controller medication has been strongly linked with better asthma outcomes, making adherence a modifiable factor and a potential target for reducing economic burden of asthma [5]. Adherence should be over 75% of the prescribed doses to influence clinical outcomes [52]. In this section, we will discuss the different kinds of adherence barriers, how to measure them, and how we should manage them.

2.2.1. Adherence barriers

A useful model for daily practice divides nonadherence into four categories [53]:

- Unwitting nonadherence: When patients misunderstand medical indications/advice. This usually occurs when there has been a lack of information and can be addressed through proper education. This adherence barrier should be detected by interviewing the parents about the prescribed treatment (what inhalator should be taken, when, and why).

  It could be thought that adherence is directly related to education on the asthma disease, but this is not the case. Consistent evidence shows that adherence to daily medication is not significantly related to knowledge about asthma; therefore, this is neither the only nor the main barrier for asthma control, but it should always be investigated during follow-up [54].

- Intentional nonadherence: This occurs when parental or patient illness perceptions or medication beliefs are in conflict with the medical advice. These cognitions have consistently proved to be a strong determinant of adherence [13, 50, 53]. Illness perceptions are built from earlier experiences and from information collected from the media and people from closer social circles. This modulates their view of necessity for treatment. For example, it is common that a patient with episodic attacks, who is asymptomatic in between attacks, perceives asthma as an intermittent disease for which daily medication is not necessary. However, if parents understand that asthma is a chronic condition with ongoing inflammation even when asymptomatic, they are more likely to recognize daily medication as a way of preventing asthma attacks. On the other hand, fear of ICS side effects could be the reason for adherence resistance [54].

  When confronted with poor adherence to the recommendation to give daily ICS, many physicians respond by repeating asthma education and re-emphasizing the importance of daily controller medication. However, as unwitting nonadherence is a minor cause of nonadherence [54], this approach is likely to be ineffective [55, 56]. Dealing with patients' and parents' perceptions is sometimes difficult, but eliciting them during follow-up visits is important to detect poor adherence. After illness or medication beliefs have been explored in a supportive and nonjudgmental way, it could be discovered that they do not correspond to the medical model of asthma. At this point, the physician's task is to discuss these perceptions from the empathy and the genuine interest of the patient's and parents' concerns. Showing this predisposition to listen has been shown to increase patient's satisfaction, which is directly related to their adherence [57]. Although these communication skills require an effort, they are very effective when used during the deliberating process in self-management, and normally an agreement is achieved, resulting in both parts being satisfied with the decision made [47, 57]. This is one of the keys of intentional adherence

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maintenance, as shaping perception and beliefs have demonstrated to help to a good asthma control [14].

- Unplanned nonadherence: Even if patients have agreed to follow daily ICS, a number of barriers can prevent them from doing so, causing what is called "unplanned" nonadherence. Examples include the lack of family routines, the time for medication competing with important activities on the child's schedule, child raising issues, and social or family complex environment (economic issues, parental psychiatric illnesses, etc.). A recent surprising finding was that excessive responsibility for medication taking was being given to the child at a relatively young age, without proper parental supervision. Self-management should not be expected until 12 years of age [51].

  Incorporating behavioral components into educational efforts to improve adherence increases their potential efficacy. Home visits may be an efficient method to collect information on such barriers, specially in patients with severe asthma. It is important to listen to patient's preferences and try to look for some room in the schedule in which remembering and using the daily medication is easy for him/her [54]. All these specifically tailored interventions could be successful and cost-effective; but until now, studies on this subject have just shown to achieve a temporary adherence improvement [58].

- Incorrect inhalation technique: Although it is not the most frequent adherence barrier [9], many patients use their inhaler device incorrectly. The first step for a successful inhalation technique is an adequate device prescription. After this, comprehensive inhaling instructions must be provided [59]. From all ways of checking inhalation technique, the patient-demonstrated technique appears to be the most effective, at least when speaking of metered dose inhaler (MDI) [59]. In the case of MDIs, it is important that the patient or parent actually demonstrates the maneuver himself/herself and to adjust the technique afterwards, if required. Not shaking the canister at the beginning of the maneuver tends to be the most common error in the inhalation technique of patients using an MDI device. On the other hand, patients using a dry powder inhaler (DPI) prepare their inhaler device correctly, but they inhale inadequately through the device, without sufficient peak inspiratory flow (PIF), which is necessary to release medication from the device. Therefore, before prescribing a DPI, it is essential to consider whether the patient will be able to do it forcefully and deeply enough. An inspiration whistle can be used for this purpose, ensuring that the patient is able to achieve a sufficient PIF. It is important to note, however, that sufficient PIF alone is not enough to guarantee for an adequate drug delivery from a DPI [60]. Poor inhalation technique is more frequent in newly referred children using a DPI than in children using an MDI/s device [56]; but there are no significant differences in the correct inhalation technique for the different inhaler devices, when all patients receive repeated inhalation instructions. This means that inhaling technique instructions would not be enough if provided once at the time of prescription. Repeating inhaling instructions can improve correct technique up to 30% [59].

Although the classification in these types of nonadherence is useful from a daily practice point of view, adherence is a complex behavioral process influenced by more interacting conscious and unconscious factors. Therefore, all effective interventions improving adherence to long-

term therapies are complex and multidimensional [55]. **Table 1** summarizes patterns of nonadherence and how to manage them.

Patterns of adherence barrier	Investigate for	How to manage them
Unwitting non-adherence	• Misunderstanding of medical indications/ advice	• Education
Intentional non-adherence	• Illness perceptions Medication beliefs	• Discussing patient/parents' perceptions • Expressing genuine interest • Partnership between physician and patient/parents • Concordance on treatment and patient/parents' goals • Stressing importance of daily ICS adherence • Self-management
Unplanned non-adherence	• Forgetting doses • Child-raising issues • Economic issues • Parental psychiatric illnesses • Excess of responsibility relaying on the child	• Establish easy routines • Avoid complicated treatment regimens • Home supervision • Child self-management achieved gradually
Incorrect inhalation technique	• Check technique • Check PIF for DPI device	• Choose suitable device • Provide patient demonstrated technique • Repeat training

PIF, peak inspiratory flow; DPI, dry powder inhaler.

Table 1. Patterns of nonadherence and how to manage them.

A proposed formula to achieve the best adherence to maintenance medication in asthma is the result of a medical team providing evidence-based education, tailored to the patient's (and parents') context, self-management education provided in an organized and repeated way (scheduled follow-up visits), and coupled with goal setting and other behavioral approaches[57].

2.2.2. Adherence measurements

Apart from self-reporting during the clinical interview, there are other ways of measuring adherence. More reliable ways could be used when poor adherence is suspected. The Medication Adherence Rating Scale (MARS) is one example. In this case, the patient responds to 10 items of the questionnaire and chooses the answer that best describes their behavior or attitude toward their medication during the past week [61]. This scale has been used previously to assess ICS adherence in adults with asthma [62]. In children, it has only been used to assess medication adherence in other chronic diseases, including pills taking [63]. However, later research has shown that its reliability is not sufficient [64].

Nowadays, validated electronically measured adherence with smart inhalers that register date and time of each ICS actuation is universally accepted to be the most reliable way of measuring adherence [61, 65]. Its use is limited by the high cost of the device, but it is particularly useful with those parents and patients who are not aware of their poor adherence, or in those cases in which the physician is not able to detect whether poor adherence is the problem for uncontrolled asthma.

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Chapter 6

Current and Future Asthma Treatments: Phenotypical Approach on the Path to Personalized Medicine in Asthma

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Additional information is available at the end of the chapter

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Abstract

Despite widely available and effective treatments, achieving asthma control is still an unmet need for many patients. One of the explanations resides perhaps in the heterogeneity of the disease. Asthma is in fact, as we understand it today, a complex syndrome made up of numerous disease variants or asthma phenotypes; when the different underlying mechanisms are identified, the more ambitious term "endotype" is used, with consequent therapeutic implications. Remarkable efforts have been made to identify the features of difficult-to-control (usually severe) asthma, which are different from those described for mild-to-moderate asthma, setting the stage for the development of new and even individualized therapies. As different drugs target different pathways, it is necessary to determine the individual profile of pathophysiological abnormalities for each patient. The most fascinating options of the new asthma treatments are the monoclonal antibodies targeted against key inflammatory cytokines, and the most proximately available treatments within the next years are discussed here. Also, current evidence and understanding of somehow older therapeutic options, such as anticholinergics, thermoplasty, or omalizumab, are reviewed from a phenotypical approach.

Keywords: asthma, mepolizumab, monoclonal antibodies, omalizumab, phenotypes, thermoplasty, tiotropium

1. Introduction

International [1] and national [2] guidelines for the management of asthma highlight the importance of finding the effective treatments for achieving and maintaining control. In spite

of the existence of uniform treatment guidelines, as well as of quite accessible and effective treatments, achieving asthma control often remains a constant challenge. Recent studies indicate that over 50% of patients with asthma are not controlled [3, 4], not even when receiving a combination of inhaled corticosteroids (ICSs) and a long-acting beta-2-agonist (LABA) [5] as controller treatment. These data suggest that the search for alternative treatments is required, particularly for patients with severe uncontrolled a-wrap id="tab1" position="anchor"> sthma.

When searching for new treatment options in asthma, it is important to remember that different drugs, particularly biological agents, act on different pathogenic pathways. So, the individual profile of physiopathological alterations of each patient should be determined to prescribe the most appropriate treatment in each case [6].

Asthma management, from both a current as well as a future risk perspective, must comprehend the stratification of patients into the recently defined phenotypes (such as clinical, inflammatory, and molecular) [7] and endotypes (such as allergic asthma, aspirin-sensitive asthma, late-onset hypereosinophilic asthma) [8], in the attempt to find a more personalized treatment for each patient. Moreover, in the last 10 years, significant efforts have been made to identify the characteristics that differentiate severe asthma from mild to moderate asthma, preparing the ground for the development of new selective treatments.

The main goal of the treatment is to achieve and maintain the control of the disease as soon as possible, to prevent chronic airflow obstruction, and to reduce mortality. The goals of the treatment, both in its current control domain and in preventing exacerbations and accelerated loss of lung function (future risk), could be achieved in most of the patients with appropriate treatment [9, 10].

2. New bronchodilators for asthma

2.1 Anticholinergics

Maintenance treatment to achieve asthma control currently includes inhaled or systemic glucocorticoids (ICS), leukotriene antagonists, LABAs, theophylline, monoclonal antibodies (mAbs) anti-IgE (omalizumab), and recently, newly included in the latest clinical practice guidelines, tiotropium bromide [1, 2]. The parasympathetic or cholinergic system is the most important bronchoconstrictor and hypersecretory neurological mechanism of the airways [11], and blocking specific muscarinic receptors is a therapeutic alternative to reduce the increase in parasympathetic activity that characterizes the main pulmonary obstructive diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Therefore, the natural alkaloids from the Solanaceae family plants (*Atropa belladonna* and *Datura stramonium*) represent one of the traditional remedies against bronchospasm. Atropine, the prototype nonselective muscarinic receptor antagonist, with "tertiary ammonium" structure, was widely used from the late nineteenth century in oral, parenteral, and inhaled forms for the treatment of asthma; however, its use is constrained by the cardiovascular side effects. Following the introduction of ephedrine and adrenaline, in the early twentieth century, atropine fell into disuse. Later, anticho-

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linergic therapy has returned to the forefront in the treatment of COPD, with the introduction of synthetic quaternary derivatives of atropine, short acting (ipratropium bromide) and long acting (tiotropium, aclidinium, umeclidinium, and glycopyrronium), the latter known under the acronym LAMA (long-acting muscarinic antagonists). The "quaternary ammonium" structure [12] makes them soluble in water and insoluble in lipids, therefore preventing the passage through biological barriers that are easily crossed by "tertiary ammonium" components, such as atropine, hence their lack of central nervous system effects; also they are poorly absorbed from the lung and gastrointestinal tract and do not inhibit the mucociliary clearance [13].

2.1.1 Tiotropium

Tiotropium bromide is the first long-acting anticholinergic agent (24 hours action), widely used for treatment of COPD. At the end of 2014, it was also approved by the FDA as an additional treatment of asthma in patients >12 years in the United States and in adult patients with asthma not controlled by the ICS in the European Union (Spiriva® Respimat). Such approval has been obtained based on sound scientific evidence on the effectiveness and safety of treatment with tiotropium in patients with mild-to-moderate and severe asthma. The major evidence is discussed below and is summarized in Table **1** [14].

Study	Patients' characteristics	Main results and Conclusions
Park et al. 2009 [15]	One hundred and thirty-eight patients with severe asthma on conventional medications and with decreased lung function.	– Forty-six of the 138 (33.3%) of patients with severe asthma were found to respond to adjuvant tiotropium bromide. – The presence of Arg16Gly in ADRB2 (coding beta-2 adrenoreceptor) may predict response to tiotropium bromide.
Peters et al. 2010 [17]	Two hundred and ten patients with poorly controlled asthma with an ICS alone.	– Tiotropium bromide, added to an IC, improved symptoms and lung function in patients with inadequately controlled asthma. – Its effects appeared to be equivalent to those with the addition of salmeterol.
Bateman et al. 2011 [16]	Three hundred and eighty-eight patients with asthma with the B16-Arg/Arg genotype whose symptoms were not controlled by ICS (moderate asthma).	– Tiotropium bromide was more effective than placebo and as effective as salmeterol in maintaining improved lung function in B16-Arg/Arg patients with moderate persistent asthma. – Safety profiles were comparable.
Kerstjens et al. 2011 [18]	One hundred patients with uncontrolled severe asthma, despite receiving treatment with high-dose ICS plus a LABA.	– The addition of once-daily tiotropium to asthma treatment significantly improved lung function over 24 hours in patients with inadequately controlled, severe, persistent asthma.

Study	Patients' characteristics	Main results and Conclusions
Kerstjens et al. 2012 [19]	Nine hundred and twelve patients (814 finished the study) with uncontrolled asthma in spite of ICS/LABA (studies PrimoTinAsthma 1 and 2).	– The addition of tiotropium (409 patients) compared with placebo (405 patients) significantly increased the time to the first severe exacerbation and provided a modest but sustained bronchodilation.

ICS = inhaled corticosteroids; LABA = long-acting beta-2-agonists.

Table 1. Summary of studies that demonstrate the efficiency of tiotropium bromide in asthma [14].

A study published in 2009 [15] showed additional improvement in lung function in patients with severe asthma when tiotropium was added to conventional treatment, according to the guidelines (LABA/ICS, theophylline, antagonists of leukotriene receptor, and oral steroids). A total of 138 severe asthmatics with decreased lung function were recruited. Tiotropium 18 μg (via HandiHaler) was added once a day, and lung function was assessed every 4 weeks. Responders were defined as those with an improvement of ≥15% (or 200 mL) in FEV1 that was maintained for at least 8 successive weeks. Of the 138 people with asthma, 46 (33.3%) responded to tiotropium.

Peters et al. [16] conducted an independent three-way, double-blind, crossover study in 210 patients with asthma to evaluate the effect of the addition of tiotropium to ICS, when compared with doubling the dose of ICS (primary superiority comparison) or adding salmeterol (secondary comparison of non-inferiority). Use of tiotropium was superior when compared with doubling the dose of ICS; it also demonstrated superiority in the secondary endpoints, including evening PEF, the proportion of asthma control days, prebronchodilator FEV1, and daily symptom scores. The addition of tiotropium was not inferior to the addition of salmeterol on all evaluated results and increased FEV1 prebronchodilator more than salmeterol. In summary, when added to an ICS, tiotropium improved symptoms and lung function in poorly controlled patients with asthma, and its effects appear to be equivalent to those obtained with the addition of salmeterol.

Bateman et al. [17] carried out a double-blind, double-dummy, placebo-controlled trial to compare the efficacy and safety profile of tiotropium (Respimat 5 μg, administered daily in the evening with the Respimat device) with that of salmeterol and placebo added to an ICS, in 16-Arg/Arg patients with asthma that was not controlled by ICS alone. The study population comprised patients aged 18–67 years, with reversibility to bronchodilators and symptoms that were not controlled by regular therapy with ICS (400–1000 μg of budesonide or equivalent maintained throughout the trial). Changes in weekly primary endpoint (PEF) from the last week of the run-in period to the last week of treatment showed that tiotropium was not inferior to salmeterol.

It has been also assessed whether tiotropium could be an effective bronchodilator in patients with severe asthma who remain symptomatic and obstructed despite maximum recommended treatment with the combination of ICS and LABA. Kerstjens et al. [18] compared the efficacy and safety profile of two doses of tiotropium (Respimat, 5 and 10 μg daily) with placebo as an

http://dx.doi.org/10.5772/62411

add-on therapy in 100 patients with uncontrolled severe asthma despite maintenance treatment with at least a high dose ICS combined with a LABA, in a randomized, double-blind, crossover study with three treatment periods of 8 weeks each. The PEF was peak FEV1 at the end of each treatment period. Peak FEV1 was significantly higher with 5 μg and 10 μg of tiotropium than placebo, whereas there was no significant difference between the two active doses. Domiciliary PEF values were higher with both tiotropium doses. Adverse events were balanced across groups, except for dry mouth, which was more common in patients taking tiotropium 10 μg. This study shows that the addition of once-daily tiotropium for asthma treatment, including a high-dose ICS combined with a LABA, significantly improves lung function over 24 hours in patients with uncontrolled severe asthma.

Subsequently, Kerstjens et al. [19] have evaluated the influence of add-on treatment with tiotropium on exacerbations, an important marker, as is well known, of asthma control. Two parallel, randomized, double-blind placebo-controlled trials (PrimoTinAsthma 1 and PrimoTinAsthma 2) were conducted between October 2008 and July 2011 in 15 countries, involving 912 patients with severe asthma and fixed airflow obstruction, who were randomized for tiotropium (Respimat, 5 μg) or placebo once daily for 48 weeks.

It was concluded that in patients with poorly controlled severe asthma despite the use of ICS and LABA, the addition of tiotropium significantly increased the time to the first severe exacerbation and provided a modest but sustained bronchodilation.

As mentioned in the introduction, we once more insist on the importance of determining the asthma phenotype: a small study (17 patients) showed that tiotropium is more effective in asthmatic smokers or non-smokers treated with medium-to-high doses of ICS if the inflammatory phenotype according to induced sputum is non-eosinophilic [20]. This suggests that perhaps early phenotyping poorly controlled asthmatic patients with high doses of ICS and even systemic corticosteroids (SC) could give tiotropium a corticosteroid-sparing effect in patients who turn out to have steroid-resistant asthma phenotypes. In fact, given its mechanism of action, the bronchodilator additive effect of tiotropium makes most sense in the following circumstances [21]: patients with asthma–COPD overlap syndrome (ACOS) [12], asthma of psychogenic origin, bronchospasm triggered by beta blockers, asthma with chronic airflow limitation, and severe asthmatics with Arg/Gly variation in codon 16 of the ADRB2 gene [15].

However, it seems that the effect of tiotropium goes beyond the bronchodilation because it has significant anti-inflammatory and antiproliferative capacities, such as reduction of hyperplasia of bronchial smooth muscle and inhibition of proliferation of fibroblasts and myofibroblasts [22]. Furthermore, in vitro studies using experimental models of asthma (ovalbumin-sensitized guinea pigs) have shown that tiotropium inhibits airway remodeling induced by allergens in a similar way to budesonide [23, 24], so its role in the management of allergic asthma may be more important than it seems at first glance.

Regarding adverse effects, tiotropium is a safe drug and is generally well tolerated, the most common side effect being dry mouth. The heart rhythm disturbances are rare (atrial fibrillation, atrial sinus, or supraventricular tachycardia). The TIOSPIR [25] study concluded that tiotropi-

um Respimat was safe in COPD patients with ischemic heart disease and/or stable arrhythmias. The study excluded patients with myocardial infarction in the past 6 months, class III–IV NYHA heart failure, potentially fatal arrhythmias, and chronic renal failure.

2.2. New combinations: ICS/LABA, LABA/LAMA, and triple therapy LABA/LAMA/ICS

Because combination therapy with ICS and LABA is the usual therapeutic option for the treatment of asthma, there is great interest in developing combinations of administration once a day, in an attempt to simplify treatment and improve treatment compliance [26], a currently achievable challenge with the new ICSs (such as ciclesonide, mometasone, and fluticasone furoate) and the emergence of new ultra-LABAs (such as indacaterol, vilanterol, and olodaterol), which can be administered in a single-daily dose. Currently, new combination therapies of ultra-LABA/ICS have been developed, are in clinical trial phases II–III, or have even recently marketed (vilanterol/fluticasone furoate), like several other LAMA–LABA combinations for the treatment of COPD: tiotropium/ olodaterol, aclidinium/ formoterol, umeclidinium / indacaterol, vilanterol / umeclidinium, and so on [27]. However, the use of some of these drugs in asthma is still being investigated (see also Table **2**).

Long-acting muscarinic antagonists (LAMA):

- Aclidinium bromide (approved for treatment of COPD)

Ultra-long-acting muscarinic antagonists (ultra-LAMA):

- Tiotropium bromide (approved for treatment of asthma and COPD)
- Glycopyrronium bromide (approved for treatment of COPD)

Ultra–long-acting beta-2-agonists (ultra-LABA):

- Indacaterol maleate (approved for treatment of COPD)
- Carmoterol hydrochloride, milveterol hydrochloride, olodaterol hydrochloride

New combinations of ultra–long-acting beta-2-agonists (ultra-LABA) and inhaled corticosteroids (ICS):

- Vilanterol trifenatate / fluticasone furoate (approved for treatment of asthma and COPD)
- Indacaterol maleate / mometasone (MGC-149)
- Indacaterol maleate / QAE 397

New combinations of LAMA or ultra-LAMA and LABA or ultra-LABA:

- Tiotropium bromide / olodaterol hydrochloride
- Indacaterol maleate / glycopyrronium bromide (QVA149)
- Umeclidinium bromide / vilanterol trifenatate (approved for COPD)
- Formoterol/ aclidinium (approved for COPD)

Triple therapy of ultra-long-acting beta-2-agonists (ultra-LABA), inhaled corticosteroids (ICS), and ultra–long-acting muscarinic antagonists (ultra-LAMA):

- Vilanterol trifenatate/fluticasone furoate/umeclidinium bromide

COPD = chronic obstructive pulmonary disease; ICS = inhaled corticosteroids; LABA = long-acting beta-2-agonists; LAMA = long-acting muscarinic antagonists.

Table 2. New bronchodilators, either available or under clinical development, with probable upcoming indication for asthma (monotherapy and combinations).

When talking about the triple combination, it refers to ICSs, such as beta-2-agonist and inhaled anticholinergics, but mainly to long-acting drugs (LAMA–LABA–ICS). The possibility of associating these three drugs can contribute to better compliance, better control of the symptoms, and improved quality of life, as well as to a decrease in exacerbations. There are several clinical studies in development: fluticasone/salmeterol/tiotropium and budesonide/formoterol/tiotropium [28]. The first triple combination formoterol/tiotropium/ciclesonide (Triohale®, Cipla) is now available in India [29], and its probable effectiveness in asthma is yet to be proven in future clinical trials.

3. Biological and other highly specialized therapies for uncontrolled asthma: Phenotype-oriented present and future options

In the last decade, significant efforts have been made to identify the characteristics of severe asthma, which are different from those described in the mild-to-moderate asthma, setting the stage for the development of new personalized therapies [7, 30]. The most promising options are represented by biological therapies, including mAbs against selective targets [10]. Later, we summarize the evidence of the only mAb that is available today to treat patients with severe asthma (omalizumab) and review those biological treatments that are currently in clinical trials, but in a more advanced stage of development and will be available for the clinical practice in the upcoming years.

3.1 Allergic asthma

3.1.1 Omalizumab: state of the art on long-term efficacy and safety in real life studies.

Omalizumab is currently approved as an additional treatment in patients older than 6 years with severe allergic asthma [31]. The antibody is an IgG1 kappa that binds to IgE and prevents it binding to FCεRI and FCεRII (IgE receptors of, respectively, high and low affinity), expressed on mast cells, basophils, and dendritic cells [32]. Several post-marketing studies have been conducted in European countries [33–37] to assess the effectiveness of omalizumab: despite obvious differences between countries, all studies confirmed the usefulness and safety of omalizumab in real-life conditions. The discontinuation rate was variable, but the lack of efficacy was less than 20%, whereas in clinical trials, it was 30–40%. A probable explanation is

that the "real" patients are more serious and less selected than those included in clinical trials. In Spain, a multicenter study was conducted within the routine clinical practice, in Pulmonology and Allergology departments, to evaluate the efficacy and tolerability of omalizumab [38]. With the participation of 30 centers nationwide, 266 patients who had received at least one dose of omalizumab, with 2 years of follow-up at least, were analyzed. The global evaluation of therapeutic efficacy (GETE) was good or excellent in most treated patients: 74.6% at 4 months, reaching 81.6% of the patients after 2 years, with statistically significant differences from baseline. Significant improvements in asthma control test (ACT), lung function, and exacerbation frequency were also demonstrated. In terms of medication, the doses of ICSs were significantly decreased, and the maintenance treatment with oral corticosteroids was suspended in many patients [38].

3.1.2. Current evidence on omalizumab efficacy in off-label uses: Non-allergic asthma, nasal polyps, and allergic broncopulmonary aspergillosis

The interaction between IgE and omalizumab prevents a fundamental step in the inflammatory cascade. The rapid decrease in the free circulating IgE leads to a progressive and significant decrease in the expression of IgE receptors on the inflammatory cells, so it is important to take into consideration certain entities in which IgE may also play a part even if the allergic etiology is not well established, such as nasal polyposis (NP) or non-allergic asthma.

While the inflammation in allergic or "extrinsic" asthma is clearly caused by outdoor allergens (such as dust mites and animal dander), in the intrinsic disease, there is no identifiable allergen, at least not by currently available methods. In this case, an unidentified exogenous antigen (without systemic sensitization), an infectious agent, or an endogenous "allergen" might be responsible for triggering the mechanism of atopy or in this case "entopy" [39]. The finding of specific IgEs against *Staphylococcus aureus* enterotoxins in patients with severe asthma, intolerance to NSAIDs and NP allowed to speculate that they were susceptible of having their airways colonized by *S. aureus*, which through the release of superantigens could trigger an inflammatory response with formation of local IgE [40]. NP may be present in asthma with or without concomitant atopy, but it is particularly associated with non-allergic aspirin-sensitive asthma and is one of the most common comorbid conditions in patients with severe asthma. NP is not a life-threatening condition, but the patients see their quality of life severely compromised and must undergo prolonged treatments with topical and systemic corticosteroids and multiple sinus surgeries in most cases. Over time, the lack of effective alternative treatments and the need to respond to these IgE-mediated diseases led professionals to use omalizumab off-label, with very promising results, as discussed later.

In 2010, a multicenter study performed in Spain described the evolution of nasal polyps in 19 patients with NP and severe asthma treated with omalizumab [41]. The average treatment time was 16 (15–28) months. Thirteen patients (68%) had undergone at least one endoscopic surgery. The size of the polyps (assessed by calculating a score of 0–8 points by means of nasal endoscopy and confirmed by CT scan) diminished significantly in both nasal cavities after the treatment. Later, Bachart et al assessed the usefulness of anti-IgE in severe or recurrent NP associated with asthma, in a prospective double-blind placebo-controlled study (24 patients)

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[42]. There was a significant improvement at 16 weeks of treatment in clinical terms: nasal congestion, rhinorrhea, and loss of smell. An overall reduction in polyp size (primary end-point, assessed using the same above-mentioned score) when compared to baseline was observed [41].

As to non-allergic asthma, in Spain it was first demonstrated, in a retrospective observational study [43], the efficacy of omalizumab in 29 patients with "non-atopic" asthma. GETE, ACT, the number of exacerbations and lung function improved significantly after treatment with omalizumab. There was no statistically significant difference in the response of the non-atopic asthmatics when compared with 266 patients with positive prick tests to usual inhalants. These results were subsequently confirmed in a prospective double-blind placebo-controlled trial [44].

Total IgE levels are a marker of immune activity in another severe lung disease, often without any effective therapeutic alternative to systemic corticosteroids: allergic bronchopulmonary aspergillosis (ABPA). The anti-IgE treatment was also evaluated in this pathology (also off-label). In 2011, a multicenter research conducted in Spain included 18 patients with ABPA from 11 hospitals [45]. Patients were followed for a median of 36 (28–42) weeks. In this series, the largest published so far, omalizumab was beneficial in reducing daytime symptoms (44%) and nighttime awakenings (22%), significantly reduced exacerbations and improved FEV1 (p = 0.03), allowing a reduction or even discontinuance of systemic corticosteroids.

3.1.3. New anti-IgE agents: ligelizumab and quilizumab

The inverse correlation between free IgE levels and asthma control, found in several studies [46], suggests that a more profound suppression of free IgE could lead to an even more marked clinical improvement, so new, more potent anti-IgE mAbs are currently being assessed in clinical trials.

3.1.3.1. Ligelizumab

QGE031B (ligelizumab) is a new anti-IgE mAb (Novartis). It is a humanized IgG1 that binds with higher affinity to the Ce3 region of IgE. QGE031 is designed for greater suppression of IgE, with a dissociation constant (Kd) of 139 pM, representing an increase in almost 50 times of the affinity for IgE when compared with omalizumab (Kd = 6–8 nM). This is hypothesized to overcome some of the limitations associated with the dosage of omalizumab and lead to better clinical outcomes in asthma.

Up to date, December 2015, we have only data from preclinical experiments, and the results of two phase I, randomized, double-blind placebo-controlled studies investigating the pharmacokinetics, pharmacodynamics, and safety of ligelizumab in atopic but otherwise healthy subjects [47]. Ligelizumab was superior to omalizumab in the suppression of free IgE and FceRI expression on surface of basophils. These effects resulted in the almost complete suppression of skin response to allergens, which was higher in extent and duration when compared with omalizumab. In the 156 patients who completed the study, no serious adverse effects were reported, and only one patient developed urticaria accompanied by systemic

symptoms. QGE031B's effectiveness is currently being evaluated in patients with allergic asthma (GINA step 4/5) in a phase IIa clinical trial, with omalizumab as an active comparator.

Quilizumab

Quilizumab (MEMP1972A, Genentech/Roche), another mAb anti-IgE, is being studied now in a phase IIb, randomized, double-blind, placebo-controlled clinical trial aimed to evaluate the efficacy and safety of three different doses (150, 300, and 450 mg, subcutaneously) in adults with allergic asthma not controlled with ICS and a second controller (NCT01582503). Quilizumab already has been proven effective in decreasing total and specific IgE in patients with allergic rhinitis (NCT01160861) and mild allergic asthma (NCT01196039), with a good safety profile [48].

3.2. Eosinophilic and Th2 high asthma

3.2.1. Anti IL-5 monoclonal antibodies: mepolizumab, reslizumab, and benralizumab

Interleukin-5 (IL-5) is a hematopoietic cytokine produced by various cells such as Th2 lymphocytes, eosinophils, basophils, mast cells, and natural killer T-cells, and it is the main eosinophil modulator cytokine [49] because it enhances eosinophil chemotaxis, activation, and degranulation, while reducing apoptosis and prolonging eosinophils' survival. The IL-5 receptor (IL-5R), expressed on both basophils and eosinophils, is made up of two subunits: an α-subunit (IL-5Rα) that is IL-5-specific and a βc-subunit (IL-5Rβc) that is responsible for signal transduction and is shared with the specific α-receptor subunits of IL-3 receptors and granulocyte–macrophage colony–stimulating factor (GM-CSF).

Two mAbs (mepolizumab and relizumab) that neutralize IL-5 and another mAb (benralizumab) that blocks the IL-5Rα have been developed and are currently being evaluated in clinical trials [50].

3.2.1.1 Mepolizumab

Mepolizumab in a fully humanized anti-IL-5 IgG1 mAb that binds to the free IL-5 with high affinity and specificity, thus preventing its binding to the α chain of the IL-5R on the eosinophil cell surface. It was the first IL-5 antagonist used in randomized, controlled trials in patients with mild asthma [51, 52] and with moderate uncontrolled persistent asthma [53]. A reduced eosinophil count was observed in both sputum and peripheral blood asthma in biopsies of bronchia and bone marrow, but with no effect on bronchial hyperresponsiveness (BHR), late asthmatic response, lung function, symptoms, or use of rescue medication whatsoever [51–53]. The reduction in the percentage of exacerbations [53] did not reach statistical significance though.

In these studies, patients were not selected according to the presence of eosinophilic airway inflammation, and the number of exacerbations, a parameter directly and causally related with

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eosinophilic airway inflammation, was not evaluated as a principal variable of the response to treatment [49]. Two new trials were subsequently performed in patients with refractory severe persistent asthma with recurrent exacerbations, who had bronchial eosinophilic inflammation [54, 55]. Both trials reported a very significant reduction in the number of exacerbations and in the dose of oral corticosteroids in the active group when compared to those in the placebo group, as well as a major improvement in asthma control questionnaire (ACQ) scores. This response was accompanied by a significant reduction in eosinophil numbers in blood and sputum.

A phase IIb multicenter study (GlaxoSmithKline) has also been performed in order to determine the optimal dose of mepolizumab and to confirm its efficacy and safety in patients with severe eosinophilic asthma (the DREAM study) [56]. A total of 621 patients were randomized to placebo or one of three mepolizumab doses (75, 250, or 750 mg respectively) in parallel groups for 1 year. Mepolizumab reduced the number of severe exacerbations by 50% approximately in all the mepolizumab groups when compared with placebo, irrespective of the dose. Also, no dose–response effect was reported. The blood and sputum eosinophil counts were also reduced, and a dose–response effect was observed for eosinophil counts in sputum. On the other hand, no changes in asthma symptoms, quality of life, FeNO or lung function were observed. The drug was safe and effective. A multivariant analysis established that blood eosinophilia and the number of exacerbations in the 12 months prior to the study only were associated with a good response to mepolizumab. A meta-analysis performed on published clinical trials with mepolizumab, including a total of 1131 patients, confirmed that in cases of eosinophilic asthma, mepolizumab reduced the number of exacerbations and improved asthma-related quality of life [57].

3.2.1.2 Reslizumab

Reslizumab, a humanized IgG2, is another IL-5 inhibitor that is administered intravenously, although it has not been studied at such extent as mepolizumab. The only published clinical trial in patients with poorly controlled eosinophilic asthma proved that patients treated with reslizumab showed a significant improvement in FEV1 and, interestingly, patients with concomitant polyposis showed better asthma control compared to the placebo group [58].

3.2.1.3 Benralizumab

Benralizumab is a humanized IgG1 mAb targeting IL-5Rα, which reduces eosinophilia by antibody-dependent cell-mediated cytotoxicity. Intravenous benralizumab has shown acceptable safety and tolerability in a phase I, dose-escalating study, with a marked reduction in circulating eosinophils [59].

In a phase I, multicenter, double-blind, placebo-controlled study, 13 patients were randomized to receive a single intravenous dose of placebo or 1 mg/kg benralizumab, and other 14 patients were randomized to receive a monthly subcutaneous dose of placebo, or either 100 or 200 mg benralizumab, for 3 months. The study concluded that both the single intravenous dose and the multiple subcutaneous doses of benralizumab reduced the percentage of eosinophils in

the bronchial biopsies and in induced sputum and suppressed eosinophil counts in the bone marrow and peripheral blood [60]. Additional studies are further required.

3.2.2. Anti IL-13 monoclonal antibodies: Lebrikizumab

IL-4 and IL-13 are key therapeutic targets in Th2 high asthma, due to their significant role in Th2 lymphocyte responses and in B lymphocyte isotype switching for IgE synthesis and also for their intervention in mast cell selection (see Figure 1). The strong evidence existing upon the involvement of this pathogenic pathway in asthma, initially ranging from genetic studies up to convincing data from animal studies, leads to the development of a wide range of biological agents aimed at these targets, including anti-IL-13, anti-IL-4Rα and anti-IL-13Rα1 mAbs, IL-4Rα/IL-13Rα1 fusion protein, IL-4/IL-13 vaccines, anti-IL-4Rα antisense oligonucleotides, and double mutein IL-4 [61]. However, although many of these drugs are under development, to date only a few have been evaluated in patients with asthma [62] (see also Table 3).

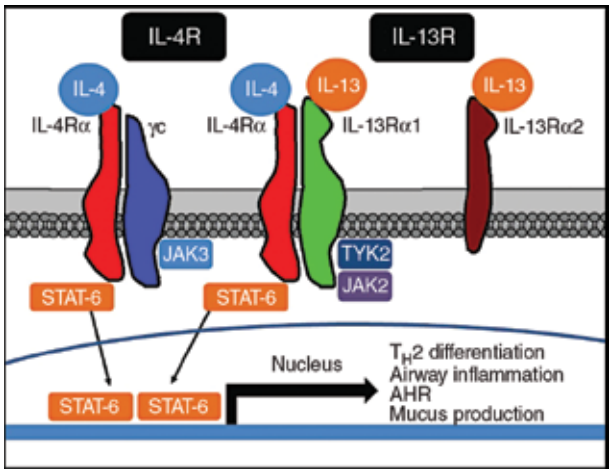

Figure 1. The IL-4/ IL-13 receptor.

		Drug	Pharmaceutical company
	mAb anti IgE	Quilizumab (MEMP1972A)	Genentech/Roche
		8D6	United BioPharma
		Ligelizumab (QGE031B)	Novartis
mAb anti IL-5	IgG1	Mepolizumab	GlaxoSmithKline
	IgG2	Reslizumab	TEVA
mAb anti IL-5	ASO anti-IL-5Rβc and anti-CCR3	TPI-ASM8	BioCentury

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		Drug	Pharmaceutical company
mAb anti IL-5Rα IgG1		Benralizumab	AstraZeneca
	mAb anti IL-13	Lebrikizumab	Roche
		Anrukinzumab	AstraZeneca
		Tralokinumab	AstraZeneca
mAb anti IL-4α/IL-13Rα1		Dupilumab	Sanofi
Other IL-4/IL-13 antagonists	mAb anti IL-4	Pascolizumab	GlaxoSmithKline
	Recombinant soluble IL-4 receptor (sIL-4 R)	Altrakincept	GlaxoSmithKline
	AcMo anti IL-4	Pascolizumab	GlaxoSmithKline
	IL-4RI-selective mutein (IL-4/Q116E)	Pitrakinra	Aerovance

ASO = "anti-sense" oligonucleotide; CCR3 = cysteine–cysteine chemokine receptor-3; IL = interleukin; mAb = monoclonal antibodies; sIL-4 R = recombinant soluble IL-4 receptor.

Table 3. Monoclonal antibodies for the treatment of asthma.

3.2.2.1 Lebrikizumab

Corren et al. [30] first studied the effects of lebrikizumab in 219 adults with moderate-to-severe persistent uncontrolled asthma. Lebrikizumab was administered subcutaneously every month for 6 months. A significant improvement in prebronchodilator FEV1 was recorded at 12 weeks in patients treated with lebrikizumab when compared to the placebo group. The study drug was significantly more effective in patients with pretreatment circulating periostin levels above the median and also in those with Th2-high phenotype (total IgE > 100 IU/ml and eosinophilia > 140/mm^3), when compared to those with Th2-low phenotype. Exacerbations were not significantly reduced in the active group compared to placebo, but when sub analyzed in the Th2-high subgroup, the rate of exacerbations was 60% lower in patients receiving lebrikizumab compared to placebo. These data suggest that therapy with anti-IL-13 antibodies may be more effective when directed to a selected subgroup of patients (i.e. Th2-high –phenotype).

3.2.3. Anti IL4R monoclonal antibodies: Dupilumab

Dupilumab (Sanofi) is a humanized mAb that targets the α-subunit of the IL-4–IL-13 shared receptor. The efficacy and safety of dupilumab in the treatment of patients with persistent eosinophilic asthma were evaluated in a phase IIa, randomized, double-blind, placebo-controlled study [63]. One hundred and five patients with moderate-to-severe persistent asthma and eosinophilia ≥300/mm^3 in blood or ≥3% in sputum were included. All patients were on moderate-to-high doses of ICS and LABA. They were randomized to receive either dupilumab 300 mg (n = 52) or placebo (n = 52), subcutaneously, once a week for 12 weeks, or until the development of a moderate or severe exacerbation (primary endpoint).

Asthma exacerbations were reduced by 87% in the active group (6% exacerbations in the patients receiving dupilumab versus 44% in the placebo group), being this difference statisti-

cally significant. Significant differences in favor of dupilumab in the time until the first exacerbation and in the risk of exacerbations were also recorded. In the dupilumab patient group, both the morning peak expiratory flow (PEF) and the asthma symptoms evaluated by the ACQ5 improved significantly. Nocturnal awakenings and the use of short-acting beta-2 agonists were also reduced.

Regarding adverse effects, more local reactions at the injection site, nasopharyngitis, nausea, and headache were reported in patients on active treatment, and there was one case of angioedema. The authors of this study emphasize the effect of dupilumab on the reduced frequency of exacerbations, even after withdrawal of ICS and LABA. Nevertheless, they admit that the definition of "exacerbation" used in their protocol does not coincide with that usually employed in clinical practice and, accordingly, recommend that larger studies should be further performed [63].

As we have seen, most new mAbs under development are directed against different targets of the Th2 pathway [62]. A summary of all these drugs is found in Table 3. Figure 2 briefly sketches the allergic inflammatory cascade, so that we might easily visualize these therapeutic targets.

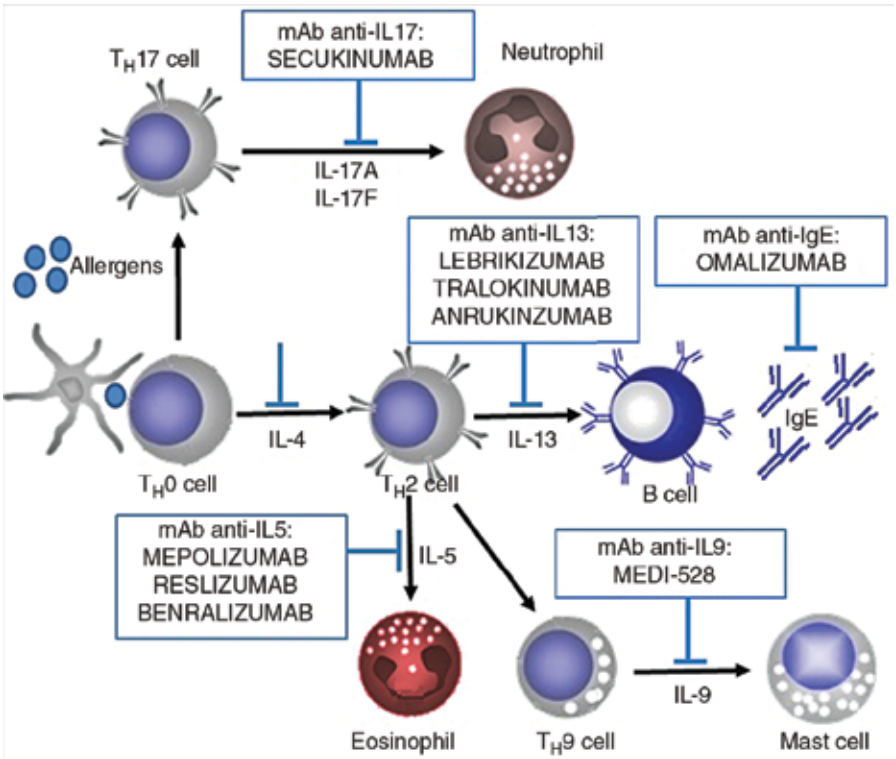

Figure 2. Therapeutic targets within the allergic cascade.

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3.3. Non-eosinophilic asthma: Neutrophilic, Th2-low asthma

3.3.1. Anti-tumor necrosis factor-α monoclonal antibodies

Unfortunately, for patients belonging to severe asthma phenotypes other than eosinophilic asthma, current therapeutic options are scarce, and many of these patients are steroid-dependent and even steroid-resistant [2]. Clinical trials with anti-tumour necrosis factor (TNF)-α mAbs (such as infliximab, adalimumab, and golimumab) have been performed with discouraging results. A study including 309 patients with severe persistent asthma, randomized to receive placebo or three different doses of golimumab (50, 100, and 200 mg), showed no significant improvement in any of the efficacy variables [64]. More importantly, the trial had to be prematurely discontinued due to serious adverse events (SAEs), namely infections and malignancies, in the golimumab group. A post-hoc analysis suggested that patients with a prestudy history of sinusitis and FEV1 reversibility (≥12%) who received golimumab (100 and 200 mg) had fewer severe asthma exacerbations, apparently associated with a dose–response effect. Perhaps, if biomarkers were developed for predicting response to anti-TNF-α agents, then they could be used for selected subgroups of patients with severe asthma, but the contradictory efficacy results and especially the potential safety concerns have prevented the performance of any additional clinical trials so far.

3.3.2. Bronchial thermoplasty

Thermoplasty is a bronchoscopic procedure that reduces the bronchial smooth muscle layer by applying heat by radiofrequency. The results of the studies showed, in patients with moderate and severe asthma, a significant improvement in their quality of life, increased disease control, and a reduction of exacerbations. These results persist for years after the procedure, without medium- to long-term secondary effects [65–67]. While new evidence is needed to identify the ideal candidate, it is currently considered to be preferably indicated in patients with severe uncontrolled asthma, with chronic airflow limitation (FEV1 > 50% and <80%), and without bronchial hypersecretion. Likewise, its application is recommended to be performed in centers with experienced and sufficiently trained endoscopists [2].

4. Conclusions and future perspectives

We are witnessing the rapid development of new molecules and also of promising new combinations in terms of efficacy, safety, and dosage for the treatment of asthma, except perhaps for treatment for a subgroup of patients with severe non-eosinophilic asthma, in which therapeutic options still remain limited. Given the heterogeneity of the disease, we consider it is important to establish the phenotype or endotype as a first step on the road to the "personalized" medicine in asthma.

From a practical point of view, in Table 4 we present the personal opinion of the authors of this chapter on the individualized" utility of the new asthma treatments, already existing or proximally available.

Asthma phenotype/endotype and its major characteristics		Therapeutic options
1. Extrinsic or allergic asthma		– Allergen avoidance, montelukast, allergen-specific immunotherapy, omalizumab – Tiotropium bromide
2. Intrinsic or non-allergic asthma		
Eosinophilic, may associate atopy or entopy	**AERD (or NERD):** Th2-high and extensive eosinophilic infiltration, potentially severe or difficult-to-control asthma, glucocorticoid-dependent/glucocorticoid-resistant asthma	– Montelukast, tiotropium bromide, aspirin desensitization – Omalizumab (anti-Th2 effect, off-label). – In the future: assess new treatments with anti-IL-5 and anti-IL-13
	Late-onset hypereosinophilic asthma: similar to AERD, increased airway remodelling, fixed airflow limitation, usually glucocorticoid-dependent asthma	– Tiotropium bromide – Omalizumab (off-label). – Future: anti-IL-5
Non-eosinophilic, non-atopic	**Non-eosinophilic asthma:** obese females, neutrophilic or paucigranulocytic inflammation, Th2-low: worse prognosis, glucocorticoid-resistant asthma	– Tiotropium bromide, ultra-LABA/LAMA – Bronchial thermoplasty. – Anti-TNF-α???*

AERD = aspirin-exacerbated respiratory disease (or Samter's triad); ICS = inhaled corticosteroids; IL = interleukin; LABA = long-acting beta-2-agonists; LAMA = long-acting muscarinic antagonists; NERD = non-steroidal anti-inflammatory drugs-exacerbated respiratory disease; Th2 = helper type 2 lymphocyte; TNF = tumour necrosis factor
** Clinical trials with anti-TNF agents (infliximab, adalimumab, and golimumab) had to be suspended prematurely due to the appearance of serious adverse events, especially severe infections and malignancies* [64].

Table 4. The path to personalized treatment of asthma insufficiently controlled with ICS/LABA: Present and future.

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Chapter 7

Genetics of Allergic Asthma and Current Perspectives on Therapeutic Management

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Additional information is available at the end of the chapter

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Abstract

Globally, more than 300 million people are asthmatics and this number has been estimated to become 400 million by 2025. Asthma is a chronic inflammatory condition, which, although has no cure, is treatable in most patients. The most common structural alterations in asthmatic airways include thickening of the epithelial and sub epithelial layers, increased airway smooth muscle mass, and angiogenesis. Several genetically controlled factors greatly influence the predisposition and severity of allergic asthma. Twin studies have attributed as much as 75% of asthma susceptibility to heredity. Particularly, genome-wide association studies (GWASs) have discovered several asthma and/or atopy susceptibility genes. Current treatment protocols for managing asthma involve the use of corticosteroids and β-agonists. Over the last 40 years, there has been a marked development-targeted therapy for asthma, such as anti-leukotrienes, anti-immunoglobulin (Ig)E, anti-tumor necrosis factor (TNF)-α, and anti-interleukins (ILs) (Th2 cytokines). To identify novel targets and to develop newer drug generations, better understanding of asthma molecular pathophysiology is required. Furthermore, the pharmacogenetic studies, focusing on better understanding of beneficial or/and adverse effects to anti-asthma drugs, will also facilitate the development of more effective and targeted treatments in specific subpopulations of patients suffering from asthma.

Keywords: asthma, asthma therapies, genetics, pharmacogenetics

1. Introduction

Asthma is an inflammatory chronic condition that has reached globally epidemic levels. Although no cure exists, symptoms are treatable in most patients [1]. Statistically, the number

of asthmatic cases has been on the rise over the past 10 years and affecting up to 10% of adults and 20% of children worldwide [2]. Globally, more than 300 million people are asthmatics, and this estimate is predicted to become 400 million by 2025 [3]. The worldwide economic burden caused by asthma is predicted to be more than that of both acquired immunod eficiency syndrome (AIDS) and tuberculosis combined together. For example, in the United States of America, the annual asthma care costs exceed US$6 billion [4]. Moreover, these numbers are due to the fact that more than 50% of asthmatic cases are poorly controlled by medication, since the response to treatments varies considerably among patients despite having similar clinical features [3, 5]. Asthma is characterized by altered and distinct clinical changes in the lung airways obstructing the flow of air into the lungs. The most prominent airway remodeling features include epithelial and subepithelial layer thickening, increased airway smooth muscle (ASM) mass, and angiogenesis [6]. Different classes of asthma therapies address one or more of the phenotypes of asthma; however, the heterogeneous nature of the disease prevents homogeneous clinical outcomes in response to the current treatment guidelines [7].

In the past two decades, the field of human genetics has evolved due to the advancements in the human genome project and high-throughput sequencing technologies [8, 9]. Currently, the advances in genetic, pharmacodynamic, and pharmacokinetic studies, analyzing responsiveness of patients to various therapies, may eventually allow to prescribe personalized treatment and to shift asthma therapies from classical standards, using mostly corticosteroids and β-adrenergic agonists, to a highly tailored approach [10]. Future genetic profiles of the population would form the basis of tomorrow's treatments in order to potentiate the required therapeutic benefits, and to diminish any possible adverse effect risks. Overall, there remains a great need for comprehensive drug research, paralleled with high-throughput genetic profiling, in order to treat asthma in a personalized or stratified manner [11].

2. Genetic control of airway hyperresponsiveness, atopy, and allergic asthma

The heritable nature of asthma has been demonstrated through various types of studies over the past decades. Family and twin studies indicate that 60–70% of asthma cases are due to genetic factors. Moreover, it has been proven that the concordance of asthma is greater among monozygotic twins rather than among dizygotic ones. Adoption studies have shown greater disease prevalence within biological relatives of the affected people compared to the adopted family [12].

Higher prevalence of allergic disease phenotypes is observed among relatives of atopic individuals compared to nonatopic individuals. Overall, the heritability estimates remain in between the range of 30–66% for airway hyperresponsiveness, 35–95% for asthma, and 35–84% for total serum IgE levels [13]. It is clear that both the inter-genetic individual differences and the degree of allergen exposure contribute to these variations in heritability. Heritability of asthma is linked to both disease susceptibility and severity. While the main concern of asthma genetic studies has been on disease susceptibility, increasing evidence shows that many genetic variants are important in asthma progression and severity as well [14]. Lung

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function tests in asthma showed that genes in the T-helper lymphocyte 1 (Th1) pathway affect asthma severity; meanwhile, T-helper lymphocyte 2 (Th2) pathway genes relate to susceptibility [14]. Based on these hypotheses, genes associated with asthma susceptibility differ from those related to asthma severity; hence, it is important to define both groups distinctly.

By knowing the genetic signature associated with allergic asthma, geneticists can help to better understand the molecular mechanism of this disease, and the shared and distinct pathways among other allergic diseases. Moreover, the genetic signature of asthma-associated genes with altered expression during the peak of asthmatic episodes may help predict the severity and response to therapy. Unfavorable response might be identified and, consequently, more targeted and personalized treatments can be considered for this complex trait. The human genome project and the ongoing advancements in sequencing technologies, both, resulted in more successful gene discovery over the last years, even in diseases as complexed as asthma. Since then, dozens of susceptibility genes were identified through a large variety of methods and rationales. *ADAM33* is the first asthma susceptibility gene to be discovered through positional cloning [15]. ADAM33 (also known as Disintegrin and metalloproteinase domain-containing protein 33) is a membrane-bound metalloproteinase enzyme that has been involved in several cellular interactions involving cell-cell and cell-matrix events [16]. Variants in this gene have been correlated to asthma susceptibility and bronchial hyperresponsiveness, but not atopy. Due to its clinical significance, *ADAM33* studies were conducted among 33 different asthmatic population samples all over the world. Additionally, numerous studies have suggested that altered *ADAM33* DNA methylation patterns could result in diversely unbalanced biological effects in the airways [17]. Studies focused on candidate genes have examined a number of genes involved in asthma and highlighted more than 100 interesting genetic spots; however, the role of those loci in asthma susceptibility remains largely unexplored [18].

Genome-wide association studies (GWASs) extensively investigate the unknown genetic bases of many intricate disorders including asthma [19,20]. In the first reported GWAS study for asthma susceptibility, Moffatt et al. [21] identified the 17q21 locus, containing several genes, for example, *ORMDL3* and *GSDMB* as being associated with childhood asthma. Importance of this region was later on replicated in numerous subsequent studies [22–24]. Expression levels of the gene *ORMDL3* are differentially regulated by distinct haplotypes in this region. This gene encodes protein acting as an inhibitor of sphingolipid biosynthesis and in general Orm family proteins were shown to be implicated in the control of sphingolipid homeostasis [25]. Dysregulated sphingolipid formation in the respiratory tract instigates airway hyperreactivity [26] although exact molecular steps are still not known. The results of these studies suggested that the mechanisms of asthma development are linked with genetically determined abnormalities in some patients resulting in their inability to control balance between oxidative and anti-oxidative reactions. The mechanisms of asthma development are linked with genetically determined abnormalities in the functioning of antioxidant defense enzymes. These alterations seem to be accompanied by a systemic imbalance between oxidative and anti-oxidative reactions with the shift of the redox state toward increased free radical production, oxidation of proteins and phospholipids, and eventually to their selective degradation.

To increase the power of detection of modest alleles due to the large sample size, the results of individual GWAS need to be gathered into a meta-analysis. The scientific literature recognizes two meta-analyses of asthma GWAS. One was done by the GABRIEL Consortium [27] of the European investigators, and the other was conducted by the EVE Consortium of the US investigators [22]. While the EVE meta-analysis included diverse subjects from different ethnic background, US and Mexico population backgrounds, the GABRIEL meta-analysis included only European subjects. Overall, these two thorough meta-analyses present a comprehensive overview of the genetic associations for asthma. Some associations are shared among different populations; by contrast, others are specific to one race. Grouping GWAS in this way increases the power of genetic detection, contrasts different ethnic groups' genotypes, and highlights the worldwide populations' genetic patterns. Overall independent GWASs have identified large number of candidate loci that deserve further testing. Replication studies help to prioritize which genes deserve further study, based on their identification in multiple populations.

Additionally, more loci were identified to be associated with asthma; these include interleukin (IL)-33 (on 9p24), *HLA-DR/DQ* (on 6p21), *IL1RL1/IL18R1* (on 2q12), *TSLP* (on 5q22), and *IL13* (on 5q31) [22,27,28]. Collectively with *ORMDL3/GSDMB* (on 17q21), these are the most remarkable and consistent loci, which are identified for asthma. Since Moffatt et al. had published the first GWAS results for asthma, identifying *ORMDL3* as a candidate gene, numerous other studies have been conducted investigating an array of phenotypes which are observed in allergic diseases. In particular, FCER1A, RAD50, and STAT6 have been associated with total serum IgE levels [29].

3. Environmental factors contributing to asthma

Parallel to genetic factors, environmental factors are also involved in the development and progression of asthma (**Figure 1**). The exposure to some environmental factors was shown to contribute not only to asthma but also to other related respiratory disorders, for example, emphysema development. By contrast, there are also some other environmental factors that seem to be solely linked to the development of asthma but not to other inflammatory or/and respiratory disorders [30]. Various studies assessed the risk factors of asthma and found evidence that allergen exposure, respiratory tract infections, gastroesophageal reflux disease (GERD), and physical and psychosocial stress might represent individual risk factors. It is important to keep in mind that some other environmental factors are protective, such as maternal diet, breastfeeding, and farming conditions [31].

Allergen exposure is the major factor impacting sensitization and constitutes the most common cause of asthmatic exacerbations in adults and children. A wide variety of inhaled allergens may trigger asthma symptoms, for example, house dust mite [32], pollens [33], cockroaches [34], and animal fur [35]. Respiratory tract infections have been implicated in asthma occurrence and exacerbation as well. Examples include infection with viruses [36,37], *Mycoplasma* [38], and *Chlamydia species* [39]. Based on the conclusions from the Japanese study, which

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included 3085 patients, the change in weather followed by smoking was identified as two leading asthma-exacerbating factors [40]. Although (passive) smoking is a predominant contributing factor for the development of asthma [41], one occupational study [42] has shown that nonsmokers might also develop asthma due to occupational air pollutant exposure.

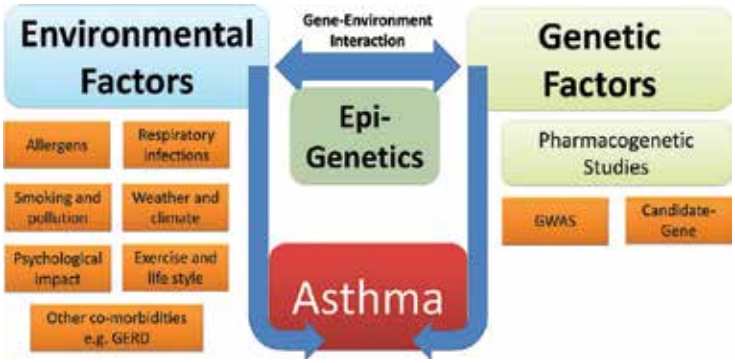

Figure 1. Environmental stress, in conjunction with genetic factors, both contribute to the development of asthma exacerbations.

Additionally, a correlation has been observed between the presence of asthma and gastroesophageal reflux-induced disease, with reports showing one-third of asthmatic patients also diagnosed with GERD [43,44]. Although the coexistence of GERD in asthmatic patients did not affect asthma severity, the airway resistance was significantly higher in asthmatic patients with GERD [45]. Some other psychosocial factors such as parental stress during childhood [46] and the socioeconomic status [47] are reported to influence allergic inflammation severity. It is estimated that psychopathology is six times more common among asthmatics, and accordingly it correlates more closely with the asthmatic quality of life, rather than with lung physiological functions [48,49]. In both directions, psychopathology is supposed to precipitate asthma or vice versa; psychopathology may develop as a consequence of asthma [50].

4. Asthma pathophysiology

Scientists tried to uncover alterations related to asthma since a long time ago. One of the oldest publications that discussed asthma pathophysiology was in 1873 [51]. Later on, in 1886, F.H. Bosworth concluded a possible relation between asthma and hay fever [52]. Clearly, it is well known that asthmatic patients suffer from reversible airway obstruction resulting from an allergen exposure, consequently releasing multiple broncoconstricting mediators that stimulate airway muscles to contract. Furthermore, airways narrow results from past and current mucus and edema occlusion [53]. The chronic inflammation and associated repair of lung airways leads to structural changes, referred to as "*airway remodeling*." Airway remodeling (**Figure 2**) usually involves lung epithelial layer injury and includes features such as subepithelial thickening, airway smooth muscle hyperplasia, and angiogenesis [6].

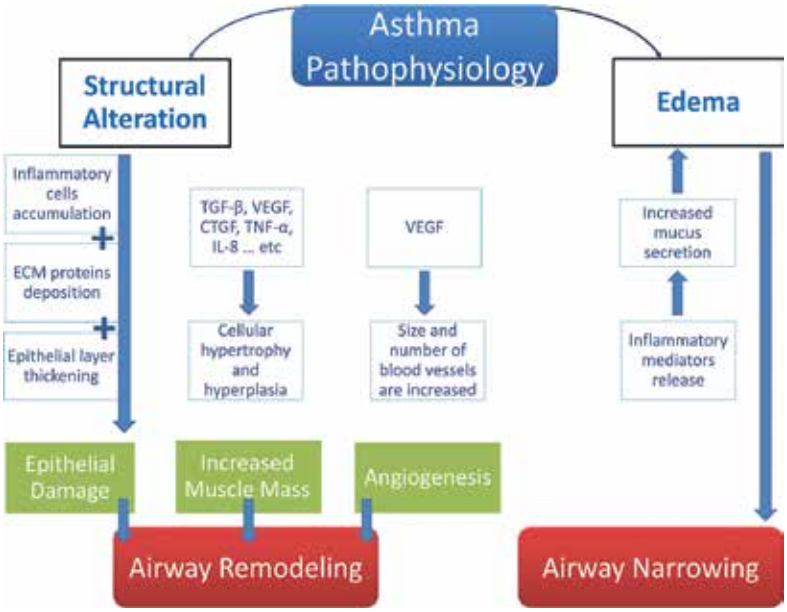

Figure 2. Schematic representation of the major events underlying asthma pathophysiology.

Asthma and COPD (chronic obstructive pulmonary disease) are now considered to be discrete respiratory disorders. Although both share several similar underlying mechanisms, driving airway obstruction in COPD, and hyperresponsiveness in asthma, core molecular pathology remains to be mostly different for both [54]. Pauwels et al. [55] defined COPD as "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of lungs to noxious particles and gases." One important reason of asthma and COPD overlap is the effect of aging. Asthma-COPD overlap syndrome (ACOS) is a medically recognized coexisting syndrome of both asthma and COPD [56]. Some other health conditions may occur more frequently in asthmatic patients. Rhinosinusitis [57], obstructive sleep apnea [58], or GERD [59] are the most common documented comorbidities. Substantially, they can contribute to the same pathophysiological process, which is already triggered by allergic response or alter asthma phenotype detrimentally. The impact of these diseases on asthma is variable and still not fully clear [60].

5. Structural alterations in asthmatic airway walls

5.1. Epithelial/subepithelial layer thickening

Epithelial changes are not unique to asthma, they are also observed, in more or less of similar manner, in lungs of smokers and cancer patients [61]. Epithelial layer damage in asthma

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includes loss of ciliated cell layer, shedding of the epithelium, goblet cell hyperplasia and growth factors, cytokine and chemokine upregulation [62].

One important feature of asthma, which has been routinely used as an asthma severity index, is the thickening of the subepithelial airways layer. The epithelial and subepithelial layer thickening is caused by the overdeposition of extracellular matrix (ECM) proteins [63]. Roche et al. observed that intensive layers of collagen sedimentation contribute to the thickened subepithelial basement membrane. Through immunohistochemistry, they have shown that the commonly involved collagen types are collagen I, III and V, and fibronectin [64]. Additionally, the cells that are responsible for ECM protein production are myofibroblasts and fibroblasts, as both are embedded in the sophisticated ECM which they secrete [65]. Meanwhile, some inflammatory cells, for example, T cells, mast cells, and eosinophils also accumulate in the submucosal layer [66]. Moreover, transforming growth factor-β (TGF-β), and some similar growth factors, is usually secreted by the lung epithelial cells echoing any ongoing lung injury, and consequently directly impress the matrix proteins' production by fibroblasts/myofibroblasts. By increasing the airway rigidity, however, Holgate et al. suggested that the airway thickening due to the ECM proteins precipitation may in fact have a remodeling protective effect via postponing long-term bronchoconstriction events [62]. Collectively, the ECM proteins, the lung structural cells (i.e., epithelial cells and fibroblasts), and the immune system inflammatory cells, all interact together and control the overall airway remodeling and fibrosis [67].

5.2. Hyperproliferation of airway smooth muscle mass

Hyperproliferation of airway smooth muscle mass is a common event in asthma and has been suggested to be implicated in its pathophysiology. Hyperplasia and hypertrophy of the ASM in the bronchial airways of asthmatics can be observed by three-dimensional (3D) morphometric studies [68]. Airways smooth muscle layer is estimated to be increased by 25–55% in nonfatal asthma and up to 50–200% in fatal asthma [69]. Meanwhile, in response to some growth factors like TGF-β, vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF), ASM cells actively participate in the remodeling process through the process of ECM synthesis [70]. ASM cells also express cellular adhesion molecules (CAMs), receptors for cytokines (e.g., tumor necrosis factor-α), Toll-like receptors, and chemokines (eotaxin, macrophage inflammatory protein 1α, and interleukin 8) presenting multiple mechanisms for the inflammatory and remodeling process [71]. Additionally, one characteristic event of the airway remodeling is the ASM cells migration toward the epithelium [72]. Since ASM cells are crucial in asthma, Zuyderduyn et al. suggested that these cells should be targeted, rather than targeting inflammation or dealing with the symptoms [73].

5.3. Angiogenesis

Accumulating evidences indicate that there is an abnormal elevation in the size and number of blood vessels, as well as microvessels vascular leakage within the bronchial tissue in remodeled airways [74]. It is assumed that VEGF strongly affects airways remodeling via its

angiogenic effects, but the exact molecular mechanism linking the increase in the VEGF expression to remodeling of the airways has not been fully understood [75].

Correlation between angiogenesis and asthma severity has also been documented. Dense vascularity occurs in severe asthmatics, followed by moderate, and then finally mild asthmatics, who experience less angiogenesis events [76]. This pattern was also observed in fatal asthmatics compared with nonfatal asthmatics [77]. While current asthma therapeutics is not directly targeting vascular remodeling, recent trials investigate some anti-angiogenic therapies as a new approach for asthma. Yuksel et al. showed that Bevucizamab, which significantly neutralizes VEGF, results in a reduced thickening of lung epithelium, a reduced ASM, and a reduced basement membrane thickness compared with untreated ovalbumin (OVA)-challenged mice [78].

6. Therapies for asthma

Modern treatments for asthma have been tested and used since the early twentieth century [79]. However, the oldest documented drug for asthma dates back to ancient Egypt. Kyphi, an incense mixture drink, was used inside the temples by the priests as a multipurpose lung medicament. There was more than one recipe for Kyphi; each may include as many as 10 herbs [80]. Following this, about 4000 years ago, Atropa Belladonna alkaloids, also called "deadly nightshade" because of their poisonous properties ("Natural Medicinal Herbs"), were derived from the leaves of thorn-apple plant and smoked by the Indians as cough suppressant [82]. Till today, natural and synthesized entities related to the tropane alkaloids class are still widely used. This includes anticholinergics (e.g., natural atropine, hyoscyamine (the levo-isomer of atropine), acopolamine, and the synthetic Ipratropium Bromide and stimulants (e.g., cocaine and hydroxytropacocaine) [83]. In 1872, one of the first papers published on asthma states that rubbing the chest of asthmatics with chloroform liniment can resolve airway constriction [84]. Adrenergic stimulants were in use for asthma over 100 years ago. In 1901, the adrenaline isolated from sheep and oxen adrenal glands was used to treat asthma [85]. The first documented publication of adrenaline as a bronchodilator therapy for asthma was written in 1903 by James Burnett, a physician in Edinburgh [86]. One year later in 1904, adrenaline was synthesized in the laboratories of Friedrich Stolz and Henry Drysdale Dakin, independently [87].

As suggested by the Global Initiative for Asthma (GINA) [88], a five-level step-down approach is widely recognized among the medical practitioners (**Figure 3**). The GINA approach assigns two types of drug classes for managing asthma:

- *Relievers* (bronchodilators) cause immediate dilatation effects on the airways obstruction, mainly by acting on lung's smooth muscle.
- *Controllers* (preventers) provide long-term control of symptoms, mainly by suppressing the underlying disease process.

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Legend:
SABA: Short acting beta agonists, ICS: Inhaled corticosteroids (arrow down = low dose, arrow up = high dose), LABA: Long acting beta agoints, LTRA: Leukotriene receptor antagonists, MX: Methylxanthines, OCS: Oral corticosteroids, OZ: Omalizumab

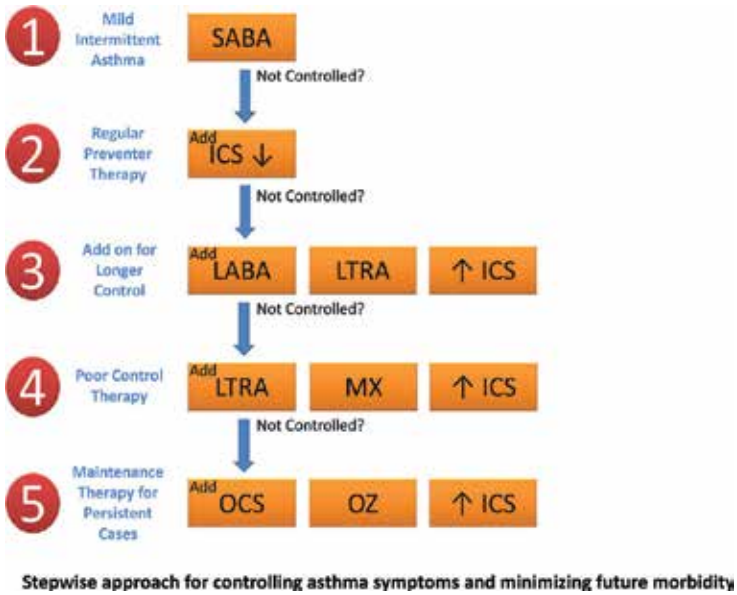

Figure 3. Stepwise approach for controlling asthma symptoms and minimizing future morbidity.

β2-agonists and anticholinergics are considered to be bronchodilator relievers. Asthma controllers include corticosteroids, anti-leukotrienes, and anti-IgE. Theophylline is casually classified as both a bronchodilator and a reliever. The following book section will briefly discuss each therapeutic class.

6.1. Corticosteroids

Nowadays, most popular protocols for managing asthma involve the use of corticosteroids and β-agonists [1]. Anti-inflammatory corticosteroids, which are one of most trusted treatments for asthma, were introduced in mid-twentieth century [79]. The principle mode of action of corticosteroids in asthma is through their direct anti-inflammatory effect in different white blood cells including T cells, mast cells, and eosinophils. Among leukocytes, corticosteroids suppress chemotaxis and adhesion, and prevent inflammatory cytokines recruitment [89]. *In vitro*, corticosteroids reduce human ASM proliferation directly [90] by stimulating p21 gene expression [91], an important regulator of cell cycle progression. Moreover, corticosteroids improve vast majority of vascular remodeling aspects in asthma, reducing angiogenesis, excess blood flow, and vascular leakage [92]. This is mainly mediated by decreasing VEGF activity within the airway wall cells [93].

Various studies describe contradicting effects of corticosteroids on the lung epithelial abnormalities in asthmatics. Dorscheid et al. [94] reported that Dexamethasone treatment resulted

in increased epithelial apoptosis and shedding. Similar results were obtained when treating guinea pigs with Budesonide, which did not improve the tracheal epithelium [95]. By contrast, some *in vivo* studies showed that inhaled corticosteroid (ICS) treatment resulted in improvement of epithelial damage in severe asthmatics [96,97].

ICS has been used around for the past couple of decades. Its idea dates back to the nineteenth century when the hand-held glass bulb nebulizer was used; however, pressurized metered-dose inhaler (pMDI) came to the clinic in 1956. After seeing his daughter's suffering while using the hand-held nebulizer, George Maison, a medical consultant at 3M Pharmaceuticals, had advocated the use of pMDI. In 1959, George Maison and Irvine Porush were awarded a patent on the first pMDI [98].

6.2 β-adrenergic agonists

Long-acting β-agonists (LABAs), for example, Formoterol [99] and Salmeterol [100], offer a longer period of bronchodilation compared to the short-acting beta agonists (SABAs), for example, Salbutamol [101] and Terbutaline [102]. LABAs persist in the airway tissues for long periods due to their lipophilic nature and they provide a good umbrella of asthma bronchodilation and control, particularly at night [99,100]. However, until recently, the medical literature lacked supporting studies reporting the positive effect of β_2 agonists on the chronic airway remodeling [103]. Addition of a β-agonist to the corticosteroid therapy allows a "steroid-sparing" effect, that is, maintains asthma control using lower doses of corticosteroids [104]. LABAs are not used as monotherapies anymore and they must be used in combination with ICS [105], because there have been cases of severe exacerbations and death when LABAs are administrated solely.

6.3. Antimuscarinic agents

Inhaled antimuscarinic agents, also known as inhaled anticholinergics, are considered another alternative bronchodilator group to β-agonists. The bronchodilation effect is functionally mediated via muscarinic receptor subtypes M1, M2, and M3, although five muscarinic receptors have been revealed in the lungs M1, M2, M3, M4, and M5 [106]. It is widely known that parasympathetic stimulation via the vagus nerve leads to immediate smooth muscle contraction and mucus secretion in the airways [107]. It is also suggested that M receptors interact with β2-adrenergic receptors (ADRB2) on the airways smooth muscle, leading to a reduced bronchodilator response of the β-agonists [108]. For years, in both adults and children, short-acting antimuscarinic agents use, for example, Ipratropium [109], has been limited to acute asthma management, in addition to inhaled SABA [110, 111]. Long-acting antimuscarinic agents, for example, Tiotropium [112], appear to have more benefits in difficult-to-control asthma. Adding Tiotropium to the standard asthma therapy significantly reduces asthma symptoms and highly increases the clinical outcomes [113, 114].

6.4. Targeted therapies

Over the last 40 years, there has been a marked increase in the development of targeted treatments for asthma—anti-leukotrienes, anti-IgE, anti-interleukins, and anti-TNF-α [115]. Obviously, as more of the biological basis of asthma is uncovered, more effective targeted asthma treatments might be developed. The list of most recently published clinical trials covering the period from 1 January 2013 to 1 January 2016, as well as the list of currently ongoing registered clinical trials that has started since 2013 for the new asthma medications are summarized in **Tables 1** and **2**, respectively.

Clinical Trial Identifier	Publication Title	Phase	Drugs	Outcome	Responsible Party	Reference
NCT01147744	Efficacy, safety, and tolerability of GSK2190915, a 5-lipoxygenase-activating protein inhibitor, in adults and adolescents with persistent asthma: a randomized dose-ranging study.	Phase 2	GSK2190915 (5-lipoxygenase-activating protein inhibitor)	GSK2190915 30-mg efficacy was demonstrated in day-time symptom scores and day-time SABA use, compared with placebo. No additional improvement on efficacy was gained by administration of greater doses than 30 mg. GSK2190915 was well tolerated.	GlaxoSmithKline	[1]
NCT00411814	A phase 1, randomized, placebo-controlled, dose-escalation study of an anti-IL-13 monoclonal antibody in healthy subjects and mild asthmatics.	Phase 1	GSK679586 (anti-IL-13)	GSK679586 showed dose-dependent pharmacological activity in the lungs of mild intermittent asthmatic patients. GSK679586 could be a potential therapeutic candidate for treatment of asthma.	GlaxoSmithKline	[2]
NCT00659659)	Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia.	Phase 1	Benralizumab (Anti-IL-5)	Single-dose I.V. and multiple-dose S.C. of benralizumab reduced eosinophil counts in airway mucosa/submucosa and sputum and decreases eosinophil counts in bone	MedImmune LLC	[3]

Clinical Trial Identifier	Publication Title	Phase	Drugs	Outcome	Responsible Party	Reference
				marrow and peripheral blood in asthmatic patients.		
NCT01007149	A proof-of-concept, randomized, controlled trial of omalizumab in patients with severe, difficult-to-control, nonatopic asthma.	Phase 3	Omalizumab (anti-IgE)	Omalizumab may have a therapeutic potential for treatment of severe nonatopic asthma.	Novartis Pharmaceuticals	[4]
NCT00971035	Dose-ranging study of lebrikizumab in asthmatic patients not receiving inhaled steroids.	Phase 2	Lebrikizumab (anti-IL-13)	Blocking IL-13 alone was insufficient to improve lung function in asthmatic patients.	Genentech, Inc.	[5]
NCT00873860	A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma.	Phase 2	Tralokinumab (anti-IL-13)	Safety profile of tralokinumab was acceptable with no serious adverse effects. Although tralokinumab treatment was associated with improved lung function, no improvement in asthma control questionnaire score was observed.	MedImm une LLC	[6]
NCT01018186	Safety and tolerability of the novel inhaled corticosteroid fluticasone furoate in combination with the β2-agonist vilanterol administered once daily for 52 weeks in patients ≥12 years old with asthma: a randomized trial.	Phase 3	Fluticasone furoate (ICS) + Vilanterol (LABA)	Fluticasone furoate/ Vilanterol (100/25 μg or 200/25 μg) administered once daily over 52 weeks was well tolerated by asthmatic patients aged ≥12 years. The overall safety profile of Fluticasone furoate/ Vilanterol did not reveal any serious adverse effects.	GlaxoSmithKline	[7]
NCT00393952	Efficacy and safety of fluticasone/ formoterol combination	Phase 3	Fluticasone propionate (ICS)	Fluticasone/formoterol combination therapy was an efficient alternative treatment option for	SkyePharma AG	[8]

Clinical Trial Identifier	Publication Title	Phase	Drugs	Outcome	Responsible Party	Reference
	therapy in patients with moderate-to-severe asthma.		Formoterol fumarate (LABA)	moderate-to-severe asthmatic patients.		
NCT01691521	Mepolizumab treatment in patients with severe eosinophilic asthma.	Phase 3	Mepolizumab (anti-IL-5)	Administration of mepolizumab (I.V. or S.C.) significantly reduced asthma exacerbations and is associated with improvements in markers of asthma control.	GlaxoSmithKline	[9]
NCT00500539	Immunogenicity and safety of omalizumab in pre-filled syringes in patients with allergic (IgE-mediated) asthma.	Phase 3	Omalizumab (anti-IgE)	Pre-filled syringe of omalizumab was not associated with immunogenicity.	Novartis Pharmaceuticals	[10]
NCT00781443	The effects of lebrikizumab in patients with mild asthma following whole lung allergen challenge.	Phase 2	Lebrikizumab (anti-IL-13)	Lebrikizumab reduced the late asthmatic response in subjects with mild asthma.	Genentech, Inc.	[11]
NCT01181895	Comparison of vilanterol, a novel long-acting beta-2 agonist, with placebo and a Salmeterol reference arm in asthma uncontrolled by inhaled corticosteroids.	Phase 3	Vilanterol (LABA)	The study failed to show a therapeutic difference between vilanterol and placebo for the primary end point. The magnitude of placebo effect may be due to increased compliance with anti-inflammatory therapy regimen during the treatment period.	GlaxoSmithKline	[12]

Clinical Trial Identifier	Publication Title	Phase	Drugs	Outcome	Responsible Party	Reference
NCT01233284	Tiotropium Respimat® in asthma: a double-blind, randomized, dose-ranging study in adult patients with moderate asthma.	Phase 2	Tiotropium (LAMA)	Administration of tiotropium Respimat® (Once-daily) add-on to medium-dose ICS improves lung function in symptomatic patients with moderate asthma, and the largest improvement was with a dose of 5 μg.	Boehringer Ingelheim	[13]
NCT00983658	OX40L blockade and allergen-induced airway responses in subjects with mild asthma.	Phase 2	huMAb OX40L (anti-OX40L)	Anti-OX40L MAb decreased serum total IgE and airway eosinophils at 16 weeks post dosing, but there was no effect on allergen-induced airway responses. This may be due to the treatment duration or dose of antibody was insufficient to have an effect on the airway responses.	Genentech, Inc.	[14]
NCT00768079	A randomized trial of benralizumab, an anti-interleukin 5 receptor α monoclonal antibody, after acute asthma.	Phase 2	Benralizumab (Anti-IL-5)	A dose of benralizumab—when added to usual care—reduced the rate and severity of asthma exacerbations experienced over 12 weeks by subjects who presented to the emergency department with acute asthma.	MedImmune LLC	[15]
NCT01369017	IL-1 receptor antagonist reduces endotoxin-induced airway inflammation in healthy volunteers.	Phase 1	Anakinra (Anti-IL-1)	Anakinra effectively reduced airway neutrophilic inflammation with no serious adverse	University of North Carolina, Chapel Hill	[16]

Clinical Trial Identifier	Publication Title	Phase	Drugs	Outcome	Responsible Party	Reference
				reactions in a model of inhaled lipopolysaccharide challenge. Anakinra is a potential therapeutic candidate for treatment of asthma.		

Abbreviations: **IL**: Interleukin; **IgE**: Immunoglobulin E; **TNF-α**: Tumor necrosis factor – α; **PDE**: Phosphodiesterase enzyme; **ICS**: Inhaled corticosteroids; **OCS**: Oral corticosteroids; **SABA**: Short-acting β-agonists; **LABA**: Long-acting β-agonists; **LAMA**; Long-acting muscarinic antagonists.

Table 1. Summary of recent published clinical trials for new drugs used in the treatment of asthma (from 1 January 2013 to 1 January 2016).

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
NCT01907763	Phase III study to assess the efficacy and safety of SOTB07 in asthma patients	Phase 3	Placebo SOTB07	Jan 2013	Assessment of the efficacy and safety of SOTB07 in asthma patients.	Interventional	Recruiting	SK Chemicals Co.,Ltd.
NCT02388997	Treatment with Omalizumab to improve the asthmatic response to an experimental infection with rhinovirus	Phase 2	Omalizumab (anti-IgE) Rhinovirus (strain 16)	Feb 2013	Determination of whether anti-IgE therapy will lead to decline in inflammatory biomarkers prior to virus inoculation, and thus reduce the severity of clinical manifestations after an experimental human RV challenge.	Interventional	Recruiting	University of Virginia
NCT01902290	Study of efficacy and safety of Brodalumab compared	Phase 2	Placebo Brodalumab (Anti-IL-17)	May 2013	Determination of the safety and efficacy of Brodalumab (AMG 827).	Interventional	Recruiting	Amgen

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
	with placebo in inadequately controlled asthma subjects with high bronchodilator reversibility							
NCT01836471	A study to assess the effect of QAW039 in nonatopic asthmatic patients	Phase 2	Placebo QAW039 ICS	May 2013	Assessment of the clinical effect of QAW039 in nonatopic asthmatics taking low-dose ICS as background therapy.	Interventional	Recruiting	Novartis Pharmaceuticals
NCT01955512	Effect of Clopidogrel on allergen challenge in asthma	Phase 2	Placebo Clopidogrel (platelets P2Y12 receptor blocker)	May 2013	Determination if the drug Clopidogrel reduces inflammation following breathing in house dust mite in people with mild asthma.	Interventional	Recruiting	University of Southampton
NCT01705964	Intramuscular epinephrine as an adjunctive treatment for severe pediatric asthma exacerbation	Phase 4	Epinephrine (IM)	Jun 2013	Determination if IM epinephrine is an effective adjunct to inhaled β_2-agonists for children with severe asthma exacerbation.	Interventional	Recruiting	University of Louisville
NCT01868061	A study of Lebrikizumab in patients with uncontrolled asthma on inhaled corticosteroids and a second controller medication	Phase 3	Placebo Lebrikizumab (anti-IL-13)	Jul 2013	Evaluation of the efficacy and safety of Lebrikizumab in patients with uncontrolled asthma despite daily administration of ICS therapy and at least 1-s controller medication.	Interventional	Recruiting	Hoffmann-La Roche
NCT01867125	A study of Lebrikizumab in patients with uncontrolled asthma who are on inhaled	Phase 3	Placebo Lebrikizumab (anti-IL-13)	Jul 2013	Evaluation of the efficacy and safety of Lebrikizumab in patients with	Interventional	Recruiting	Hoffmann-La Roche

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
	corticosteroids and a second controller medication				uncontrolled asthma despite daily treatment with ICS therapy and at least 1-s controller medication.			
NCT01841281	L-arginine in severe asthma patients grouped by exhaled nitric oxide levels	Phase 2	Placebo L-Arginine (Nitric oxide precursor)	Aug 2013	Identification of the benefit from supplemental L-arginine therapy in adult severe asthma cohort.	Interventional	Recruiting	University of California, Davis
NCT01912872	Study to assess the efficacy and safety of Omalizumab treatment on ICS reduction for severe IgE-mediated asthma (MEXIC)	Phase 4	Omalizumab (anti-IgE) Budesonide Formoterol (LABA)	Nov 2013	Assessment of the efficacy and safety of Omalizumab treatment during 12 months to reduce the use of ICS in pediatric and adult patients with severe IgE-mediated asthma inadequately controlled with high doses of corticosteroids.	Interventional	Recruiting	Novartis Pharmaceuticals
NCT02041221	Pharmacology study of Sun Pharma Advanced Research Company Limited's S0597	Phase 1 Phase 2	Placebo S0597	Jan 2014	Evaluation of safety, tolerability, pharmacokinetics, and pharmacodynamics of S0597 by oral inhalation.	Interventional	Not yet recruiting	Sun Pharma Advanced Research Company Limited
NCT02049294	Study of the prednisone-sparing effect of Xolair (Omalizumab) in patients with Prednisone-dependent asthma with	Phase 2 Phase 3	Omalizumab (anti-IgE) Placebo Normal Saline	Mar 2014	Investigation whether addition of Omalizumab enables a reduction in the dose of prednisone in patients with asthma	Interventional	Recruiting	McMaster University

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
	eosinophilic bronchitis				and eosinophilic bronchitis.			
NCT01987492	A study of Lebrikizumab In patients with severe asthma who depend on oral corticosteroids	Phase 2	Placebo Lebrikizumab (anti-IL-13)	Mar 2014	Evaluation of the efficacy of Lebrikizumab compared with placebo as measured by the ability of patients to achieve lower daily doses of OCS in patients with severe corticosteroid-dependent asthma.	Interventional	Recruiting	Hoffmann-La Roche
NCT02075008	Long-term safety study of QGE031 in patients with allergic asthma who completed study CQGE031 B2201	Phase 2	QGE031	Mar 2014	Assessment of long-term safety of QGE031 during 12 months of treatment in asthma patients who completed study CQGE031B2201.	Interventional	Recruiting	Novartis Pharmaceuticals
NCT02075255	Efficacy and safety study of Benralizumab to reduce OCS use in patients with uncontrolled asthma on high-dose inhaled corticosteroid plus LABA and chronic OCS therapy	Phase 3	Placebo Benralizumab (anti-IL-5)	Apr 2014	This trial is to confirm if Benralizumab can reduce OCS dependence (after dose optimization) in patients who are uncontrolled on high-dose ICS-LABA, and chronically dependent on OCS as part of their regular asthma controller regimen.	Interventional	Recruiting	AstraZeneca
NCT02135692	A Phase 3a, repeat dose, open-label, long-term safety study of Mepolizumab in asthmatic subjects	Phase 3	Mepolizumab (anti-IL-5) Standard of Care	May 2014	Collection of clinical data for long-term use and further assessment of efficacy in patients	Interventional	Recruiting	GlaxoSmithKline

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
					with loss of asthma control.			
NCT02126865	Multiple rising oral doses of BI 1060469 in healthy subjects and mild asthma patients	Phase 1	Placebo BI 1060469	May 2014	Investigation of the safety and tolerability of repeated rising doses of BI 1060469 in healthy male and female subjects and in asthmatic male and female patients.	Interventional	Recruiting	Boehringer Ingelheim
NCT02161757	A Phase 3 study to evaluate the efficacy and safety of Tralokinumab in adults and adolescents with uncontrolled asthma (STRATOS1)	Phase 3	Placebo Tralokinumab (Anti-IL-13)	Jun 2014	Evaluation of the efficacy and safety of Tralokinumab in adults and adolescents with asthma inadequately controlled on ICS plus long-acting β_2-agonist.	Interventional	Recruiting	AstraZeneca
NCT02104674	A study evaluating the efficacy and safety of Lebrikizumab in adult patients with mild to moderate asthma	Phase 3	Placebo Lebrikizumab (anti-IL-13) Montelukast	Jun 2014	Assessment of the efficacy and safety of Lebrikizumab in adult patients with mild to moderate asthma treated with SABA therapy alone.	Interventional	Recruiting	Hoffmann-La Roche
NCT02066298	Steroids In eosinophil-negative asthma (SIENA)	Phase 3	Placebo Mometasone Tiotropium (LAMA)	Jul 2014	Determination if patients who are persistently non-eosinophilic differ in their benefit from inhaled corticosteroid treatment compared to patients who are not persistently non-eosinophilic.	Interventional	Recruiting	Milton S. Hershey Medical Center
NCT020	A study	Phase 2	Placebo	Nov 2014	Evaluation	Interve	Recr	Hoffmann-

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
99656	evaluating the effects of Lebrikizumab on airway eosinophilic inflammation in patients with uncontrolled asthma		Lebrikizumab (anti-IL-13)		of the effects of Lebrikizumab on airway eosinophilic inflammation in patients with uncontrolled asthma on inhaled corticosteroids and a second controller medication.	ntional	uiting	La Roche
NCT02258542	A safety extension study to evaluate the safety and tolerability of Benralizumab (MEDI-563) in asthmatic adults and adolescents on inhaled corticosteroid plus LABA (BORA)	Phase 3	Benralizumab (anti-IL-5)	Nov 2014	Characterization of safety profile of Benralizumab administration in asthma patients who have completed one of the three predecessor studies: D3250C00017, D3250C00018, or D3250C00020.	Interventional	Recruiting	AstraZeneca
NCT02296411	Efficacy of LAMA added to ICS in treatment of asthma (ELITRA)	Phase 2	Placebo CHF 5259 Glycopyrrolate bromide (LAMA)	Nov 2014	Evaluation of the safety and superiority of the glycopyrrolate bromide (CHF 5259 pMDI) versus placebo on top of QVAR® pMDI, in terms of lung functions parameters.	Interventional	Recruiting	Chiesi Farmaceutici S.p.A.
NCT02293265	Cross-sectional study for identification and description of severe asthma patients	Phase 3	Mepolizumab (anti-IL-5) Omalizumab (anti-IgE) Reslizumab (anti-IL-5)	Dec 2014	The potential overlap of patients eligible for treatment with Mepolizumab, Omalizumab and/or Reslizumab will be estimated.	Interventional	Recruiting	GlaxoSmithKline
NCT02281318	Efficacy and safety	Phase 3	Placebo Mepolizumab	Dec 2014	Evaluation of the	Interventional	Recruiting	GlaxoSmithKline

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
	study of Mepolizumab adjunctive therapy in participants with severe eosinophilic asthma on markers of asthma control		(anti-IL-5) Standard of Care		efficacy and safety of Mepolizumab adjunctive therapy in participants with severe eosinophilic asthma on markers of asthma control.			
NCT02322775	Study to evaluate the efficacy and safety of Benralizumab in adult patients with mild to moderate persistent asthma	Phase 3	Placebo Benralizumab (anti-IL-5)	Feb 2015	Confirmation of the safety and clinical benefit of Benralizumab administration in asthma patients with mild to moderate persistent asthma.	Interventional	Recruiting	AstraZeneca
NCT02382510	Multiple ascending dose study of TRN-157 in stable mild and moderate asthmatics	Phase 2	Placebo TRN-157 Tiotropium (LAMA)	Feb 2015	Determination of the safety and bronchodilator activity of TRN-157 in approximately 54 mild and moderate asthmatics.	Interventional	Recruiting	Theron Pharmaceuticals, Inc.
NCT02315131	Study in healthy volunteers and COPD patients to evaluate the efficacy and safety of inhaled TV46017	Phase 1	Placebo TV46017	Mar 2015	Assessment of the safety profile and duration of bronchodilation of a single dose of inhaled TV46017.	Interventional	Not yet recruiting	Teva Branded Pharmaceutical Products, R&D Inc.
NCT02124226	Low-dose Methotrexate for reduction GINA 5 medications in chronic severe asthma	Phase 3	Placebo Methotrexate	Apr 2015	Investigation of the role of an add-on immunological modifier in patients with chronic severe asthma.	Interventional	Not yet recruiting	Universita degli Studi di Catania
NCT02377427	Pharmaco kinetics and pharmaco dynamics of Mepolizumab administered subcutaneously in children	Phase 2	Mepolizumab (anti-IL-5)	Apr 2015	Assessment of the pharmacokinetics and pharmacodynamics of Mepolizumab in children aged 6–11 years with severe	Interventional	Not yet recruiting	GlaxoSmithKline

Clinical Trial Identifier	Title	Phase	Drugs	Start Date	Purpose	Study Type	Recruitment Status	Responsible Party
					eosinophilic asthma.			
NCT02336425	Efficacy and safety of QGE031 compared with placebo in patients aged 18–75 years with asthma	Phase 2	Placebo QGE031	Apr 2015	The study will assess the safety and efficacy of different dose levels of QGE031 in asthma patients.	Interventional	Not yet recruiting	Novartis Pharmaceuticals
NCT02427165	Comparison of RPL554 With placebo and Salbutamol in asthmatic patients	Phase 2	Placebo RPL554 (PDE-3/4 inhibitor) Salbutamol	Apr 2015	Assessment of the effects of RPL554 compared with Salbutamol and placebo in patients with chronic asthma.	Interventional	Not yet recruiting	Verona Pharma plc
NCT02422121	Effect of RNS60 on the late-phase asthmatic response to allergen challenge	Phase 2	Placebo RNS60 Budesonide	May 2015	Evaluation of the effects of multiple doses of inhaled RNS60 and Budesonide on the late-phase asthmatic response to allergen challenge in patients with mild asthma.	Interventional	Not yet recruiting	Revalesio Corporation
NCT02571660	Efficacy of vitamin D on the clinical management of pediatric patients with asthma	Phase 3	Vitamin D (Low- and high-supplementation doses)	Oct 2015	Evaluation of vitamin D supplementation on exacerbation and clinical control of asthma.	Interventional	Not yet recruiting	Hospital General Naval de Alta Especialidad - Escuela Medico Naval

Abbreviations denote: **IL**: Interleukin; **IgE**: Immunoglobulin E; **PDE**: Phosphodiesterase enzyme; **ICS**: Inhaled corticosteroids; **OCS**: Oral corticosteroids; **SABA**: Short-acting β-agonists; **LABA**: Long-acting β-agonists; **LAMA**; Long-acting muscarinic antagonists.

Table 2. Summary of recent ongoing clinical trials for new drugs used in the treatment of asthma (started in the past 3 years).

6.4.1. Anti-leukotrienes

Leukotrienes are lipid eicosanoids with a wide range of biological activities. They are derived from arachidonic acid through the enzymatic action of 5-lipooxygenase, and play a crucial role

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in asthma inflammatory pathogenesis, and in other allergic diseases such as allergic rhinitis, rhinosinusitis, atopic dermatitis, and urticaria [116]. Leukotrienes class includes three main types: cysteinyl leukotrienes (CysLTs), LTB4, and LTG4. LTG4 is the metabolite of LTE4 in which the cysteinyl moiety has been oxidized to an α-keto-acid [117]. Since, very little is known about the LTG4-putative leukotriene, most clinical research studies focus on CysLTs and LTB4. CysLTs are strong bronchoconstrictors that powerfully affect airway remodeling, whereas LTB4 is a strong chemoattractant for most leukocyte subsets [118]. Over the last 20 years, since leukotriene antagonists were introduced to the clinic for asthma management, Montelukast [119, 120] and Zafirlukast [121] are the most frequently used drugs in this class.

6.4.2. Anti-IgE

At the moment, Omalizumab, which is the only approved targeted monoclonal antibody against IgE, is used to treat allergic asthma in clinical practice. It can significantly decrease serum IgE levels (up to 99%) within 2 h following subcutaneous administration, and diminish serum, sputum, and tissue eosinophilia [122]. Recently, Omalizumab has also been reported to have steroid-sparing effect, reducing the rate of asthma exacerbations up to 50%, and hence improving the quality of life [123]. However, nearly 45% of patients treated with Omalizumab had adverse reaction at the local injection site, which is considered the most commonly observed adverse event for Omalizumab. Some other minor upper respiratory tract infections and sinusitis have also been reported as well. Patients treated with Omalizumab display a very low (0.09%) frequency of anaphylaxis reaction. Importantly, there are no data reporting any correlation between cancer and Omalizumab treatment [124].

6.4.3. Anti-ILs

Three interleukin pathways are of physiological importance for asthma: IL-5, IL-9, and IL-4/IL-13 pathways. IL-5 is pivotal for both eosinophil differentiation and maturation in the bone marrow. Subsequently, it controls eosinophil mobilization, activation, and survival [125]. Hence, antagonizing IL-5 has been proposed to be beneficial for asthma therapy, particularly for predominantly eosinophilic asthma. A number of anti-IL-5 and anti-IL-5 receptor monoclonal antibodies are in the process of development for allergic diseases: Reslizumab [126], Mepolizumab [127], and Benralizumab [128]. IL-9 is one of the T-helper 2 (Th2) pro-inflammatory cytokines that promote mast cell proliferation and T-cell growth [129]. In mouse models, IL-9 causes several common features of chronic asthma: excessive mucus production, eosinophilic airway inflammation, smooth-muscle cell hyperplasia, and aryl hydrocarbon receptor (AHR) [130]. Currently, a phase IIb clinical trial evaluates the efficacy and safety of subcutaneous Medi-528, a humanized IgG1 anti-IL-9 mAb, in adults with uncontrolled asthma (NCT00968669). Activated mast cells, eosinophils, basophils, and dendritic cells secrete IL-4 and IL-13. IL-4 and IL-13 both play an important role in asthma mainly by enhancing IgE production. They also control mast cells' growth and development, eosinophil recruitment, and AHR [131]. The first trial aimed at antagonizing the IL-4 used a soluble recombinant human IL-4 receptor antagonist (IL-4RA), altrakincept, which blocked the binding of IL-4 to

its cellular receptors [132]. Several humanized IL-13-neutralizing antibodies have entered asthma phase I/II clinical trials—anrukinzumab [133], QAX576 [134], and CAT354 [135].

6.4.4. Anti-TNF-α

TNF-α, a cytokine produced by Th1 cells and macrophages, has diverse biological functions. TNF-α shows crucial, and previously extensively documented, role in Crohn's disease, rheumatoid arthritis, and psoriasis pathogenesis. The association between TNF-α increase and these disease progressions had inspired studies aiming to extend anti-TNF-α therapies also for the treatment of severe asthma and COPD [136]. Infliximab and Golimumab, two anti-TNF-α mAbs, and Etanercept, a decoy soluble TNF-α receptor, are both able to biologically neutralize TNF-α cytokine, and blunt the immune response, thereby abolishing TNF-α effects in asthma [137].

7. Pharmacogenetics of asthma

The US Food and Drug Administration definition for pharmacogenomics is "the study of variations in DNA and RNA characteristics as related to drug response" [138]. "*Pharmacogenomics*" differs from "*Pharmacogenetics*" in that the former is concerned with the whole genome, its components, and regulators, while the latter is focused only on the DNA sequences of individual gene. Thus, in sense, pharmacogenetics is thought to be a subset of pharmacogenomics [139].

Because it is a complex trait, the drug response to asthma is diversely heterogeneous even among patients with apparently similar clinical profiles [7]. It is estimated that up to 50% difference in therapeutic response has been attributed to genetic variations between individuals [140]. Although several possible mechanisms have been postulated, genetic variants affect the pharmacogenetic response to drugs in two different ways:

1. *Pharmacodynamic genetic variations* are variations in which the receptor binding the drug ligand or another member of the drug target pathway is altered resulting in different drug effect. Most of the current pharmacogenetic research fall into this mechanistic category. Populations are stratified into responders and nonresponders, and then analyzed for DNA polymorphisms, which distinguish these two groups apart.
2. *Pharmacokinetic genetic variations* are related to altered uptake, distribution, and/or metabolism of the administered drug. Fewer examples fall in this category; however, the most common research subfield here is the area of investigating drug-catabolizing or -excreting enzymes. An important example here is the cytochrome *P450* (CYP450) family, a widely recognized metabolizing enzyme with several variable pharmacogenetic patterns.

Single nucleotide polymorphism, SNP, denoted by a reference sequence (rs) number, represents a class of polymorphism that is derived from a one-base point mutation in which a single nucleotide is substituted with another one. SNPs may be located in the gene regulatory

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or coding regions, and so it may affect the gene expression in more than one way; however, in majority of cases, most discovered SNPs do not change the gene function in a significant manner [141]. Consequently, it is essential to investigate whether the DNA sequence variances would actually cause significant functional impacts (i.e., resulting in an altered observed biology), or is a linkage disequilibrium marker of another DNA variant, which is the real cause of the response variability, or is generally nonsignificant. Because of its strong importance, since 15 years, catalogs of SNPs have started to outline the most common genetic polymorphisms among different population groups [141, 142], and this process has attracted more attention during the last couple of years [143].

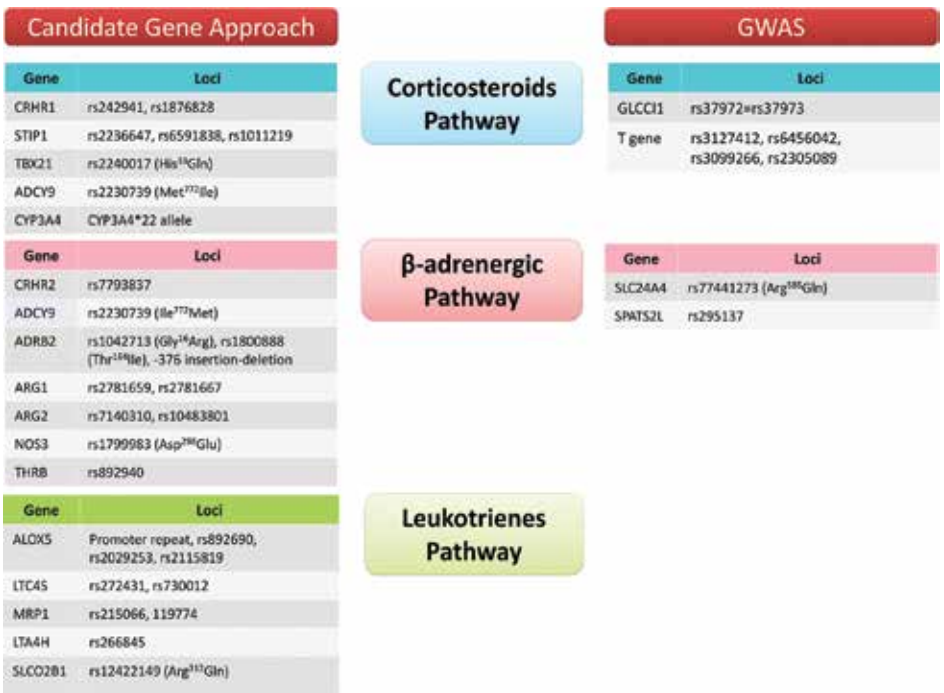

Figure 4. Pharmacogenetically significant genes with relevance to corticosteroids, β-adrenergic, and leukotriene biological pathways. Left side: Candidate gene approach studies, Right side: GWAS (Genome Wide association studies).

All genes contain huge number of SNPs and copy-number variations (CNVs). CNVs are another form of structural variations, which account for 13% of the human genome bulkiness, and manifest as kilo-to-mega bases of deletions or duplications [144]. Conjointly, it is challenging to outline which polymorphism is influencing the treatment response and which are not relevant. Two major approaches declaiming this challenge have been practiced so far: candidate gene approach and GWAS. As it combines transcriptomic, proteomic and metabolomic profiling traits, a third approach, the integrative system biology approach, had led to a more comprehensive pharmacogenetic view [3]. To differentiate, candidate gene approach is based on a prior evidence according to the knowledge of the drug pharmacody-

namics or/and pharmacokinetics, by contrast, GWAS methodology identifies new associations with the null hypothesis being that no associations exist. GWAS picks the variations which are associated with observable phenotypes by scanning SNP markers that tag, via linkage disequilibrium, the complete human genome. GWAS and integrative system biology approaches are modern tools contributing to the recent advancements of genotyping and statistical technologies.

Current pharmacogenetic studies of the corticosteroids, β-adrenergic, and leukotriene pathways are mostly candidate gene studies, with some GWAS, however, altogether have identified several genetic loci in strong association with therapeutic responsiveness to asthma. **Figure 4** summarizes the pharmacogenetically significant genes with relevance to the corticosteroids, β-adrenergic, and leukotriene biological pathways.

7.1. Corticosteroid pathway pharmacogenetics

In cytosol, the glucocorticoids bind to their corresponding glucocorticoid receptor, forming a hetero-complex that is activated by ligand binding, and translocate into the nucleus. In the nucleus, this complex binds to the glucocorticoid response elements in some target genes' promoter region resulting in their expression regulation. The core role of glucocorticoids is mediated via activating the transcription of anti-inflammatory genes, and suppressing the transcription of pro-inflammatory genes [145, 146]. The glucocorticoid pharmacogenetic studies formerly focused on candidate gene approach. Those candidate genes covered functions related to the corticosteroid biosynthetic pathway, the hetero-complex receptor formation, and the related chaperone proteins.

Corticotrophin-releasing hormone *(CRHR1)*, stress-inducible protein 1 *(STIP1), TBX21, CYP3A4, GLCCI1*, T gene, and *FBXL7* are the most up-to-date potential pharmacogenetic biomarker targets for predicting patients' response to ICS [147]. Studies of the corticotrophin-releasing hormone gene are considered to be as one of the oldest and remarkable footsteps in asthma pharmacogenetics. CRHR1 protein, also known as CRF1, is the primary receptor controlling the adrenocorticotropic hormone release; hence, it plays a pleiotropic and vital role in steroid actions. A candidate gene study of *CRHR1* in 1117 asthmatics administrating ICS therapy, from three clinical cohorts, revealed two SNPs (rs242941 and rs1876828) associated with different response in lung functions [148]. Tantisira et al. [148] found that *CRHR1* gene variation was frequently related to augmented therapy response in each of the three studied cohorts. Since 2004, *CRHR1* gene studies opened the doors for all other corticosteroid pharmacogenetics and the possible future therapeutic outcomes.

STIP1 or *HOP* (abbreviated for Hsp70-Hsp90-Organizing Protein) gene mainly functions to reversibly link Hsp70 and Hsp90 together as a co-chaperone [149]. *STIP1* pharmacogenetic studies in one adult cohort revealed three SNPs (rs2236647, rs6591838, and rs1011219) within this heat shock-organizing protein and related to improved lung response during ICS therapy [150]. *STIP1* rs2236647 variant analysis in healthy and asthmatic children showed that this SNP could serve as an asthma marker for choosing the population who receives corticosteroid therapy[151]; however, further replication studies should be held to confirm those results.

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One significant aspect of pharmacogenomics is that it investigates the interactions with genes of other pathways. *TBX21* gene is one good example for observing the ICS response outside the glucocorticoid pathway. *TBX21* is one of the conserved genes of a family sharing a common DNA-binding domain; the T-box encodes T-box transcription factor TBx21 protein. Tbx21 protein is a Th1 (T-helper1) transcription factor, which regulates one of the Th1 cytokine expression, interferon-gamma (IFNG). In 2004, a nonsynonymous SNP rs2240017 ($His^{33}Glu$) in the *TBX21* gene was linked to improvements in bronchial hyperresponsiveness or "broncho-protection" in response to ICS in individuals participating in the Child Asthma Management Program (CAMP) cohort [152]. This finding was also observed in an independent Korean cohort in 2009 [153]. Thus, *TBx21* may be an important determinant pharmacogenetic candidate gene for predicting asthmatics' response to inhaled corticosteroid therapies.

In 2005, another example demonstrated the glucocorticoid pathway interactions with one other pathway. *ADCY9*, adenylyl cyclase type 9, gene encodes a membrane-bound enzyme in the β2-adrenergic receptor pathway, which catalyzes the production of cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). This candidate gene contains a pharmacogenetic nonsynonymous SNP, $Met^{772}Ile$, which was correlated to enhanced Salbutamol (SABA) bronchodilator effects only in patients treated with ICS [154]. An independent Korean cohort replicated the trial, using Formoterol (LABA) treatment in combination with ICS, and confirmed those results [155].

Cytochromes P450s belong to a heme cofactor-containing superfamily of metabolizing enzyme proteins that potentially control the metabolism of drug (i.e., pharmacokinetics), and consequently treatment response in many diseases. For asthma, *CYP3A4*, *CYP3A5*, and *CYP3A7* candidate genes have been studied among a retrospective analysis of 413 asthmatic children treated with the ICS Fluticasone propionate [156]. The three candidate CYPs of all subjects were genotyped for nine SNPs. Results showed that asthmatics with the *CYP3A4*22* allele demonstrated a significant symptom control compared with those lacking that allele. This study included a small number of participants (n = 20), so further large-scale replication is required.

Tantisira et al. [157] conducted the first pharmacogenetic GWAS for ICS treatment in asthma and identified an SNP (rs37972) in the promoter of the glucocorticoid-induced transcript-1 gene (*GLCCI1*), which significantly associates with lung functions. Replicated in four independent populations (935 persons in total), this candidate SNP was linked to substantial decrements in the response to the ICS in asthmatics. The wild-type allele homozygotes (CC) showed greater forced expiratory volume in 1 s (FEV1) in response to the ICS compared with those identified with the homozygote variant allele (TT). Another functionally correlated SNP (rs37973) in the promoter of the same gene was further validated within *in vitro* studies [157]. Results showed declined luciferase reporter activity in cells with the minor allele. *GLCCI1* GWAS outlines that drug response to asthma treatment is subjected to wide inter-individual variation, and GWAS would uncover more novel pharmacogenetic associations in the future. Tantisira et al. conducted a second GWAS among 418 asthmatics randomized to ICS treatment from the Childhood Asthma Research and Education (CARE), Asthma Clinical Research Network (ACRN), and CAMP trial cohorts. The *T-gene* (encoding the Brachyury transcription

factor protein) compromised two SNPs (rs3127412 and rs6456042) that were associated, out of the successfully genotyped 47 SNPs, with altered lung function response to ICS [158].

7.2. β-adrenergic receptor pathway pharmacogenetics

β2-adrenergic receptor gene remains to be the most studied pharmacogenetic loci among the beta-agonist pathways. *ADRB2* gene has several polymorphic variants that were discovered in multi-ethnic genetic asthma cohorts [159, 160]. ADRB2 protein is a cell membrane-spanning receptor that binds epinephrine, but not norepinephrine, unlike the other adrenergic receptors, and consequently mediates both smooth muscle relaxation and bronchodilation [161, 162]. Early ADRB2 studies showed that $Gly^{16}Arg$, a prevalent coding variant of the amino acid at position 16 of ADRB2, is associated with altered bronchodilator response to SABAs [163].

The BARGE (Beta-Agonist Response by Genotype) study [164], held by the National Heart, Lung and Blood Institute Asthma Clinical Network, was one of the first genotype "stratified" pharmacogenetic studies for asthma. In this study, only $Gly^{16}Arg$ homozygotes for ADRB2 were included (i.e., Arg/Arg and Gly/Gly). Participants were randomly receiving either intermittent or regular albuterol, and then crossed over to receive the alternative treatment dose. For statistical stratification, this study ensured that the Arg^{16} homozygotes, who are less frequent, were appropriately randomly distributed to both SABA intermittent and regular protocols. Compared to Gly^{16} homozygotes, the BARGE study showed that the Arg^{16} homozygotes were good responders only to acute intermittent SABAs rather than to long-term regular treatments, a finding that does not coincide with the current clinical asthma treatment guidelines [165] which recommend SABA as for on-demand intermittent usage. Since the 16th amino acid of ADRB2 controls regular response to albuterol, bronchodilator medications other than SABAs would be more appropriate for Arg/Arg asthmatics.

Collectively, the BARGE study [164], along with some other pharmacogenetic studies [163, 166–168] of $Gly^{16}Arg$ and SABAs' exposure, provided insights for further studies [169–171] on LABAs. In contrast to SABAs, a large cohort [169] of 2250 asthmatics, randomly assigned to formoterol plus budesonide, demonstrated no pharmacogenetic action due to *ADRB2* variation on therapeutic response. Furthermore, a multicenter trial [170] showed that asthmatics with both Arg/Arg and Gly/Gly genetic signatures had improved airway functions, if they received combination treatment with Salmeterol and ICS, when compared with ICS therapy alone. Similarly, the results of another prospective trial cohort [171] of 544 subjects, also randomized by genotyping, demonstrated no evidence of any pharmacogenetic action due to *ADRB2* variation in response to Salmeterol. Together, these findings, confirmed among several asthma populations, suggest that in contrast to SABAs, asthmatics can still be treated with LABAs plus ICS irrespective of their genotyping status.

Genetic variants' occurrences among different ethnic groups are quantified by their percentage of allele frequencies. Usually, frequent and common variants have only little or modest impacts on disease susceptibility and, subsequently, therapeutic response. On the other hand, as the variant is characterized to be rare or more "private," its effect size on disease progression and therapeutic response dramatically increases [172]. Early *in vitro* studies had investigated a rare polymorphism of *ADRB2* within the fourth transmembrane domain, the $Thr^{164}Ile$ variant. For

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the Ile^{164} genotype, results showed significant lowering in Gs-protein signaling and different SABA- and LABA-binding affinities [173, 174]. While the Thr^{164}Ile polymorphism is pointed out to be a rare coding variant (i.e., <5%), population studies showed that this variant is more common in non-Hispanic white populations [159, 160], a finding that requires further pharmacogenetic investigation in different and larger populations. To replicate results, a study of two large Copenhagen population cohorts [175], with more than 55,000 participants, was held to investigate the relation of Thr^{164}Ile variation and lung responses. Among the general population, the Copenhagen study reported that the Thr^{164} genotype was associated with decreased FEV1, diminished lung function, and increased the overall COPD risk.

In addition to ADRB2 Gly^{16}Arg and Thr^{164}Ile variants, the (-376 In-Del) polymorphism was extensively studied as another significant pharmacogenetic *ADRB2* variant. Presented primarily among African Americans and Puerto Ricans [159, 160], the 24-bp promoter insertion at −376, related to the start codon, is associated with asthma-related hospitalization in asthmatics treated with LABA [160]. Altogether, these variants, being unique to different populations, highlight the increasing need of personalized-based treatments.

Adenylyl cyclase type 9, encoded in humans by *ADCY9* gene, is a membrane-bound enzyme that catalyzes the formation of cyclic AMP from ATP. ADCY9 is a widely abundant adenylyl cyclase, and it is stimulated via beta-adrenergic receptor activation [176]. Ile^{772}Met is a coding variant of *ADCY9* gene that has been associated with both acute FEV1 bronchodilation in response to SABAs [154] and long-term FEV1 response for LABAs [155]. CRHR2 (which is more commonly known as CRF2) is a type-2 G protein-coupled protein receptor for the corticotropin-releasing hormone [177]. Out of the 28 studied SNPs in *CRHR2*, five SNPs were significantly correlated with acute bronchodilator response in one, or frequently more than one, cohort. Among those, variant rs7793837 was associated with altered SABA response in all three cohorts of the *CRHR2* study containing 607, 427, and 152 participants, respectively [178].

Different variants of *ARG1* (Arginase 1) and *ARG2* (Arginase 2) show altered acute response to SABAs, while the endothelial nitric oxide synthase (*NOS3*) shows altered acute response to LABAs. NO (nitric oxide), an endogenous vasorelaxing bronchodilator, is generated by the action of NOS3 on L-arginine. Since ARG1 and ARG2 are metabolizing L-arginine, so it is expected that the entire three genes, *ARG1*, *ARG2*, and *NOS3*, might be implicated in asthma pharmacogenetics. Combined association evidence, surviving Bonferroni correction for multiple testing from the CAMP four asthma cohorts [179], points to SNP rs2781659 in *ARG1*. C-allele homozygotes for SNP rs2781667 in arginase 1 showed significantly less response to the inhaled corticosteroid treatments [180]. Arginase-2 variants rs17249437 and rs3742879 correlated with increased airway obstruction and airway hyperresponsiveness, and lower reversibility of airway constriction following treatment with beta-2 agonists [180]. A small candidate gene study [181] of *NOS3* had revealed one possible variant (Asp^{298}Glu) correlated with lung function response to ICS/LABA combined treatment; however, this result still needs to be replicated in larger cohorts. *THRB* [182], *SLC24A4* [183], *SLC22A15* [183], *SPATS2L* [184], and SNPs (rs892940, rs77441273, rs1281748/rs1281743, and rs295137, respectively) show promising loci for further pharmacogenetic investigations.

7.3. Leukotriene pathway pharmacogenetics

Relative to the corticosteroid and β-adrenergic pathways, the cysteinyl leukotriene pathway pharmacogenetic studies are generally fewer and have smaller sample sizes. The oldest of these studies [185], held in 1999, had investigated the tandem repeat polymorphism in *ALOX5* promoter. Among 114 asthmatics, it has been shown that the *ALOX5* promoter repeat is associated with altered lung functions in response to a 5-LO inhibitor [185]. It has been shown in children that those who had more or less than five repeats (3, 4, and 6) of the *ALOX5* promoter-binding motif experienced increased urinary leukotriene E4 (the terminal cysteinyl leukotriene metabolite) concentrations and reduced FEV1 baseline than the wild-type genotype with five repeats [186]. Further pharmacogenetic studies revealed that the *ALOX5* promoter polymorphism, along with the *ALOX5* SNPs rs892690, rs2029253, and rs2115819, influences leukotriene pathway antagonist therapy [187–190]. Moreover, variants of *LTC4S*, encoding Leukotriene C4 synthase, and *MRP1* (or *ABCC1*), encoding multidrug resistance-associated protein 1, have been linked to lung function response while treatment with Zileuton and Montelukast [189, 190].

$Arg^{312}Gln$, rs12422149, which is a coding variant in *SLCO2B1* (solute carrier organic anion transporter family member 2B1 gene), has been related to symptom control during Montelukast therapy. This fact was due to the interindividual variability of carrier-mediated Montelukast transport in the intestines, and consequently its plasma levels [191]. By contrast, two other studies, probably due to their small sample sizes, were unable to replicate similar *SLCO2B1* pharmacokinetic effects [192, 193]. Overall, larger replicate cohorts, for the leukotriene pathway identified loci, are still needed.

8. Current and future challenges facing asthma pharmacogenetics

As demonstrated above, there has been fundamental progress in the field of asthma pharmacogenetics; however, these efforts have not yet been introduced into clinical practice to guide physician. There are several reasons that account for this gap. Most important is the limited number of asthma pharmacogenetics-focused GWAS, which would compare common candidate gene methodology that would allow combining all patients from small cohorts studied. Small sample sizes prevent any expansion of the pharmacogenetic research of asthma, which needs a large number of subjects for statistical significance. Along with limited cohort size, study defects due to poor ancestry structuring and stratification substantially result in replication inconsistencies. Furthermore, genes interact together in networks; therefore, simply attributing phenotypic variation to individual genes is not appropriate. Epigenetics studies investigate the changes in gene activities, which are heritable to the subsequent generations, but are independent of any DNA sequence alterations [194, 195]. Epigenetic tuning of the genes associated with asthma has a significant impact on determining the drug response. Several mechanisms, related to epigenetics, are currently being investigated for both biomarker tagging and therapeutic innovation intervention [196]. Moreover, epigenetic changes have the ability to override the genetic effects of time, environment, tissue specificity,

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and other conditions such as age and gender of a patient, nutrition and hygiene, and intestinal microflora, which all highly influence the drug response in addition to the genetic factors. The collective impact of all combination of these factors requires the application of complicated algorithm that could take into consideration each of these factors and their interplay. The prospective genetic profile of an asthmatic should compromise a set of common and rare variants, on ancestral basis, which will be predictive of the pattern of his/her therapeutic responsiveness to different treatment options. The current human variant catalog continues to grow in an exponential manner because of the lower costs associated with whole genomic sequencing. Despite the steep decline in sequencing costs, the technology of sequencing, in terms of speed and quality, enormously increases. The future pharmacogenetic profile would also predict any possible adverse response associated with the chosen line of treatment. Genetic biomarkers are needed to warn the physician about any potential adverse side effects which can be life threatening. It is very important for typical genetic profiling to also consider gene-gene and gene-environment interactions. Gene-gene interactions are predominately crucial in the framework of combination therapies, for example, ICS and β-adrenergic agonists. Interactions between the surrounding environment and the patients' genes are assumed to be an additional element, because environmental stress, apart from the genetic makeup, contributes to the development of asthma exacerbations. Future pharmacogenetic directions need to cover also the pharmacokinetic side of the patient profile. Altered drug absorption, metabolism, distribution, or excretion extensively influence drug dosing and even drug selection. All in all, the complete asthma pharmacogenetic catalog has many aspects to cover, before being introduced into the clinical practice.

9. Conclusion

Asthma is a complex respiratory and immune disease. Inadequate (or exaggerated) ability of genetically predisposed individuals to control inflammation, induced by innate and environmental factors, results in asthma. Further, studies using allergic asthma and atopy models enable to better understand several interacting gene products and variable responsiveness of asthmatic subjects to current therapies. Eventually, thorough investigation of the complexity of asthma might lead to successful designing of personalized therapies for patients suffering from allergic asthma.

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Chapter 8

Managing Bronchial Asthma in Underprivileged Communities

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Additional information is available at the end of the chapter

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Abstract

The definition of a primary care facility is the site where the first patient contact occurs. In developing countries, primary healthcare (PHC) facilities are officially healthcare centers, hosting World Health Organization (WHO) programs for tuberculosis and chronic respiratory diseases. In addition, there are many other providers of PHC services, which include emergency departments, general outpatient clinics in public hospitals. Asthma patients may present for treatment in any of these primary care facilities. An international study achieved by the Union showed that 51% of asthma patients in Syria are treated in emergency departments only, many developing countries like Sudan, Algeria, and some African countries had the same use of ER. There are questions about the quality of care provided in these clinics with regard to their adherence to Global Initiatives for Asthma (GINA) guidelines. Evidence suggests that there may be over prescribing of oral corticosteroids and antibiotics and under-prescription of inhaled corticosteroids, so there is a need to improve practice bringing it more into alignment to international guidelines. It may be considered by many that it is not possible to follow guidelines in developing countries and those that have economic and political pressures. However, a pilot program to test the feasibility of a providing systematic follow-up of uncontrolled asthma patients in a general free of charge hospital in Syria showed that it is possible to achieve asthma control following to GINA guidelines even in very deprived community. The same was mentioned by the Union in an international survey for other developing African and Mediterranean countries. WHO launched programs for non-communicable disease including chronic respiratory disease: Practical Approach to lung Health (PAL) and Package of essential no communicable (PEN) disease interventions for primary health care in low-resource settings. The IPCRG also worked on how to improve implementation of guidelines. We will provide the results and following evidence-based recommendations from our field surveys in developing countries, as well comment on international programs. Although much progress has been realized in the diagnosis and management of asthma in developed nations, progress in one variant of asthma, inner city asthma, has been slow. Inner city asthma is that variant of the disease which afflicts residents of urban environments with low socioeconomic conditions, poor housing, and rampant

environmental risks. This variant of asthma appears to be more severe, associated with increased psychological burden as well as morbidity and mortality, has a diverse array of predisposing factors, and poses significant challenges in management and treatment. One important aspect of treatment is education which leads to the participation of the patient and the families in the care resulting in a more favorable outcome.

Keywords: asthma, primary care, WHO, chronic respiratory diseases, essential drugs for asthma, inner city, developed nations

Background

This chapter deals with asthma in developing nations and touches on one variant of asthma in the developed world which has significant resemblance to its counterpart in developing nations. The first section is derived from studies in Syria, while the second from the United States.

1. Introduction - Managing bronchial asthma in primary health care

According to the International Study for Asthma and Allergies in Childhood (ISAAC), comparing phase one in 1994 to three in 2000–2003, asthma prevalence expressed by wheezing the last 12 months in 13–14 years old is increasing in developing countries. Asthma prevalence is higher in developed countries, but asthma is more severe in developing countries [1].

The definition of a primary care facility is the site where the first patient contact occurs [2]. If we take Syria as an example of developing county, primary healthcare (PHC) facilities are officially healthcare centers under the control of the Ministry of Health, which among other services provided, host World Health Organization (WHO) programs for tuberculosis and chronic respiratory diseases [3, 4]. In addition, there are many other providers of PHC services which include emergency departments(ED), general outpatient clinics in public hospitals, school health clinics, clinics in workplace settings, and internal and general clinics within the private sector.

Asthma patients may present for treatment in any of these primary care facilities in Syria. A multicenter survey of primary care clinics revealed that 13% of patients aged over 6 years attended with asthma [5]. Another study showed that 51% of asthma patients are treated in ED only, and only 9% are treated in primary care centers [6]. Many developing countries like Sudan, Algeria and some African countries had the same use of ED [6]. The same under-utilization of community primary healthcare services has also been observed in China in 2014 [7].

There are questions about the quality of care provided in these clinics with regard to their adherence to Global Initiatives for Asthma (GINA) guidelines. Evidence suggests that there may be over prescribing of oral corticosteroids and antibiotics and under-prescription of

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inhaled corticosteroids [5, 6], so there is a need to improve practice bringing it more into alignment to international guidelines.

It may be considered by many that it is not possible to follow guidelines in developing countries and those that have economic and political pressures. However, a pilot program to test the feasibility of a providing systematic follow-up of uncontrolled asthma patients in a general free of charge hospital in Syria showed that it is possible to achieve asthma control following to GINA guidelines even in very poor community [8]. The same was mentioned by the Union in an international survey for other developing African and Mediterranean countries [9], the same in a recent study in Sudan [10].

A Ministry of Health—WHO program for non-communicable diseases in Syria including an intensive courses for asthma and COPD for GPs in health centers has been launched since the beginning of 2015 in a pilot site from Syria. However, unlike developed countries, in Syria, presentations are very personalized, there are no established accredited modules or curriculum for continuing medical education for primary care physicians and nurses. In order to make optimum improvements in care across the country, we need to ensure that high-quality training interventions are made available for healthcare providers who are working in a primary care setting, aiming to increase both their competence and confidence in the essentials of asthma management. This program needs to incorporate accredited educational materials, a process for monitoring and continuous evaluation, and collaborative efforts with an international agency such as the Global Alliance against Chronic Respiratory Diseases (WHO–GARD www.who.int/gard) [8]. A survey conducted by the International COPD Coalition gave the same conclusions about absence of curriculum for education for asthma and COPD [11].

Primary healthcare services are free of charge in Syria, and other developing countries hosting WHO programs, and are staffed by full-time qualified nurses who are supported by part-time physicians, who also provide services in the private sector. In addition, some patients who are able to pay for healthcare may refer themselves directly to private pulmonologists, without being referred from primary care. Since 2006, WHO launched programs for asthma and COPD at primary care level in Syria and other developing countries, and training was undertaken on site [3–5, 8].

In this paper, we share our experience in developing countries and will present first field surveys in developing countries. Second, we will comment on the international programs of WHO and International Primary Care Respiratory Group (IPCRG). And third, we will give our evidence-based recommendations.

2. Discussion

2.1. Field surveys and what we learned

Asthma is under-diagnosed in primary care [5, 6, 8, 12–14]. The Global Alliance against chronic respiratory disease (GARD–WHO: www.who.int/gard) survey on chronic respiratory diseases prevalence and risk factors in Syria revealed that although 27% of the 1599 patients

surveyed had evidence of reactive airway disease and reversible obstruction by spirometry, but only 13% had been diagnosed as asthma by the primary care practitioners. Indicating that 50% were under-diagnosed [4]. This finding is not unique to Syria. Under-diagnosis has also been reported in the same GARD survey in Cape Verde [12]. There are several hypotheses for these high rates of under-diagnosis. The condition's variability of symptoms, misdiagnosis such as an infection, some may be mislabeling of patients as COPD when in fact, they may have uncontrolled asthma [5, 15], increasing workload, and demand on services and limited experienced doctors. It is important for practitioners to follow standard diagnostic procedures and good history taking such as recurrent symptoms (wheezing, cough, difficult breathing, and chest tightness) and the presence of triggers. In addition, practitioners should obtain objective tests and look for reversibility of peak expiratory flow rate (PEFR) expressed as increase of 20% and 60 l/min, or 12% increase in forced expiratory volume in one second (FEV1) and 200 ml after short-acting beta agonists (SABA), or a decrease in these measurements after exercise testing. Practitioners could also rely on variability of PEFR or FEV1 between two visits. In situations where spirometry or peak flow meter results are not available, or patients are under 5 years of age, practitioners should rely on clinical history and treatment trials [16]. WHO recommend peak flow meter to be available in the most remote healthcare centers, and referral for spirometry in healthcare centers or hospitals at central level is available [3, 4].

Asthma is under-treated and under-controlled: The GARD–WHO multicentre national survey 2010 for chronic respiratory diseases [5] revealed that only 25% of inhaled corticosteroid (ICS) prescriptions included adequate doses according to the GINA guidelines. In addition, 46% of patients received oral corticosteroids which could be avoided if ICS were prescribed in PHC according to the guidelines. Similarly, 56% of asthma patients received oral antibiotics without a clinical indication. Another important issue is that 56% of asthma patients surveyed have FEV1 < 80% after bronchodilators, which points to poor control [5, 16].

Despite asthma being poorly diagnosed and treated, it is possible to observe improvements in asthma control to the level of published guidelines in underserved areas. In a pilot study conducted on economically deprived patients in an underserved area, we systematically followed up patients with uncontrolled asthma in a general free of charge outpatient clinic in a public hospital 2006–2007 [8]. A trained postgraduate medical student asked every patient questions about the parameters of asthma control, measured PEFR, and ensured prescription of ICS at their first presentation. The student also taught patients proper inhaler technique and educated them about how to avoid risk factors. Weekly follow-up data were collected by the GP. After 3 months, 44 of 66 patients who had not been followed up previously were properly controlled. We conclude that GINA guidelines could be realistic even in underserved areas. In 2006, an international multicenter survey of the Union showed that implementation of asthma guidelines was possible in primary care in developing countries [13]. The same recently in Sudan (2014): In a new model specialized center for asthma, a survey aiming at describing the epidemiological and clinical characteristics of asthma patients concluded that most patients had abnormal spirometry with more than half having an FEV1 that is 60% or less of their predicted normal reading. The majority improved with combined treatment (Formoterol,

http://dx.doi.org/10.5772/62990

budesonide) with 60% normalizing their spirometry highlighting the feasibility and applicability of specialized asthma care centers in resource-poor countries [10].

The global initiative for asthma guidelines are based on the level of control [16] such that for each asthmatic presenting to a primary healthcare facility, the general practitioner (GP) should ask standard questions about the clinical control of asthma including:

- Frequency of symptoms
- Need for inhaled bronchodilators
- Frequency of night awakening
- Exercise limitation
- Number of exacerbations.
- values of PEFR or FEV1

To improve care, training should emphasize that: Patients with uncontrolled asthma need to be prescribed low-dose or medium-dose inhaled corticosteroids, educated on inhaler technique, and referred to higher level of care for further assessment if not controlled after follow-up visits [3–5, 16], and referred back to primary care for long-term follow-up and education.

2.1.1. Review of international WHO programs and other programs

WHO–MOH programs: Three programs have been introduced for chronic respiratory diseases (CRD) in Syria since 2006 [3, 4, 11, 16]:

1. Practical Approach to Lung Health (PAL) program, integrating asthma, and COPD care in the National Tuberculosis program, adopting for the first time a referral policy and initiating a respiratory disease dispensary at central level equipped with peak flow, oximetry, and spirometry.
2. The Package for the essential needs for non-communicable diseases (NCD) at primary care level in low resources settings: WHO–PEN program. Integrating all major NCD including CRD at primary care level.
3. GARD program www.who.int/gard Survey on CRD risk factors and prevalence. And the resulting evidence based guidelines, and training. WHO country office is involved.

2.1.2. Other programs

1. The GINA world Asthma day program: Conferences in World Asthma Days are improving asthma care: Make health workers in PHC familiar with asthma symptoms, asthma control, peak flow meter, and corticoid inhalers.
2. Civil societies, in collaborating with the International COPD Coalition www.internationalcopd.org, are helping patients with CRD and allergy is wide-spreading patient education for asthma and COPD, and health education.

3. Educational Program for nurses about asthma and COPD: Two national workshops were run for this purpose in 2004 and 2007 by the Education for health center of Excellence—UK in Syria www.educationforhealth.org, but also in Bangladesh, and many other developing countries, aiming to introduce the role of nurses in national programs.

4. The new intensive course of WHO–MOH mentioned above for non-communicable diseases in Syria and other EMRO countries.

2.1.3. International Primary Care Respiratory Group (IPCRG)

The IPCRG tried to resolve the question on how to deal with asthma and COPD in primary care in developing countries, elaborating a symptomatic approach and algorithms, but they recommend providing primary care in developing countries with PEF and Inhalers [17]. While PAL–WHO went further (Spirometry and referral) [4].

Research priority to improve asthma management in primary care have been investigated by the IPCRG, 2009: Conclusion, primary care research should include awareness about local asthma triggers like biomass fuel, early diagnosis, and management in remote areas where there are no tools for diagnosis, the reliability of medication trial, how to overcome taboos about cultural misbelieves and inhalers, how to make essential drugs available, and how to adapt and evaluate guidelines implementation [18].

PEN–WHO opted for an integrated approach with other NCD; the approach was symptoms and PEAK flow measurements; primary care physician prescribes ICS if asthma; and treat acute attacks with oral corticosteroids and inhaled bronchodilators; referral rules to confirm diagnosis or help for better long-term treatment are stated if failure of control at follow-up visit. Necessary tools are PEF, oximetry, oxygen, and nebulisers or inhalers via spacer [3]

PAL–WHO, integrating CRD with tuberculosis program was very ambitious, referral and spirometry were recommended [4], but unfortunately, there were discontinuity and no evaluation process for the implementation of these programs, in conflict zones.

GARD–WHO was a success with the survey, and following publications and guidelines [5]

2.2. The core messages from the field surveys and international national programs

To empower the role of PHC in controlling asthma and lessen related mortality. Core messages are as follows:

1. The first consultation with the uncontrolled patient is crucial. It is vital that the correct diagnosis is made and good education is delivered. The correct treatment should be initiated at this time which will be an appropriate dose of inhaled corticosteroid and a short-acting reliever inhaler (Bronchodilator). Inhaler technique needs to be taught and the initiation of a self-management plan including what to do in an emergency and whom to contact. A follow-up appointment is important, and the PHC should consider a referral if not controlled during a follow-up visit [19]

http://dx.doi.org/10.5772/62990

2. Prescription of oral corticosteroids on discharge from emergency room, if not hospitalized, is recommended. By contrast, expectorants and antibiotics are often unnecessarily prescribed [5, 13, 19].

3. Methodical follow-up leads to control and is feasible even in poor settings by using standard guideline-based protocols and follow-up records. [8, 13]

4. The core equipment and medicines that health facilities should have include peak flow meter, pulse oximetry, bronchodilator inhalers, spacers (which could be built up in very poor settings/plastic bottle), [20–22], nebulizers and solution of short acting beta agonists (SABA) and if possible anticholinergics, oxygen extractor or oxygen bottle, and systemic corticosteroids.

5. Short-acting beta-agonists (via spacer, which can be built in poor settings using a plastic bottle) is as effective as nebulizer to relieve symptoms, except for very severe attacks [21–23]. The asthma-trained nurses or community healthcare workers key role is to educate patients, particularly in inhaler technique and self-management [23]. This is common practice in many developed countries and the UK and Australia have been leaders in developing nurses to fulfill this role. In the UK, much of the asthma management in primary care is provided by appropriately trained nurses [23].

6. Every uncontrolled asthma patient in spite prescription of ICS and regular follow-up in Primary care should be referred to a specialist or well-trained physician with an interest in asthma and placed in an asthma management plan and then referred back to the PHC for follow-up.

7. A toolbox for general practitioners and nurses should consist of the following:

   - Questions related to symptoms suggestive of asthma diagnosis to face under-diagnosis
   - Asthma control test for initial evaluation and follow up
   - Use of peak flow and table of values
   - Educational photos about inhaler and spacer use (Photos of all available inhalers to educate.)
   - What to do in an emergency
   - Education on how inhaled corticosteroids reverse inflammation
   - A patient self-management plan

8. Every follow-up visit should be the occasion to improve the partnership with patients according to their literacy level of education and cultural believes. A follow-up record to monitor clinical control, PEFR, compliance, and trigger avoidance should be filled at every visit. Education for inhaler technique should be part of every follow-up visit.

9. Including these core elements on the role of primary care in curriculum of medical schools, nursing schools, and pharmacies is recommended.

10. WHO programs of NCD, PEN, and PAL have been introduces in PHC dispensaries in many developing countries including Syria, and training for those programs was done at pilot sites, but the humanitarian crisis in Syria and other conflict zones discontinued progress. Effort should be done to continue [3, 4].

11. Conferences and World Asthma Days as recommended by GINA are improving asthma care: Make health workers in PHC familiar with asthma control, peak flow meter, and corticoid inhalers, but there is a great need for more education, which can also be provided online.

12. Patient organizations are playing a role on patient education and providing free medications [11].

13. According to the GARD Survey results, training sessions for the essential on asthma and COPD are needed for health workers at primary care level. This needs to adapt educational materials.

14. The list of essential medications for asthma, especially inhaled corticosteroids [24], is not available in all countries; in Sudan, a survey pointed this issue [25]. In a general review, researchers reported [26]: Another issue is in some developing countries, the essential medication as listed by WHO is not available. Health services in low-resource countries are poorly adapted to treating chronic diseases. Designed to respond episodically to acute disease, almost all historical investment has focused on infectious diseases. Crucial to the successful management of chronic diseases is an infrastructure designed to support proactive management, providing not only an accurate diagnosis, but also a secure supply of cost-effective drugs at an affordable price. When in very poor health systems, ICS are not available, a variety or a phenotype of severe asthma prevails (defined by WHO as the non-treated severe asthma) [27], while the ATS/ERS definition for severe asthma is asthma refractory to ICS.

15. The WHO issued a guide on prevention and control of non-communicable diseases in: Guidelines for primary healthcare in low-resource settings: 2012, and urged developing countries to follow the directives for chronic respiratory disease as well [28].

3. Inner city asthma - Introduction

Inner city asthma is a variant of asthma that afflicts patients who reside in some of the poorest neighborhoods of some urban localities [29]. These patients frequently have economic and financial difficulties and reside in housing projects that are environmentally poor with increased likelihood of pollution [30]. Several studies have demonstrated that these factors coupled to barriers to appropriate asthma care, as well as reliance on emergency care, poor medication compliance, limited availability of primary and specialty asthma care and poor communication between patient and physicians are responsible for the unique nature of this entity [31–33]. Because of these factors, the character of inner city asthma may be different from

http://dx.doi.org/10.5772/62990

that observed in other localities. Different patterns of prevalence, predisposing factor, severity, morbidity, mortality, and management have been observed.

4. Prevalence

Over the past several decades, a gradual increase in the prevalence of asthma has been noted in several industrialized countries [29]. Although some of this increase may be related to changes in health insurance policies resulting in better coverage and increased access to care and improvements in diagnostic testing, other factors may be involved. Some of the increase is attributed, in part, to a gradual increase in prevalence of asthma in individuals of lower socioeconomic status who reside in inner cities [29, 34]. For example, statistical analysis shows that between the years 2001 and 2010, rates of asthma in adolescents in the United States of America have increased at a rate of around 1.4% reaching 9.5% in 2010 [34]. Careful analysis of this phenomenon indicates that this increase was most realized among various minority groups including African-Americans and Hispanics.

African-American children are reported to have a rate of asthma per population that is 1.6 times the level observed in white children [34, 35]. Some Hispanic groups, like children originally from Puerto Ricco, have been reported to have asthma prevalence that is almost 2.4 times that of white children [35, 36]. The prevalence of asthma among children in some Chicago neighborhoods is estimated to be as high as 44%, with the highest rate observed and reported in neighborhoods with a higher proportion of residents of African-American and Hispanic ancestry [37]. In one district of New York City, asthma prevalence was reported 13.2% for Puerto Ricans [38]. Racial background is not the only factor responsible for this disparity. Asthma prevalence varies among various localities with those localities with low income levels manifesting an increase in prevalence irrespective or racial and ethnic mix [29, 33].

5. Severity

Numerous studies have shown that inner city asthma, especially among children, is characterized by increased intensity and poor response to therapy [39]. It is not clear why this population of asthmatics is more difficult to control. Some investigators speculate that a myriad of factors may be involved including environmental, socioeconomic, psychosocial, behavioral, or genetic ones [39, 40]. Other authorities believe that the poor control may be related to inappropriate asthma management practices, limited access to care, poor compliance with therapy, and limited communication between physicians and patients [41, 42]. The practical implications of these observations are that these children have a higher rate of hospital admissions [43] and their condition at the time admission is serious and is frequently described as near fatal [44] which refers to a group of individuals predisposed to acute respiratory failure from their disease state with acidosis and altered mental status.

The increased acuity of inner city asthma has several short-term and long-term implications. The collective cost resulting from loss of work and productivity as well as absenteeism from

school and work is hard to measure. The added cost of overutilization of healthcare facilities and emergency department adds to the financial implications of this phenomenon. Overconsumption of pharmaceutical agents and other supportive agents and procedures further increases the overall cost. Finally, the impact on the general health of the individuals, stunted growth, and development of long-term respiratory impairment adds to the overall societal impact.

6. Predisposing factors

The factors contributing to the high prevalence of asthma among inner city residents are varied. Cohen et al. [45] suggest that a key factor in this regard is the poor access to healthcare that patients in urban environments experience. This may be related to a limited number of physicians and healthcare facilities as well as limited availability of safe transportation. Limited access to care has a negative impact on most clinical conditions including the availability of effective prenatal care. Another major predisposing factor which increases the prevalence of asthma in residents of inner cities is exposure to tobacco smoke [45]. Tobacco smoke is known to affect the rate of lung growth, clearance of secretions, and defense mechanisms against particulate matter and infectious agents.

Studies have shown that children living in urban environments have a higher rate of emergency room visits and lower use of inhaled corticosteroids [46]. This may be related to lower rates of diagnosis as shown by the 1999 National Health Interview Study [34]. Specific factors that have been examined include poverty with reduced access to and quality of care [47]. The resultant additional health issues such as prematurity [48, 49] and obesity [29, 50] further confound the problem. In addition, poor housing [51] with exposure to indoor pollutants and environmental tobacco [52] plays a significant role in aggravating the condition. Finally, the psychological impact of the disease in the setting of poor resources worsens the perception among patients, impacts coping, and results in further deterioration in symptomatology [53, 54].

7. Morbidity and mortality

In general, inner city asthma has a higher index of severity, is associated with increased morbidity, and has a higher mortality rate than asthma outside the inner city milieu [29]. Several criteria may be used to evaluate the level of acuity. These include, among others, emergency room visits, hospitalizations, office visits, time lost from work, and absenteeism from school. Research shows that inner city asthma is associated with increased morbidity in each of these criteria. The national database report of 2006 indicated that around 3.5 million visits to physician offices, half a million visits to hospital outpatient departments, an equivalent number of visits to an emergency department, and over 150,000 hospitalizations were related to asthma in this population [55].

http://dx.doi.org/10.5772/62990

Inner city asthma is associated with increased mortality when adjusted for the level of acuity. As far as mortality is concerned, there were 167 deaths from asthma in 2005 among children and adolescents [29, 55]. Interestingly, African-Americans demonstrate a sevenfold increase in mortality, around threefold increase in emergency department visits, threefold increase in hospitalization, but 20% lower nonemergency ambulatory visits than white children. Data on Hispanic children show emergency department visits to be twice that of whites [55].

8. Management

Management of inner city asthma places additional demands on patients and healthcare providers alike. Patients need to be maintained on the usual asthma medications including short-acting and long-acting bronchodilators, inhaled corticosteroids, leukotriene antagonists, and possibly methyl xanthines. In addition, one has to focus on eliminating or minimizing the effect of the predisposing factors listed in the previous paragraphs. Specifically, dealing with poor housing, indoor pollutants, and environmental tobacco makes it necessary for patients and their families to invest in home improvement projects that are costly and demanding. Since this may be beyond the capabilities of several patients, this poses a significant burden on the public health and social safety networks in various cities. These services are already oversubscribed and have limited resources.

Several interventions have focused on the fact that patient and family education are critical to the process of managing and controlling inner city asthma. Patients and their families need to learn the components of quality care so that they can participate in their own care. An important component of this strategy is making sure patients, and their families have the requisite knowledge to reach healthcare providers in a timely and structured manner. Therefore, efforts should focus on educating patients to improve their ability to acquire the knowledge needed to navigate the healthcare system. A family-based intervention performed by a trained counselor has been shown to improve care and decrease morbidity [56]. School-based asthma education is also effective [57]. Emphasis should also be placed on reducing environmental triggers such as the use of pest control [58] services and reduction of exposure to tobacco smoke [59] and weatherizing homes to decrease mold and moisture [60, 61] Good management of inner city asthma requires the same kind of proactive care that has been shown to be effective in other situations. These include guidelines-driven care and assured access to the appropriate controller medication [62] and the addition of a biologic such as omalizumab in selected cases [63].

9. Summary and conclusions

To the pulmonologist, inner city asthma is a complex and challenging entity that requires consolidated management efforts and a comprehensive approach that includes awareness of the unique predisposing factors, the increased acuity, and the need to focus on improved access

to care. A multifaceted approach that targets a wide array of risk factors including allergens, indoor pollution, housing quality, and external sources of pollution, such as neighborhood trash collection receptacles must be utilized. Guidelines-based management that is effective in other types of asthma may not be sufficient to provide adequate control. In addition, the structure of the healthcare system must change to allow the needed access and the recognition and management of social factors which impact on asthma management.

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Chapter 9

Asthma-COPD Overlap Syndrome (ACOS): Current Understanding and Future Perspectives

Irina Bobolea and Luis Alejandro Pérez de Llano

Additional information is available at the end of the chapter

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Abstract

This chapter resumes our current understanding of asthma–chronic obstructive pulmonary disease (COPD) overlap syndrome (ACOS), pretending to offer a comprehensive approach for the practicing physician, and provides some future perspectives on this entity.

Although different studies recognize the presence of ACOS, the detection, diagnosis, and treatment of these patients in clinical practice are not always simple and are subject to different interpretations. These patients are of special interest, because they are usually excluded from clinical trials with new medications, and also represent a clinically very important and quite prevalent population, with particular characteristics: more respiratory symptoms, frequent exacerbations, and worse health-related quality of life. They are also characterized by an increase in comorbidity and a greater consumption of health care resources compared to patients with only asthma or COPD alone.

There are currently no universally accepted, validated criteria for the diagnosis of ACOS. The differences between clinical guidelines are discussed here (GINA 2014, GEMA 2015, and GOLD 2014). However, to obtain clear and validated criteria, we think that further research about the underlying mechanisms is needed.

Several potential pathways that might lead to the adult presentation of ACOS are revised. The therapeutic recommendations of the Spanish consensus guideline for patients with overlap phenotype COPD–asthma are provided, and other possible future therapies are discussed in this chapter.

Keywords: ACOS, ACOS criteria, ACOS treatment, asthma, COPD

1. Introduction

Although different studies recognize the presence of asthma–chronic obstructive pulmonary disease (COPD) overlap syndrome (ACOS), the detection, diagnosis, and treatment of these patients in clinical practice are not always simple and are subject to different interpretations and controversies. These patients are of special interest, because they are usually excluded from clinical trials with new medications for asthma and also represent a clinically very important and quite prevalent population, apparently with particular characteristics: more respiratory symptoms, frequent exacerbations, and worse health-related quality of life [1–5]. They are also characterized by an increase in comorbidity and a greater consumption of health care resources compared to patients with only asthma or COPD alone. There are currently no universally accepted, validated criteria for the diagnosis of ACOS. Also, clinical trials are necessary to verify the response to treatments of this group of patients.

2. Definition of ACOS

ACOS is the coexistence of two distinct diseases in the same individual: asthma and COPD. Whether this concept is clinically relevant or not depends on its capacity to describe an entity with differentiated pathogenic mechanisms, prognostic particularities, and potentially specific treatment options. The recently updated Spanish COPD guidelines [6] acknowledge the existence of a syndrome that overlaps characteristics of COPD and asthma, and it proposes a differential treatment.

When considering how to define this entity, existing definitions of asthma and COPD should be taken into account. The new definition of COPD according to GOLD 2014 includes subtle changes regarding the previous definitions, integrating the findings of recent evidence [7]. For instance, there is no longer mention of reversibility, and it emphasizes the role of exacerbations and comorbidities. Thus, COPD is a common, preventable and treatable disorder, defined by persistent airflow obstruction that is mostly progressive, characterized by a chronic inflammatory response in the airways and lungs to noxious particles and gases; exacerbations and comorbidities generally contribute to the severity of the disease in individual patients.

On the other hand, in 2014, GINA [8] defined asthma as "a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation". The similarities between asthma and COPD definitions are obvious, but none of their features are pathognomonic, and all of them might be present in individual patients.

For operative purposes, in 2014, GINA and GOLD published a joint document on ACOS [9]. ACOS was defined as the presence of persistent airflow limitation with several features usually associated with asthma and others usually associated with COPD. The document presents the characteristics of asthma and COPD listed separately and suggests that ACOS may be the

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diagnosis when a similar number of characteristics of both asthma and COPD are identified in a given patient. The joint task force also recommends a stepwise approach to the diagnosis. It uses clinical, spirometric and radiographic findings to help delineate if an adult patient is most likely suffering from asthma or COPD or fulfills enough shared features to be considered within ACOS. This definition and the diagnostic criteria differ from other guidelines, for instance, the Spanish expert report from 2012 [10].

The different diagnostic criteria proposed so far are discussed in Section 5. However, to obtain any clearer and validated criteria, further research about underlying mechanisms is needed.

3. Prevalence of ACOS

The exact prevalence of ACOS is unknown. In general, the literature on ACOS has been mostly retrospective and observational, and the studies focused on asthma or COPD populations. It is well known that studies on asthma are usually performed in populations of children or young adults, where the prevalence of COPD is negligible, whereas studies on COPD are usually performed in elderly populations, where the prevalence of asthma is low. The COPDGene study found a prevalence of 13% of ACOS [2]. These patients may have a different clinical natural history, with more frequent and severe exacerbations (odds ratio [OR] 3.55), and different treatment response, which led to recommend early introduction of inhaled corticosteroids (ICS) in these patients. These figures are similar to those reported in the PLATINO study [11]: 12% prevalence for the ACOS phenotype and more risk of exacerbations in these patients (OR 3.01). The inconsistencies and discrepancies that exist upon reported data on prevalence can be in part explained by the absence of a consistent definition and diagnostic standards. In comparison to previous studies that have considered selected groups of patients, such as COPD patients, the study published by de Marco et al [12] assessed the prevalence of ACOS in the general population. They found that this prevalence ranged from a minimum of 1.6% (95% confidence interval (CI): 1.3%–2.0%) in the 20–44 years old age group to 4.5% (95% CI: 3.2%–5.9) in the 60–84 years old age group.

4. Pathogenic mechanisms

Several potential pathways might lead to the presentation of ACOS in adults. One such pathway begins in early-onset asthma. Smoking habit later in life might lead to development of fixed airflow limitation and COPD in many of these patients. A second potential pathway recognizes patients with a lifetime smoking history, subsequent COPD, and late-onset features of asthma (adult-onset eosinophilic asthma and aspirin-exacerbated respiratory disease) (see also Figure 1).

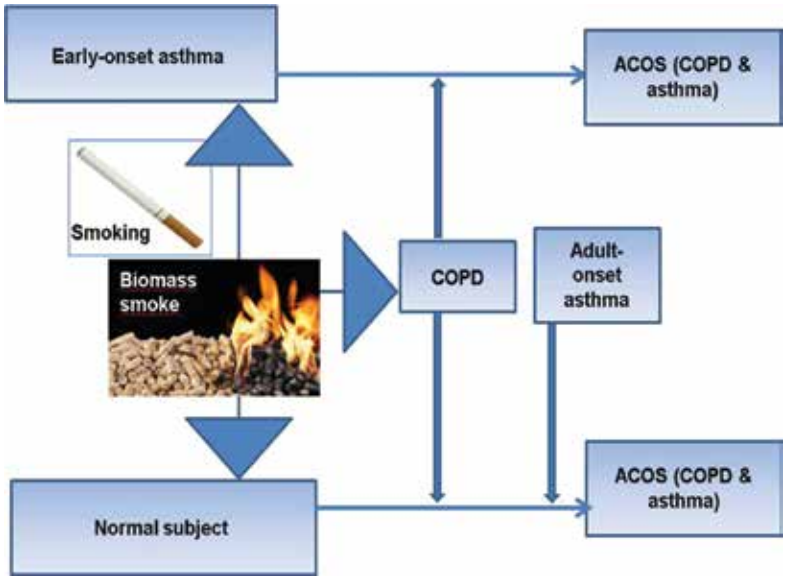

Figure 1. Pathogenic pathways leading to ACOS development.

Although no previous studies have addressed the underlying mechanisms of inflammation in ACOS, there is convincing evidence that eosinophils play a pivotal role, similar to what it is found in asthma with a Th2-high profile. Different studies have demonstrated that the presence of significant eosinophilia in an induced sputum sample predicts a good response to ICS, both in patients with COPD and ACOS [13–15]. On the other hand, the presence of more number of neutrophils in sputum has been recently associated with a worse prognosis in asthmatics [16]. Since both asthma and COPD are inflammatory diseases that affect the bronchial tree, it is to be expected to find, in patients with ACOS, some evidence of the Th-1 pattern (characteristic of COPD) and some evidence of Th-2 pattern (characteristic of asthma). The current search for reliable biomarkers of Th1 and Th2 inflammation hopefully will provide additional information in the upcoming years.

Previous studies defined two new asthma molecular phenotypes, namely Th2 high and Th2 low [17]. The Th2–high gene signature includes chloride channel accessory protein 1 (CLCA1), SERPINB2, and periostin (encoded by POSTN), a secreted 90-kDa extracellular matrix protein that is induced by interleukin (IL)-4 and IL-13 in airway epithelial cells and lung fibroblasts. All three genes are induced in bronchial epithelial cells by recombinant IL-13 treatment in vitro, and the expression of these genes correlates with IL-13 and IL-5 expression in the bronchial mucosa, airway and peripheral eosinophilia, airway remodeling, and clinical responsiveness to ICS treatment, but not with atopy. Even more so, periostin seems to become an emerging noninvasive biomarker associated with eosinophilic inflammation, Th2-high molecular phenotype, and airway remodeling, and has potential utility in patient selection for emerging asthma therapeutics targeting Th2 inflammation. A study by Jia et al. [18] identified serum periostin as a systemic biomarker of airway eosinophilia in severe, uncontrolled

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asthmatics belonging to the BOBCAT cohort (Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma). In a logistic regression model, serum periostin was the single best predictor of sputum and tissue eosinophilia, showing superiority to blood eosinophils, IgE, and FeNO. Mean periostin levels were significantly higher in "eosinophil-high" when compared with "eosinophil-low" patients, as defined by sputum or tissue eosinophil measurements. Using 25 ng/mL serum periostin as an arbitrary cutoff, eosinophil-low and eosinophil-high patients from the BOBCAT study were effectively differentiated, with a positive predictive value of 93% [18]. Moreover, in 62 patients diagnosed with severe asthma, Bobolea et al. [19] found that periostin levels were higher in patients with fixed airflow limitation than in patients with variable airflow limitation (69.76 vs 43.84 ng/ml, $p < 0.05$) and in patients with eosinophilic phenotype than in patients with mixed granulocytic phenotype (61.58 vs 37.31 ng/ml, $P < 0.05$). However, in a cohort of patients with a broad spectrum of asthma severities, Wagener et al. [20] found that blood eosinophils had the highest accuracy in the identification of sputum eosinophilia. In this study, serum periostin was not able to distinguish eosinophilic from noneosinophilic airway inflammation. Therefore, in view of the differing positions, the exact role of periostin in the diagnosis of Th2 bronchial inflammation remains to be determined.

5. Diagnosis of ACOS

The specific criteria for diagnosis of this special syndrome had never been established until 2012, when an expert panel meeting of Spanish key opinion leaders agreed unanimously to confirm the existence of this patient profile and to establish a set of criteria for its diagnosis [10], which has been posteriorly adapted within the Spanish COPD Guideline, although not yet validated. The initial definition of ACOS proposed by the Spanish consensus group is as follows: the diagnosis of ACOS is made when two major criteria or one major and two minor criteria are met. The major criteria include a very positive bronchodilator (BD) test (increase in forced expiratory volume in 1 second [FEV1] >15% and >400 mL), eosinophilia in sputum, and personal history of asthma. Minor criteria include high total IgE, personal history of atopy, and positive BD test (increase in FEV1 >12% and >200 mL) on two or more occasions.

The new Finnish guidelines for the treatment of COPD proposed the same criteria for the diagnosis of ACOS as the Spanish guidelines, with the addition of an elevated FeNO higher than 50 parts per billion as a major criterion and a peak flow follow-up typical for asthma as an additional minor criterion [21]. The latest Czech Republic guidelines, published in 2013, also include ACOS with its own diagnostic criteria, similar to the Spanish recommendations [22]. These are, however, quite restrictive criteria and represent a very conservative approach until more evidence about the characterization of ACOS becomes available from large clinical trials or prospective studies. In fact, two recent studies in Spain, using the previous criteria, identified that only between 5% and 6% of the patients fulfilled the criteria for ACOS, in patients with smoking-related COPD [23, 24]. This percentage is clearly below the expected number of individuals sharing the characteristics of asthma and COPD, according to epidemiological data.

In fact, in a Spanish survey performed among pulmonology specialists, selected as experts in asthma and COPD, aimed at collecting their opinions about ACOS and their attitudes in regard to some case scenarios of ACOS patients, only 34.6% of the specialists surveyed were in agreement with the Spanish criteria, and 30.8% were in an intermediate position between agreement and disagreement. The main aspect highlighted by 76.9% of the specialists was that these criteria had to be validated in prospective studies [25].

On the other hand, the GINA–GOLD approach to diagnosis of ACOS [9] is deliberately descriptive and perhaps not very suitable for clinical applications, but recognizes that this entity, just like asthma and COPD, comprises a heterogeneous group of disorders.

The characteristics that might support the diagnosis of ACOS according to GINA–GOLD are as follows:

1. – Usually age 40 years or older, but may report symptoms in childhood or early adulthood.

2. – Respiratory symptoms, including exertional dyspnea, are persistent, but variability may be prominent.

3. – Airflow limitation that is not fully reversible, but often with evidence of significant current or historical variability.

4. – Persistent airflow limitation.

5. – Frequently, a history of doctor-diagnosed asthma (current or previous), allergies and a family history of asthma, and/or a history of noxious exposures, as seen in COPD.

6. – Symptoms are partly but significantly reduced by treatment. Progression is usual, and treatment needs are high.

7. – Exacerbations may be more common than in COPD, but are reduced by treatment.

8. – Comorbidities can contribute to clinical and functional impairment.

9. – COPD-like findings on the chest X-ray.

10. – Typically eosinophilia (with or without associated neutrophilia) in sputum.

Taking into consideration all these previously proposed criteria, the most recent Spanish guideline on the management of asthma (GEMA 2015) [26] proposes an algorithm designed to guide physicians in their routine clinical practice. The existence of patients who fulfill the criteria for COPD – adult smokers with respiratory symptoms and post-BD FEV1/FVC <0.7 – and who present characteristics of asthma, such as high reversibility of airflow, signs of bronchial and systemic eosinophilic inflammation, history of atopy, or even a previous diagnosis of asthma before the age of 40 years, has been definitely recognized. This approach also includes, as a novelty when compared with all the others, an oral corticosteroid test with prednisone to assess reversibility of the bronchial obstruction. If FEV1/FVC remains below 70% after a BD test, a methacholine test and the presence or absence of biomarkers of Th2 inflammatory response should help the clinician to distinguish between COPD and ACOS [17]. The algorithm is summarized in Figure 2.

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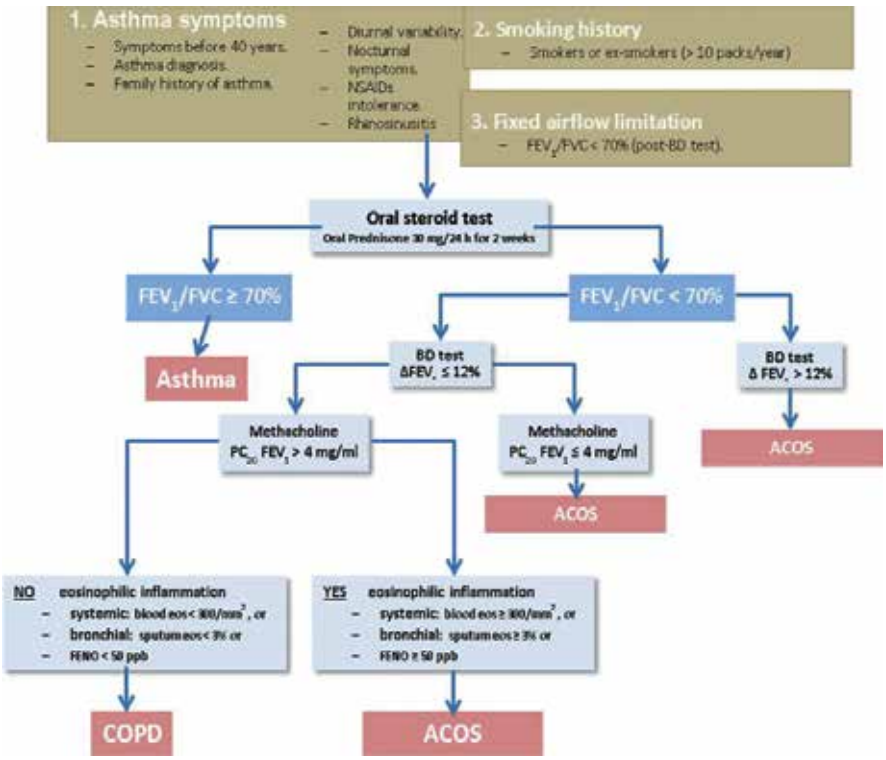

Figure 2. Diagnostic algorithm for ACOS according to GEMA 2015 [26].

BD-test: bronchodilator test; eos: eosinophils.

6. Prognostic implications

It has been reported that patients with ACOS have more frequent exacerbations, are more likely to have a severe exacerbation requiring hospitalization, use more respiratory medications, and have the highest reported pulmonary symptoms. More importantly, patients with ACOS also report worse quality of life than those with either disease alone [27, 28].

However, other authors found that the ACOS phenotype was not clinically different at baseline, in other than the specific criteria used to define it, than patients with no criteria for ACOS. Interestingly, survival after 1 year of follow-up was significantly better in patients with ACOS [29]. To explain such a discrepancy, it should be mentioned that this population included 72% of patients with mild-to-moderate disease, which differs from previous publications with more severe COPD, and of note, 63% of patients with ACOS were receiving ICS, which likely contributed to ameliorate the clinical differences, when compared to the non-ACOS group.

The prognostic significance of having a diagnosis of ACOS must be further assessed in the light of a prospective cohort study, designed to compare the outcomes of COPD patients, asthmatics, and individuals with both diseases.

7. Treatment of ACOS

The present approach to pharmacotherapy for ACOS includes trial and error and extrapolation from results of investigations, in particular subpopulations of asthma or COPD patients. The first option recommended by the Spanish consensus guideline for patients with overlap phenotype COPD-asthma is to add ICS to the treatment for COPD [10], as it is also indicated in the Finnish and Czech guidelines [21, 22]. The GINA–GOLD document also indicates that the default position in the case of ACOS should be to start treatment accordingly for asthma, and recommends the LABA/ICS combination, with special attention to avoid the use of LABAs in monotherapy. In the Spanish survey among expert pulmonologists mentioned before [25], 88.5% of the participants agreed that ACOS requires a different treatment compared to COPD, starting with LABA/ICS and stepping up to triple therapy (LABA/ICS+LAMA) in severe cases.

However, these and other recommendations are not based on high-quality data, because patients with ACOS have been classically excluded from pharmacological clinical trials both in asthma and in COPD. As a consequence, there is no clear information about the response of these patients to most of the current pharmacological therapies. The only clinical trial performed to date in patients with ACOS studied the spirometric effects of tiotropium in individuals with concomitant COPD and asthma. Improvement in lung function and a reduction in rescue medication were observed with tiotropium [30].

However, the main interest in differentiating ACOS from COPD lies in the different response to ICS.

Kitaguchi et al. [15], in a retrospective study with a small sample size, found that COPD patients with asthmatic symptoms had high peripheral and sputum eosinophil counts and better reversibility response to treatment with ICS. These findings reproduce those of other studies, in which COPD patients with a positive BD test, or with a positive hyperreactivity test, or with sputum eosinophilia, have been shown to be more responsive to ICS than those without these features [31–36]. Therefore, it seems logical to consider ICSs as the cornerstone of treatment in ACOS with the addition of long-acting β-agonists in those patients who remain symptomatic or suffer recurrent exacerbations.

In addition to the only clinical trial of Magnussen et al., more recent literature has demonstrated the efficacy of long-acting muscarinic antagonists (LAMA), such as tiotropium, in people with asthma with persistent bronchial obstruction [37, 38]. With all these recent data in mind, the Spanish guideline GEMA 2015 recommends the combination of an ICS with a long-acting β-agonist as the first therapeutic choice in patients with ACOS, leaving open the option to add a LAMA if the patients remain uncontrolled [26].

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Biological treatments that target Th2-related pathways (omalizumab, anti–IL-5, and perhaps anti–IL-4/-13) might also be effective in ACOS, and they warrant further investigation. This is also the case for drugs that target predominantly neutrophil-driven mechanisms, such as roflumilast.

8. Discussion: ACOS as a phenotype or endotype of obstructive airway disease.

Asthma and COPD are themselves heterogeneous disorders that comprise several phenotypes and endotypes. If we admit that ACOS should be characterized by the presence of inflammatory features of both COPD (mainly Th1) and asthma (mainly Th2), we could argue that COPD patients with sputum eosinophilia and with asthma, with a mixed neutrophilic–eosinophilic pattern are, in fact, patients with ACOS, regardless of their clinical presentation. So changing our point of view, from an initial clinically based classification of obstructive airways diseases to another centered on inflammatory underlying mechanisms (endotypes), would allow us to tailor and optimize treatments, leaving behind the rigid categorization of patients into existing diagnostic labels of either asthma or COPD, which do not fully recognize the molecular and clinical heterogeneity of chronic obstructive airway diseases [39].

We need to move toward a new taxonomy of airway diseases that takes into account the underlying pathogenic mechanisms. In this new scenario, ACOS would be an endotype, like early-onset allergic asthma or emphysema. However, things are not so straightforward, and there are several important issues that complicate the settling-in of this new approach:

- Sputum cell count, the method of reference to measure and identify bronchial inflammation, is technically complex and time consuming. At this moment, we do not have substitute reliable biomarkers to identify the bronchial underlying inflammatory mechanisms in a particular patient.
- There is not a complete identification between a particular and well-recognized clinical phenotype (e.g. late-onset eosinophilic asthma) and an exclusive inflammatory pattern (e.g. eosinophilic). In a large cross-sectional study of patients with airway disease, D'Silva et al. [40] reported the cellular profile of over 4,000 induced or spontaneous sputum samples. In the ACOS group, eosinophilic bronchitis was seen in 35%, neutrophilic bronchitis in 19%, and a mixed inflammatory pattern in 10%. In COPD, the phenotypes were respectively 18%, 34%, and 7% and, in asthma alone, 26%, 14%, and 6%. These data bring to the table the heterogeneous nature of airway inflammation in asthma and COPD.
- The inflammatory pattern can vary over time, either spontaneously or as a result of treatment.

It is to be expected that in a near future, advances in the identification of inflammatory patterns can help us to adequately classify patients with chronic obstructive airway disease and offer them the best therapeutic option in each case.

9. Conclusions

ACOS is the coincidence of asthma and COPD in the same individual. Prospective studies are required to analyze the underlying inflammatory mechanisms in this entity. So we think that longitudinal studies are required to validate the diagnostic criteria and identify biomarkers of the disease. It also remains to be determined if it has different prognostic and therapeutic implications. If so, perhaps in the future, ACOS will be considered a distinct endotype of chronic obstructive airway disease. There is also a lack of studies to further clarify the best therapeutic options for patients with ACOS. At this moment, it seems reasonable to consider the combination of an ICS and a long-acting β-agonist as the first-choice therapy for these patients.

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Chapter 10

Complementary Therapy with Traditional Chinese Medicine for Childhood Asthma

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Additional information is available at the end of the chapter

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Abstract

Asthma is a heterogeneous disease that is typically characterized by chronic airway inflammation and obstruction of airflow; it frequently presents in early childhood and is the leading chronic disease in children in the western world. This review presents a brief description of the pathophysiology of asthma and summarizes recent research results on the mechanisms of action of anti-asthma Chinese herbal medicine commonly used in clinical practice. Other interventions of traditional Chinese medicine (TCM), such as acupuncture, tai chi, and meditation are also briefly discussed. We believe that this contribution is theoretically and practically relevant because the prevalence of asthma is increasing and, in addition to standard treatment, the use of complementary therapy is increasing and there is increasing scientific evidence demonstrating that TCM has potential for the treatment of childhood asthma.

Keywords: Childhood asthma, traditional Chinese medicine, Acupuncture, complementary and alternative medicine

1. Introduction

Asthma is a heterogeneous disease that is typically characterized by chronic airway inflammation and obstruction of airflow. Asthma is defined by a history of respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough [1]. Both these symptoms and airflow limitation characteristically vary over time as well as in intensity. These variations are often triggered by external factors, such as exercise, allergen or irritant exposure, change in the weather, or viral respiratory infections [2]. Symptoms and airway limitation may resolve with or without medication and may sometimes be absent for weeks or months at a time.

Asthma, a life-long condition, frequently presents in early childhood and is the leading chronic disease in children in the Western world. Although the prevalence of childhood varies widely across the world as described in the Phase III ISAAC study [3], most studies have reported that this prevalence has increased in recent decades [4–6]. This increase has been associated with a rise in atopic sensitization and other allergic disorders, such as eczema and rhinitis [6]. Approximately 25.9 million Americans (including 7.1 million children) had asthma in 2011, which equates to a rate of 84.8 per 1,000 in the population. The highest prevalence rate was seen in those in the 5–17 years of age bracket (105.5 per 1,000). Overall, the rate in those under the age of 18 years (94.9 per 1,000) was significantly greater than that in those over 18 years (81.6 per 1,000). The current asthma prevalence rate for boys under 18 years (101.7 per 1,000) was 16% higher than the rate among similarly aged girls (87.8 per 1,000) [7]. In 2008, the condition accounted for an estimated 14.4 million lost school days in children and 14.2 million lost work days in adults. Asthma is thus a leading cause of activity limitation and amounts to $56.0 billion in health care costs annually in the United States [7].

Approximately 80 percent of children with asthma develop symptoms before 5 years of age, but the disease is frequently misdiagnosed or not suspected, particularly in infants [8]. Coughing and wheezing are the most common symptoms of childhood asthma. Breathlessness, chest tightness or pressure, and chest pain have also been reported [1, 2]. Descriptors may vary between cultures and by age; for example, children may be described as having heavy breathing [2]. Confirmation of the diagnosis of asthma in children requires a careful review of a child's current and past medical history, family history, as well as a physical examination.

Asthma is characterized by variable expiratory airflow limitation. Pulmonary function tests are sometimes needed to diagnose asthma and to rule out other possible causes of the symptoms. Spirometry is the most common pulmonary function test; it measures the flow and volume of air blown out after a child takes a very deep breath and then forcefully exhales. The important parameters derived from spirometry include forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), flow between 25% and 75% of the vital capacity (FEF 25–75%), and peak expiratory flow rate (PEFR) [9]. The greater the variation in lung function, or the more times excess variation is seen in a patient with respiratory symptoms, the more likely the diagnosis is to be one of asthma. The FEV_1/FVC ratio is normally >0.75–0.80 and usually exceeds 0.90 in children [10]. In asthma, at least once during diagnostic process, the FEV_1 is low, confirming that the FEV_1/FVC ratio is reduced. Generally, an increase in FEV_1 of >12% of that predicted after inhalation of a rapid-acting bronchodilator and/or average daily diurnal peak expiratory flow (PEF) variability exceeding 13% indicates that a child has asthma [2]. In young children, in whom lung function testing is not feasible, including most preschool children, asthma is defined by the presence of variable respiratory symptoms.

Traditional Chinese medicine (TCM), particularly herbal medicine, has been used for the treatment of asthma for hundreds of years, as documented in the *Yellow Emperor's Inner Canon* (*Huangdi Neijing*) and the *Essential Prescriptions from the Golden Cabinet* (*Jin Gui Yao Lue*). In Taiwan, Chinese herbal medicine is commonly used as complementary and alternative therapy for the treatment of atopic diseases such as asthma, allergic rhinitis, and atopic

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dermatitis. The medicines used for the prevention and treatment of asthma have received much attention in recent years. The cellular and molecular details of the underlying mechanisms of action of Chinese herbal medicine efficacious for treating asthma are just beginning to be understood.

This chapter presents a brief description of the pathophysiology of asthma and summarizes recent research results on the mechanisms of action of anti-asthma Chinese herbal medicine commonly used in clinical practice. Other interventions of TCM, such as acupuncture, *tai chi*, and meditation, are also briefly discussed.

2. Pathophysiology and pathogenesis of asthma

Asthma can be classified as atopic/allergic (extrinsic), which is the most common form, or nonatopic/nonallergic (intrinsic) asthma, which is more rare, has a later onset, and tends to be more severe than atopic asthma [11]. Atopic asthma involves inflammation mediated by specific IgE antibodies directed against common environmental allergens, whereas nonatopic asthma involves inflammation and airway constriction mediated by local production of IgE antibodies that are possibly directed at bacterial or viral antigens. The pathophysiology of nonatopic asthma is very similar to that of atopic asthma, but it is not caused by exposure to an allergen [2, 11].

The gross pathology of asthma reveals significant overinflation of the lungs [12]. Microscopically, this overinflation of lungs is manifest as marked distension of the alveoli. Notable airway smooth muscle (ASM) hyperplasia, basement membrane thickening, mucous gland hyperplasia, mucosal epithelium sloughing, and tissue edema are also seen [12]. This increase in muscle mass, mucous gland tissues, and tissue edema leads to a thickened airway wall, with a resultant decrease in airway caliber [12, 13]. These structural changes have been described as remodeling, a term used to define complex morphological changes that involve all of the structures of the bronchial wall [12, 13].

The initiation of bronchial epithelial damage by environmental agents (allergens, viruses, irritants, etc.) or their inflammatory products activates a sequence of events that amplify the inflammation and induce airway remodeling [13, 14]. Bousquet et al. suggested that asthma pathophysiology involved overlapping interactions of smooth muscle dysfunction, airway inflammation, and airway remodeling [13]. The inflammatory, physiological, and structural factors that contribute to the pathogenesis of asthma will be described below.

2.1. Airway inflammation

Inflammation plays a central role in the pathophysiology of asthma [15]. Airway inflammation remains a consistent pattern throughout the distinct phenotypes of asthma (e.g., intermittent, persistent, exercise-associated, aspirin-sensitive, or severe asthma) [16]. Airway inflammation involves an interaction of many cell types and multiple mediators with the airway, which eventually results in the characteristic pathophysiological features of asthma. The principal

cells involved in airway inflammation are mast cells, eosinophils, epithelial cells, macrophages, and activated T lymphocytes [12, 13].

T lymphocytes play an important role in the regulation of airway inflammation through the release of numerous cytokines. Airway inflammation in asthma may indeed represent a loss of the normal balance between Th1 and Th2 lymphocytes [12, 16]. Th1 cells produce interleukin (IL)-2 and interferon gamma (IFN-γ), which are critical in the defense mechanisms of cells in response to infection. Th2 cells, in contrast, generate a family of cytokines (IL-4, IL-5, IL-6, IL-9, and IL-13) that can stimulate the growth, differentiation, and recruitment of mast cells, basophils, eosinophils, and B-cells, all of which are involved in humoral immunity and in the allergic response [13, 14, 16].

IgE plays an essential role in type I hypersensitivity, which results in various allergic diseases, such as allergic asthma, most types of sinusitis, allergic rhinitis, food allergies, and specific types of chronic urticaria and atopic dermatitis [17]. Antigen-specific IgE is partly responsible for the initiation of an allergic response in asthma. IgE primes the IgE-mediated allergic response by binding to Fc receptors expressed on the surface of mast cells, basophils, eosinophils, monocytes, macrophages, or platelets in humans [18]. Antigens cross-link to the IgE on mast cells, which then release bronchoconstricting mediators (histamine, cysteinyl-leukotrienes, prostaglandin D2) and further amplify the inflammatory response by damaging local tissue and attracting other lymphocytes [17]. IL-4 produced by Th2 cells stimulates IgE production in B-lymphocytes and expression of vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells, whereas IL-5 stimulates eosinophil differentiation and mobilization to inflammatory sites [13, 16]. Circulating eosinophils enter the area of allergic inflammation and begin migrating to the lung by rolling, through interactions with selectins, and eventually adhere to the endothelium by means of binding between integrins and members of the immunoglobulin superfamily of adhesion proteins, namely VCAM-1 and intercellular adhesion molecule 1 (ICAM-1) [13, 16]. As the eosinophils enter the matrix of the airway through the influence of various chemokines, such as monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1α), eotaxin or RANTES, and cytokines, their survival is prolonged by IL-4 and granulocyte-macrophage colony–stimulating factor (GM-CSF) [13, 16]. Upon activation, the eosinophils release inflammatory mediators, such as leukotrienes and granule proteins, which injure airway tissues [19]. In addition, eosinophils can generate GM-CSF to prolong and potentiate their survival and thereby contribute to persistent airway inflammation [16]. Eosinophils are the most characteristic cells accumulated in asthma and allergic inflammation; their presence is often related to disease severity. Eosinophils are recruited or activated by IL-5, the eotaxin family of chemokines, via the eosinophil-selective chemokine receptor CCR3, and by Toll-like receptors (TLRs). Activated eosinophils produce lipid mediators, such as leukotrienes and platelet-activating factor, which mediate smooth muscle contraction; toxic granule products (e.g., major basic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, or eosinophil cationic protein) that can damage airway epithelium and nerves; and cytokines, such as GM-CSF, transforming growth factors (TGF)-α and β, and interleukins, which may be involved in airway remodeling and fibrosis [13]. Recently, Th regulatory cells that exclusively produce IL-17 cytokines (TH17 cells) have been

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identified in patients with severe asthma [19]. The involvement of TH17 responses in the pathogenesis of asthma has been shown by the overexpression of IL-17 mRNA in the airways of asthma model mice [19]. It is now suggested that TH17-related cytokines play a critical role in airway remodeling and may be involved in interactions with structural cells [13, 19].

2.2. Airway remodeling and ASM dysfunction

The histopathologic changes of airway remodeling include damage or loss of the normal pseudostratified structure of airway epithelium, an increase in the proportion of mucous-producing goblet cells, fibrotic thickening of the subepithelial reticular basement membrane or "lamina reticularis," increased numbers of myofibroblasts, increased vascularity, increased ASM mass, and increased extracellular matrix [20]. These structural changes contribute to bronchial wall thickening, alterations in the physiological consequences of smooth muscle contraction, or loss of airway-parenchymal interdependence [13, 20].

Epithelial alterations in asthma include epithelial shedding, destruction of ciliated cells, goblet cell hyperplasia, upregulation of growth factor release, and overexpression of receptors, such as the epidermal growth factor receptors [21]. Loss of epithelial surface and the resultant denudation of the basement membrane may decrease this protective effect, thereby increasing the propensity for allergic insult to the airway [21]. A second important feature of airway remodeling is subepithelial fibrosis, which has been consistently reported in asthma of all levels of severity, in patients with atopic rhinitis, and even in children with treatment-resistant asthma [21]. Subepithelial fibrosis occurs in the lamina reticularis, immediately below the basement membrane, resulting in thickening of the basement membrane just below the epithelium [22]. In the asthmatic airway, fibroblasts are activated and differentiate into myofibroblasts, which secrete proinflammatory mediators and extracellular matrix proteins, including collagens I, III, and V; fibronectin; tenascin; lumican; and biglycan [21, 22]. Asthmatic airway fibroblasts promote fibrosis though expression of a higher ratio of tissue inhibitor of metalloproteinase (TIMP)-2 to matrix metalloproteinase (MMP)-2, resulting in increased matrix deposition [21]. MMPs are a family of proteases implicated in collagen degradation. MMP-2, MMP-3, MMP-8, and MMP-9 have been associated with asthma [20]. Among these, MMP-9 levels have been reported to be significantly higher in the sputum of patients with asthma than in that of control subjects [20–23].

Respiratory ASM cells are the critical effector cells that modulate airway tone [22]. In asthmatic airways, smooth muscle mass is increased due to a coordinated increase in the size (hypertrophy) and number (hyperplasia) of ASM cells [21, 22]. ASM remodeling is considered to be the primary cause of airway obstruction [21]. ASM cells participate in the inflammatory and remodeling process through the expression of cellular adhesion molecules, receptors for cytokines (e.g., TNF-α), chemokines (RANTES, eotaxin, MIP-1α, and IL-8), and TLRs [21]. Additionally, the migration of ASM cells toward the epithelium contributes to remodeling. A wide range of inflammatory mediators, such as TNF-α, IL-1b, and IFN-γ, have been shown to induce the expression of ICAM-1 and VCAM-1 on cultured ASM cells [21]. The surface expression of cellular adhesion molecules by ASM cells might be pivotal in regulating interactions with a variety of inflammatory cells, including eosinophils and T cells [21].

Additionally, accumulating evidence has indicated an abnormal increase in the number and size of microvessels within bronchial tissue in remodeled airways [21]. This occurs mainly below the basal lamina, in the space between the muscle layer and the surrounding parenchyma [21]. An imbalance between vascular endothelial growth factor (VEGF) and angiopoietin-1 has been shown to be involved in these abnormalities [21]. In fact, VEGF acts by increasing the permeability of these abnormal blood vessels, resulting in vessel dilation and edema, which contribute to airway narrowing [21, 22]. In addition to providing nutrition to the airways, these vessels are the source of inflammatory cells and plasma-derived mediators and cytokines [21].

3. Conventional treatment of childhood asthma

The optimal treatment of childhood asthma depends upon a number of factors, including the child's age, the severity and frequency of asthma attacks, and the ability to properly use the prescribed medications [2]. For the vast majority of children, asthma treatment can control symptoms, allowing the child to participate fully in all activities, including sports. Identifying and avoiding asthma triggers, the factors that set off or worsen asthma symptoms, are essential for preventing asthma flare-ups [2]. Common asthma triggers generally include allergens (such as dust, pollen, and furred animals), respiratory infections, irritants (such as tobacco smoke, chemicals, and strong odors or fumes), physical activity, certain medicines (such as beta blockers, aspirin, or other nonsteroidal anti-inflammatory medications), and emotional stress [2]. After identifying potential triggers of asthma, the parent and health care provider should develop a plan to deal with the triggers. If possible, the child should completely avoid or limit exposure to the trigger [2].

The long-term goals of asthma management are to achieve good symptom control and to minimize future risk of exacerbation, fixed airflow limitation, and side effects of treatment [2]. In control-based asthma management, pharmacological and nonpharmacological treatment is adjusted continuously in a cycle that involves assessment, treatment, and review of the response [2]. Asthma severity is determined by considering the following factors: the symptoms reported over the previous 2 to 4 weeks, the current level of lung function (FEV_1 and FEV_1/FVC values), and the number of instances of exacerbation requiring oral glucocorticoids per year [2]. The classification of severity in children aged 5–11 years or in adolescents over the age of 12 years is similar to that in adults [2]. The severity in children under the age of 4 years, however, is classified somewhat differently and includes intermittent, mild persistent, moderate persistent, and severe persistent asthma [2].

3.1. Categories of asthma medications

Medication for asthma is mainly divided into two categories: controller medications and reliever (rescue) medications [2]. Controller medications, such as inhaled corticosteroids (ICS) and long-acting beta-adrenoceptor agonists (LABA), are used for regular maintenance treatment [24–26]. These medications reduce airway inflammation, control symptoms, and reduce future risks, such as exacerbations and decreased lung function. Reliever medications,

http://dx.doi.org/10.5772/62333

such as short-acting beta-2-adrenoceptor agonists (SABA), are provided to all patients for as-needed relief of breakthrough symptoms, including during worsening of asthma or exacerbations [24–26]. They are also recommended for short-term prevention of exercise-induced bronchoconstriction [2]. Reducing and, ideally, eliminating the need for reliever treatment are both an important goal in asthma management and a measure of the success of asthma treatment. Add-on therapies for patients with severe asthma may be considered when patients have persistent symptoms and/or exacerbations, despite optimized treatment with high-dose controller medications (usually a high-dose ICS and a LABA) and treatment of modifiable risk factors [2].

The initiation of asthma therapy in a stable patient who is not already receiving medications is based upon the severity of asthma in the individual. Patients with mild intermittent asthma are best treated with an inhaled SABA, which should be taken as needed for the relief of symptoms [2]. Patients in whom triggering of asthmatic symptoms can be predicted (e.g., exercise-induced bronchoconstriction) are encouraged to use their inhaled beta agonist approximately 10 min prior to exposure, to prevent the onset of symptoms [2]. For mild persistent asthma, the preferred long-term controller is a low-dose ICS [2]. Regular use of ICS reduces the frequency of symptoms (and the need for SABAs for symptom relief), improves the overall quality of life, and decreases the risk of serious exacerbations [2]. Alternative strategies for treatment of mild persistent asthma include leukotriene receptor antagonists, theophylline, and cromoglycate [2, 26].

For moderate persistent asthma, the preferred therapy is low doses of ICS plus an inhaled LABA, or medium doses of ICS [2]. Alternative strategies include adding a leukotriene modifier (leukotriene receptor antagonist or lipoxygenase inhibitor) or theophylline to low-dose ICS [2]. For severe persistent asthma, the preferred treatments are medium (Step 4) or high (Step 5) doses of ICS, in combination with an inhaled LABA [2]. In addition, for patients who are inadequately controlled on high-dose ICS and LABAs, the anti-IgE therapy omalizumab may be considered, if there is objective evidence of sensitivity to a perennial allergen (by allergy skin tests or in vitro measurements of allergen-specific IgE) and if the serum IgE level is within the established target range [2].

4. Status and purpose

Currently, according to the guidelines published by the Global Initiative for Asthma (GINA), conventional medicines are the mainstay for managing asthma; these include steroids, beta-2 adrenergic agonists, leukotriene modifiers, theophylline, and anti-IgE therapies [2]. However, current conventional medications for childhood asthma are not yet satisfactory. The side effects of long-term use of steroids and beta-2 adrenergic agonists are major concerns for parents, in that growth, bone turnover, and adrenal gland function may be suppressed under, particularly higher doses of steroids [24, 25]. Due to the chronic and potentially life-threatening nature of asthma, and the lack of definitive preventive and curative therapies, many families look to complementary and alternative medicine (CAM) for treatment. CAM is popular in the

treatment of asthma and encompasses many therapies, including mind–body techniques, nutritional manipulation, dietary and herbal supplements, TCM (including acupuncture), exercise, manual therapies, and homeopathy [26]. Reportedly, CAM is commonly used in children who have mild or moderate persistent asthma, those receiving high-dose ICS, and patients who experience poor symptom control or require frequent physician visits, including emergency room visits [26]. One retrospective longitudinal cohort study showed that initiation of CAM treatment does not decrease future adherence to conventional asthma medications, suggesting that alternative or integrative medicine use does not necessarily compete with conventional asthma therapies [27]. As CAM use becomes more prevalent, it will become increasingly important for physicians attending to asthmatic children to be aware of CAM use. TCM is the major component of CAM therapies used in the United States and Taiwan. TCM is one of the oldest medical practices in the world and has played an important role in preventing and treating diseases in China for centuries, where it is still used as a monotherapy or as part of an integrated medicine approach. Evidence has increased showing the efficacy of TCM for the treatment of childhood asthma. Below, we explore complementary therapy, involving TCM therapy for childhood asthma.

5. Chinese herbal formulas use in children with asthma

TCM formulas have been used to treat asthma for centuries. A number of well-controlled clinical studies of several TCM formulas, including modified Mai-Men-Dong-Tang (mMMDT, five herbs), Ding-Chuan-Tang (DCT, nine herbs), and STA-1 (the combination of Mai-Men-Dong-Tang and Liu-Wei-Di-Huang-Wan, 10 herbs), and anti-asthma herbal medicine intervention (ASHMI, three herbs) provided evidence of clinical efficacy, safety, and immunomodulatory effects [24]. Typically, the traditional TCM formulas that are prescribed combine several single herbs to treat a specific disease. Recent research [25] from the National Health Insurance Research Database (NHIRD) in Taiwan has revealed the core herbal treatments for children with asthma. The most commonly used herbal formulas for the treatment of childhood asthma are Ma-Xing-Gan-Shi-Tang and Xiao-Qing-Long-Tang; the former is used for excess heat congested in the lung, whereas the latter is used for the exterior wind-cold with internal accumulation of retained fluid in the lung. These herbal formulas (shown in Table 1) and several single herbs commonly used for the treatment of childhood asthma are described below. The immunomodulatory effects of suppressing Th2 cells and decreasing subsequent cytokine secretion of these herbal remedies will also be investigated. Other commonly prescribed formulas that are used mainly to relieve asthma-related symptoms, such as productive cough (Xing-Su-San, Zhi-Sou-San), coughing with a sore throat (Yin-Qiao-San), and nasal congestion (Xin-Yi-Qing-Fei-Tang, Cang-Er-Zi-San, Shin-Yi-San), do not fall within the scope of this review.

5.1. ASHMI

ASHMI is the first herbal medicine to receive approval for phase I and II clinical trials as a US Food and Drug Administration investigational new drug (IND No. 71526) for treating asthma.

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Formula	Composition	Possible mechanisms
ASHMI	Ling Zhi (*Ganoderma Lucidum*), Ku Shen (*Radix Sophorae Flavescentis*), and Gan Cao (*Radix Glycyrrhizae*)	Decreases Th2 response; increases IFN-γ levels; decreases IL-4, IL-5, IL-13, eotaxin, TNF-α, total and specific IgE levels; reduces AHR, mucous production, neutrophilic and eosinophilic inflammation; improves FEV_1 and PEF [24, 28–30]
Modified Mai-Men-Dong-Tang (mMMDT)	Mai Men Dong (*Radix Ophiopogonis*), Ban Xia (*Rhizoma Pinelliae*), American Ren Shen (*Radix Panacis Quinquefolii*), Gan Cao (*Radix Glycyrrhizae*), and Lantern Tridax (*Herba Tridacis procumbentis*)	Antitussive effect, bronchial dilation via beta-2 adrenergic effect; decreases IL-4, total IgE, and specific IgE; reduces AHR; improves FEV_1 [24, 31–33]
STA-1	Mai Men Dong (*Radix Ophiopogonis*), Ban Xia (*Tuber Pinellia*), American Ren Shen (*Radix Panacis Quinquefolii*), Gan Cao (*Radix Glycyrrhizae*), Shu Di Huang (*Radix Rehmanniae Preparata*), Mu Dan Pi (*Cortex Moutan Radicis*), Shan Zhu Yu (*Fructus Corni*), Fu Ling (*Poria*), Ze Xie (*Rhizoma Alismatis*), and Shan Yao (*Radix Dioscoreae*)	Reduces symptom scores, systemic steroid dose, airway inflammation, AHR, total IgE, and specific IgE; improves FEV_1 [34, 35]
Ma-Xing-Gan-Shi-Tang	Ma Huang (*Herba Ephedrae*), Xing Ren (*Semen Armeniacae Amarum*), Shi Gao (*Gypsum Fibrosum*), and Gan Cao (*Radix Glycyrrhizae*)	Antitussive effect, beta-2 adrenergic effect; reduces neutrophilic inflammation [36, 37]
Xiao-Qing-Long-Tang	Ma Huang (*Herba Ephedrae*), Gui Zhi (*Ramulus Cinnamomi*), Ban Xia (*Rhizoma Pinelliae*), Gan Jiang (*Rhizoma Zingiberis*), Xi Xin (*Herba Asari*),Wu Wei Zi (*Fructus Schisandrae*), Bai Shao Yao (*Radix Paeoniae*), and Gan Cao (*Radix Glycyrrhizae*)	Decreases Th2 response; increases IFN-γ; decreases IL-4, IL-5, IL-10, IL-13, IgE, RANTES, eotaxin, and MCP-1 levels; suppresses histamine release, reduces airway inflammation, remodeling, and immunomodulation; bronchial dilation, partial beta-2 adrenergic effect [38–40]
Ding-Chuan-Tang	Ma Huang (*Herba Ephedrae*), Gan Cao (*Radix Glycyrrhizae*), Ban Xia (*Rhizoma Pinelliae*), Bai Guo (*Semen Ginkgo*), Kuan Dong Hua (*Flos Farfarae*), Sang Bai Pi (*Cortex Moris*), Su Zi (*Fructus Perillae*), Xing Ren (*Semen Armeniacae Amarum*), and Huang Qin (*Radix Scutellariae*)	Improves AHR, symptoms, and medication; reduces eosinophilic inflammation; beta-2 adrenergic effect [41, 42]

Table 1. Herbal formulas frequently used for asthmatic children

ASHMI is composed of the aqueous extracts of Ling Zhi (*Ganoderma lucidum*), Ku Shen (*Sophora flavescens*), and Gan Cao (*Glycyrrhiza uralensis*) [24]. ASHMI improved lung function (FEV_1), reduced symptom scores, and decreased beta-2-adrenoceptor agonist use, to a degree similar as that achieved by prednisone in adults with moderate to severe asthma, but without the adverse effect of prednisone on adrenal function and with no overall immune suppression. Individually, Ling Zhi, Ku Shen, and Gan Cao extracts and ASHMI (the combination of individual extracts) inhibited production of IL-4 and IL-5 by murine memory Th2 cells and that of eotaxin-1 by human lung fibroblast cells [28]. ASHMI synergistically inhibited eotaxin-1 production as well as Th2 cytokine production. In another mouse model of asthma, ASHMI also reduced the levels of ovalbumin (OVA)-specific IgE and Th2 cytokines, including IL-4, IL-5, and IL-13 in the lung, and increased IFN-γ secretion [29]. Moreover, ASHMI markedly reduced airway hyperresponsiveness (AHR), mucous production, neutrophilic inflammation, and TNF-α, IL-8, and IL-17 levels and also decreased eosinophilic inflammation and Th2 responses in vivo [30].

5.2. Modified Mai-Men-Dong-Tang

Mai-Men-Dong-Tang is a herbal TCM that has been used for the treatment of bronchitis, bronchial asthma, and cough. The compositions of Mai-Men-Dong-Tang are Mai Men Dong (*Ophiopogon japonicus*), Ban Xia (*Pinellia ternata*), Ren Shen (*Panax ginseng*), Gan Cao (*Glycyrrhiza uralensis*), Da Zao (*Ziziphus jujuba*), and Geng Mi (*Oryza sativa*). Mai-Men-Dong-Tang was shown to have an antitussive effect, based on improved airway clearance. The pharmacological effect of this antitussive effect is suggested to involve the inhibition of C-fibers, bronchodilation, anti-inflammatory effects, suppression of mucosal excretion, and augmentation of surfactant secretion [31]. Mai-Men-Dong-Tang was shown to potentiate beta-adrenergic function in ASM, which may reflect the efficacy on AHR and asthma [32]. mMMDT contains five herbs, including Mai Men Dong (*Radix Ophiopogonis*), Ban Xia (*Rhizoma Pinelliae*), American Ren Shen (*Radix Panacis Quinquefolii*), Gan Cao (*Radix Glycyrrhizae*), and Lantern Tridax (*Herba Tridacis procumbentis*) [33]. mMMDT was shown to decrease serum total IgE and house dust mite-specific IgE significantly and downregulate the expression of IL-4 in allergen-sensitized mice. The effect of mMMDT on changes in FEV_1 was studied as the first efficacy end point, given its validity for monitoring airway obstruction, which showed significant improvement in FEV_1 in patients treated with mMMDT [33]. Moreover, mMMDT also relieved asthma symptoms, including coughing, wheezing, and breathlessness [33].

5.3. STA-1

STA-1 is a combination of mMMDT (four herbs) and Lui-Wei-Di-Huang-Wan (six herbs) [34]. The four herbs of mMMDT comprise Mai Men Dong (*Radix Ophiopogonis*), Ban Xia (*Tuber Pinellia*), American Ren Shen (*Radix Panacis Quinquefolii*), and Gan Cao (*Radix Glycyrrhizae*) without Lantern Tridax (*Herba Tridacis procumbentis*). The six herbs of Lui-Wei-Di-Huang-Wan are Shu Di Huang (*Radix Rehmanniae Preparata*), Mu Dan Pi (*Cortex Moutan Radicis*), Shan Zhu Yu (*Fructus Corni*), Fu Ling (*Poria*), Ze Xie (*Rhizoma Alismatis*), and Shan Yao (*Radix Dioscoreae*). STA-1 was able to inhibit mite-induced IgE synthesis, reduce inflammation-associated

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accumulation of eosinophils and neutrophils in the airway, and relieve AHR in a murine model [35]. Clinical evaluation of STA-1 in the treatment of mild-to-moderate chronic asthma revealed a significant reduction of symptom scores, systemic steroid dose, total IgE, and specific IgE in patients treated with STA-1 [34]. Furthermore, STA-1 also improved lung function (FEV_1) as compared with placebo after 6 months' treatment and with only minimal side effects [34].

5.4. Ma-Xing-Gan-Shi-Tang

Ma-Xing-Gan-Shi-Tang, a TCM, has been used in the treatment of bronchial asthma for several centuries. Ma-Xing-Gan-Shi-Tang consists of Ma Huang (*Herba Ephedrae*), Xing Ren (*Semen Armeniacae Amarum*), Shi Gao (*Gypsum Fibrosum*), and Gan Cao (*Radix Glycyrrhizae*). A murine cough model, induced by sulfur dioxide gas, was used to investigate the antitussive effect of Ma-Xing-Gan-Shi-Tang [36]. Both Ma Huang and Xing Ren inhibited cough induction in a dose-dependent manner. However, Ma-Xing-Gan-Shi-Tang, which contains Ma Huang and Xing Ren, showed stronger antitussive effects than the individual crude drugs [36]. In a guinea pig model of allergic asthma, Ma-Xing-Gan-Shi-Tang was efficacious in stimulation of beta-2-adrenoceptors on bronchial smooth muscle and had an anti-inflammatory effect, involving inhibition of neutrophil infiltration into the airway [37]. Ma-Xing-Gan-Shi-Tang is typically indicated in syndromes involving wind-heat on the lung or stagnated wind-cold that has turned into heat and that stayed in the lung. In Taiwan, asthma triggered by respiratory tract infection among asthmatic children is much more common than that triggered by cold exposure and weather change. Asthma triggered by respiratory tract infection is the most important indication for Ma-Xing-Gan-Shi-Tang [25].

5.5. Xiao-Qing-Long-Tang

Xiao-Qing-Long-Tang (XQLT) has been widely used clinically for the treatment of allergic diseases, including bronchial asthma and allergic rhinitis. XQTL consists of Ma Huang (*Herba Ephedrae*), Gui Zhi (*Ramulus Cinnamomi*), Ban Xia (*Rhizoma Pinelliae*), Gan Jiang (*Rhizoma Zingiberis*), Xi Xin (*Herba Asari*), Wu Wei Zi (*Fructus Schisandrae*), Bai Shao Yao (*Radix Paeoniae*), and Gan Cao (*Radix Glycyrrhizae*).

XQLT was shown to reduce bronchial inflammatory cell infiltration and airway remodeling in repetitive *Dermatogoides pteronyssinus*-challenged mouse model of chronic asthma [38]. XQLT inhibited *D. pteronyssinus*-induced total IgE and *D. pteronyssinus*-specific IgG1 in serum and changed the Th2-bios in bronchoalveolar lavage fluid (BALF) by inhibiting the activation of nuclear factor-Kappa B (NF-κB). The same study also showed that XQLT treatment increased the protein levels of IL-12, but decreased that of TNF-α, TGF-β1, IL-5, IL-6, and IL-13 by inhibiting expression of the genes including IL-10, IL-13, eotaxin, RANTES, and MCP-1 in the lung. Moreover, collagen assays and histopathology indicated that XQLT reduces airway remodeling in the lung [38]. XQLT treatment could inhibit the secretion of IL-5 in the serum and downregulate mRNA expression of genes encoding eotaxin, RANTES, and MCP-1 in lung tissues, which may contribute to a reduction in eosinophils and monocytes recruited to the airway.

Studies on the OVA-sensitized allergic airway inflammation model in mice revealed that XQLT significantly inhibited the antigen-induced immediate asthmatic response and late asthmatic response in actively sensitized mice. XQLT was shown to reduce the production of Th2-associated cytokines, IL-4 and IL-5, and to restore the production of the Th1 cell-associated cytokine, IFN-γ [39]. Anti-OVA IgE antibody levels were reduced in the BALF of sensitized mice after oral administration of XQLT [39]. Furthermore, XQLT was shown to have an anti-asthmatic effect, which is partly mediated by stimulation of beta-2-adrenoceptors, leading to bronchorelaxation; furthermore, XQLT inhibits the infiltration of eosinophils into the airway [40].

5.6. Ding-Chuan-Tang

Ding-Chuan-Tang (DCT), another TCM, has been used in the treatment of bronchial asthma for several centuries. DCT is composed of nine herbs, including Ma Huang (*Herba Ephedrae*), Gan Cao (*Radix Glycyrrhizae*), Ban Xia (*Rhizoma Pinelliae*), Bai Guo (*Semen Ginkgo*), Kuan Dong Hua (*Flos Farfarae*), Sang Bai Pi (*Cortex Mori*), Su Zi (*Fructus Perillae*), Xing Ren (*Semen Armeniacae Amarum*), and Huang Qin (*Radix Scutellariae*). According to TCM principles, this decoction is frequently prescribed for children with coughing, wheezing, and chest tightness.

One study of a murine OVA-sensitized allergic airway inflammation model revealed that DCT significantly inhibited the increase of eosinophils in the airway and caused concentration-dependent bronchorelaxation via a beta-2 adrenergic effect [41]. A randomized, double-blind clinical trial [42] conducted to assess the add-on effect of DCT showed that AHR significantly improved after weeks of DCT treatment compared with that after placebo use. In addition, patients in the DCT group also showed superior clinical improvement and used less medication than in the placebo group. This study suggested that addition of DCT to conventional treatment could further improve AHR, even in patients with well-controlled asthma. However, this study did not find a significant reduction in IgE levels and FEV_1 with DCT treatment, as compared to placebo [42].

6. Chinese single herbs use in children with asthma

We described several single herbs frequently used for asthmatic children in Taiwan (shown in Table 2), including Zhe Bei Mu (*Fritillaria thunbergii*), Xing Ren (*Semen Armeniacae Amarum*), Huang Qi (*Astragalus membranaceus*), Qian Hu (*Peucedanum praeruptorum Dunn*), Gan Cao (*Glycyrrhiza uralensis*), Sang Bai Pi (*Cortex mori radicis*), Ban Xia (*Pinellia ternate*), Bo He (*Mentha haplocalyx*), Da Huang (*Rheum palmatum*), Jie Geng (*Platycodon grandiflorum*), Huang Qin (*Scutellaria baicalensis*), and Yu Xing Cao (*Houttuynia cordata Thunb.*).

6.1. Zhe Bei Mu

Zhe Bei Mu is used as an antitussive therapy and expectorant in TCM. Its extract inhibited histamine release from rat peritoneal mast cells in a concentration-dependent manner. Moreover, it also inhibits the production of inflammatory cytokines (IL-6, IL-8, and TNF-α) in

http://dx.doi.org/10.5772/62333

human mast cell line-1 (HMC-1) cells and components of the mitogen-activated protein kinase (MAPK) pathway in mast cells [43].

6.2. Xing Ren

Xing Ren has long been used in TCM to control acute lower respiratory tract infection and asthma as a result of its expectorant and anti-asthmatic activities. Xing Ren was shown to have anti-asthmatic activity and selectively inhibit the Th2 response in a mouse model by decreasing eosinophils and IL-4 in the airway [44].

6.3. Huang Qi

Huang Qi has a long history of medicinal use for asthma treatment in China. It increases metabolism and stimulates tissue regeneration, and it is used to treat colds, allergies, digestive problems, and fatigue in TCM. Huang Qi was shown to inhibit the Th2 response. It significantly reduced AHR, eosinophil counts, and IL-4, IL-5, and IL-13 levels and increased INF-γ levels in BALF. Histological studies showed that Huang Qi markedly decreased inflammatory infiltration, mucus secretion, and collagen deposition in lung tissues. $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) play a significant role in the regulation of asthma, and the induction of allergen-specific Tregs has become one appealing strategy for asthma therapy [45–47]. Huang Qi was shown to increase the population of $CD4^+CD25^+Foxp3^+$ Tregs and promote *Foxp3* mRNA expression in a rat model of asthma [47]. This suggests that the anti-asthmatic effects of Huang Qi are at least partially associated with $CD4^+CD25^+Foxp3^+$ Tregs.

6.4. Qian Hu

Qian Hu is a TCM commonly used for the treatment of asthma. Its major constituents, coumarins, were presumed to be responsible for its efficacy. Qian Hu was shown to reduce AHR and airway eosinophilic inflammation significantly, improve pathologic lesions of the lungs, reduce levels of IL-4, IL-5, and IL-13 in BALF and OVA-specific IgE in serum, inhibit the expression of TGF-β1 in lungs, and upregulate levels of IL-10 and IFN-γ in BALF, as well as the percentage of $CD4^+CD25^+Foxp3^+$ regulatory T cells in the spleen [48, 49]. This suggests that Qian Hu has great therapeutic potential for the treatment of allergic asthma.

6.5. Gan Cao

Gan Cao, commonly called "licorice," is one of the most commonly used herbs in TCM. Airway eosinophilic inflammation is a major feature of allergic asthma. Eotaxin-1 is involved in the recruitment of eosinophils to sites of antigen-induced inflammation in asthmatic airways. Licorice flavonoids can inhibit eotaxin-1 secretion by human fetal lung fibroblasts in vitro [50]. Licorice flavonoids also significantly reduced eosinophilic pulmonary inflammation, serum IgE, IL-4, and IL-13 levels but also increased IFN-γ production in lung cell cultures in response to antigen stimulation [51]. Glycyrrhizic acid is the main bioactive ingredient of licorice and has been shown to exert anti-asthmatic effects by modulating Th1/Th2 cytokines (IL-4, IL-5, IL-13 inhibition, IFN-γ increase in BALF) and enhancing $CD4^+CD25^+Foxp3^+$ Tregs in OVA-

sensitized mice [52]. Histological studies demonstrated that glycyrrhizic acid substantially inhibited OVA-induced eosinophilia in lung and airway tissues [52].

6.6. Sang Bai Pi

Sang Bai Pi (*Cortex mori radicis*), the root epidermis of *Morus alba* L., has been traditionally used for cough treatment in TCM. In OVA-induced asthma model mice [53], Sang Bai Pi significantly reduced AHR, inhibited the production of histamine and IgE in serum, and decreased airway eosinophil infiltration in BALF and lung tissue. Sang Bai Pi significantly attenuated the secretion and mRNA levels of Th2 cytokines, such as IL-4, IL-5, and IL-13. In addition, Sang Bai Pi significantly increased mRNA expression of IFN-γ, a Th1 cytokine. Furthermore, Sang Bai Pi can exert anti-asthmatic effects by enhancing $CD4^{+}CD25^{+}Foxp3^{+}$ Tregs.

6.7. Ban Xia

Ban Xia is a commonly used Chinese herb, with high bioactivity against cough and vomiting, and eliminating the stagnation of phlegm. Ban Xia significantly attenuated OVA-induced influx of the total number of leukocytes, eosinophils, neutrophils, macrophages, and lymphocytes into the lungs, decreased airway mucus production, and attenuated levels of IL-4, IL-5, IL-13, and TNF-α, in a dose-dependent manner. Ban Xia also significantly reduced the plasma levels of histamine, total IgE and OVA-specific IgE [54, 55].

6.8. Bo He

Bo He has been reported to have pharmacological effects, including the lowering of body temperature and relaxation of the muscles of the digestive tract. The herb is traditionally used for the treatment of high fever, mild chills, cough, thirst, and sore throat and to combat nausea, vomiting, and flatulence. Bo He significantly inhibited eosinophils, neutrophils, lymphocytes, macrophages, and total cells in BALF of OVA-challenged mice. Bo He also decreased specific IgE and Th2 cytokines, such as IL-4 and IL-5, in BALF and lung tissue. Airway inflammation and hyperreactivity in asthma are likely to involve oxidative stress to the lung, and excess production of reactive oxygen species (ROS) by immune cells may play an important role in airway injury. An increase in the generation of ROS in the airway and BALF has been noted in OVA-induced asthma models. Bo He has been shown to reduce the ROS in the BALF of asthmatic model mice, as did montelukast, which has been used widely as an anti-asthmatic drug [56].

6.9. Da Huang

Da Huang is used to cure stomach illness and as a "cathartic" to relieve severe constipation as well as a poultice for fevers and edema caused by inflammation. Emodin, one of the major compounds of Da Huang, displays a number of biological activities, such as anti-microbial, immunosuppressive, anti-inflammatory, anti-tumor, and anti-atherosclerotic activities. Moreover, emodin attenuates mast cell-dependent passive anaphylactic reactions in IgE-sensitized mice. Emodin has also been shown to reduce IgE and Th2 cytokine levels in OVA-

http://dx.doi.org/10.5772/62333

induced asthma mice. The inhibition of AHR by emodin may be associated with the reduction of IL-4, IL-5, and IL-13 production and eosinophilia aggregation into the lungs [57].

6.10. Jie Geng

Jie Geng is commonly used as a cough suppressant and expectorant for treatment of common colds, cough, sore throat, tonsillitis, and chest congestion. An aqueous extract of Jie Geng inhibited OVA-specific IgE levels in BALF. Inflammatory cell infiltration and mucus hypersecretion were also inhibited by Jie Geng extracts. Furthermore, Jie Geng extracts decreased the generation of ROS in BALF, as well as NF-κB nuclear translocation in OVA-induced asthma mouse model [58]. Jie Geng is abundant in saponins, which inhibit IgE antibody-induced increases in IL-4 and TNF-α expression in RBL-2H3 cells. Saponins suppressed dinitrophenyl (DNP)–IgE antibody–induced phosphorylation of Syk, and further downstream, Changkil saponins (CKS) also inhibited the phosphorylation of Akt and MAPKs [59].

6.11. Huang Qin

Huang Qin is one of the most widely used medicinal herbs for the treatment of inflammation. Ethanol extracts of Huang Qin may effectively suppress inflammation by downregulating the expression of various inflammatory mediators (such as histamine) and reducing the production of inflammatory cytokines (such as IL-8 and TNF-α) as well as MAPK activation [60]. Skullcapflavone II is a flavonoid derived from Huang Qin (*Scutellaria baicalensis*). Skullcapflavone II significantly reduced AHR, airway eosinophilia, Th2 cytokine production, and TGF-β1 levels in BALF and lungs in an OVA-induced asthma mouse model [61].

6.12. Yu Xing Cao

Yu Xing Cao is also used in folk medicine for diuresis and detoxification and for its anti-viral, anti-bacterial, and anti-leukemic activities. It has been used for the treatment of cough, pneumonia, bronchitis, uteritis, eczema, herpes simplex, acne, and chronic sinusitis. Ethanol extracts of Yu Xing Cao downregulate the expression of IL-4, IL-5, thymus and activation-regulated chemokine (TARC), and CCR4 receptor but do not have the same effect on IFN-γ [62].

Herbal name (Latin name)	Pictures	Possible mechanisms
Zhe Bei Mu (*Fritillaria thunbergii*)		Inhibits mast cell recruitment; decreases serum IL-6, IL-8, and TNF-α; and inhibits MAPK pathway [43]

Herbal name (Latin name)	Pictures	Possible mechanisms
Xing Ren (*Semen Armeniacae Amarum*)		Decreases Th2 response; reduces IL-4 levels, eosinophilic inflammation, and AHR [44]
Huang Qi (*Astragalus membranaceus*)		Decreases Th2 response; increases IFN-γ level; decreases IL-4, IL-5, IL-13, and TGF-β1 levels; reduces eosinophilic and neutrophilic inflammation; reduces AHR, collagen deposition, and mucus secretion; increases $CD4^+CD25^+FoxP3^+$ regulatory T cells [45–47]
Qian Hu (*Peucedanum praeruptorum Dunn*)		Decreases Th2 response; increases IFN-γ and IL-10 levels; decreases IL-4, IL-5, IL-13, specific IgE, and TGF-β1 levels; reduces AHR, eosinophilic inflammation; increases $CD4^+CD25^+FoxP3^+$ regulatory T cells [48, 49]
Gan Cao (*Glycyrrhiza uralensis*)		Decreases Th2 response; increases IFN-γ; decreases IL-4, IL-5, IL-13, specific IgE, and eotaxin-1 levels; reduces eosinophilic inflammation; increases $CD4^+CD25^+FoxP3^+$ regulatory T cells [50–52]
Sang Bai Pi (*Cortex mori radicis*)		Decreases Th2 response; decreases IL-4, IL-5, IL-13, and specific IgE levels; reduces AHR, eosinophilic inflammation, and histamine release; increases $CD4^+CD25^+FoxP3^+$ regulatory T cells [53]

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Herbal name (Latin name)	Pictures	Possible mechanisms
Ban Xia (*Pinellia ternate*)		Reduces IL-4, IL-5, IL-13, specific IgE, and TNF-α levels; reduces eosinophilic inflammation and mucus production [54, 55]
Bo He (*Mentha haplocalyx*)		Decreases Th2 responses; decreases IL-4, IL-5, and specific IgE levels; reduces eosinophilic inflammation and mucus production [56]
Da Huang (*Rheum palmatum*)		Decreases IL-4, IL-5, IL-13, and specific IgE levels; reduces eosinophilic inflammation [57]
Jie Geng (*Platycodon grandiflorum*)		Decreases serum IgE, ROS scavenger; decreases IL-4 and TNF-α; decreases Syk-dependent cascades; inhibits MAPK and Akt pathways [58, 59]
Huang Qin (*Scutellaria baicalensis*)		Restores serum IL-8 and TNF-α; inhibits MAPK pathways; decreases Th2 response; decreases IL-4, IL-5, IL-13, specific IgE, and TGF-β1 levels; reduces AHR and eosinophilic inflammation [60, 61]

Herbal name (Latin name)	Pictures	Possible mechanisms
Yu Xing Cao (*Houttuynia cordata Thunb.*)		Decreases Th2 response; decreases IL-4, IL-5, and TARC levels [62]

Table 2. Single herbs frequently used for asthmatic children

7. Acupuncture use in children with asthma

Acupuncture is a TCM therapeutic approach involving the stimulation of points on the body by using needles. For thousands of years, acupuncture has been used to treat several conditions, including asthma. Other methods of stimulation are traditionally used, such as electroacupuncture, laser acupuncture, and transcutaneous electrical nerve stimulation. There is evidence that acupuncture can reduce eosinophils in peripheral blood and decrease secretory IgA (sIgA) and total IgA levels in the saliva and nasal secretions of patients with allergic asthma [63]. The role of eosinophil activation in asthma has been well documented. sIgA is a potent stimulus for eosinophils and represents the main trigger for eosinophil degranulation. After acupuncture treatment, the reduction of sIgA levels and the decrease in the numbers of eosinophils may be associated with the amelioration of eosinophilic inflammation in patients with allergic asthma [63]. The numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes in the peripheral blood were significantly increased, without significant cortisol changes, in patients with allergic asthma treated by acupuncture [63]. It has been shown that electroacupuncture is prominent in promotion of $CD4^+CD25^+FoxP3^+$ Tregs in an OVA-induced experimental model [64]. Furthermore, acupuncture has also been shown to inhibit AHR, eosinophils, neutrophils, specific IgE, Th1 cytokines, and the NF-κB pathway in OVA-induced experimental asthma [65].

8. Acupuncture point application

Acupuncture point application therapies, combining Chinese herbal medicine and acupuncture points, have been extensively applied for the treatment of allergic rhinitis (AR) and asthma [66]. Summer acupuncture point application treatment, also known as San-Fu-Tie or San-Fu-Jiu, is one type of direct moxibustion administered in the summer through the direct application of an irritating herbal paste to acupuncture points. The basic herbal prescription of San-Fu-Tie is usually composed of Bai Jie Zi (*Semen Sinapis Albae*), Xi Xin (*Herba Asari*), Gan Sui

(*Radix Kansui*), and Yan Hu Suo (*Rhizoma Corydalis*) [66, 67]. These herbs are ground into a powder, mixed, and made into paste using stale ginger juice. The standard acupoints include Fei-shu (BL-13) and Feng-men (BL-12), the meridians named Taiyang Bladder Meridian of Foot [67]. Numerous studies have shown significant efficacy through acupoint stimulation in treatment of asthma, such as improvement of lung function, a decrease in cytokines (IL-4, IL-6, IL-8, and IL-10), and restoring the Th1/Th2 balance toward Th1 [66, 67]. Few adverse effects have been reported, except for mild skin allergy, or local swelling and blisters.

9. Mind–body exercise

Mind–body exercise, such as *tai chi*, yoga, and meditation, may benefit people with chronic diseases. *Tai Chi Chuan* (*tai chi*), a Chinese traditional mind–body exercise with low-to-moderate exercise intensity, is thought to improve cardiopulmonary function in patients with chronic disease. *Tai Chi Chuan* has been shown to improve pulmonary function of asthmatic children [68]. Yoga training was reported to improve pulmonary function tests (FEV_1 and PEFR), quality of life, and decrease in the weekly number of asthma attacks, scores for drug treatment, and peak flow rate [69–71]. Meditation has also been shown to be a useful adjunct for treating asthma [72].

10. Further research

There is increasing evidence for the efficacy of TCM or other complementary therapies in the treatment of children with asthma, but this is still insufficient evidence for making recommendations about the value of TCM as an asthma treatment, as well-designed double-blind, randomized clinical trials are lacking.

11. Conclusion

Asthma is the leading cause of chronic illness and missed school days among childhood. In addition to standard treatment, the use of complementary therapy is increasing. TCM is a popular CAM in East Asia and throughout the world. There is increasing scientific evidence demonstrating that TCM has potential for the treatment of childhood asthma.

12. Abbreviations

AHR: airway hyperresponsiveness

ASM: airway smooth muscle

BALF: bronchoalveolar lavage fluid(s)

FEV_1: forced expiratory volume in 1 second

FVC: forced vital capacity

GM-CSF: granulocyte-macrophage colony-stimulating factor

ICAM-1: intercellular adhesion molecule 1

ICS: inhaled corticosteroids

IFN-γ: interferon gamma

IL: interleukin

LABA: long-acting beta-adrenoceptor agonists

MAPK: mitogen-activated protein kinases

MCP: monocyte chemotactic protein

MIP: macrophage inflammatory protein

MMP: matrix metalloproteinase

NF-κB: nuclear factor-Kappa B

OVA: ovalbumin

PEFR: peak expiratory flow rate

RANTES: regulated on activation, normal T cell expressed and secreted

SABA: short-acting beta-2-adrenoceptor agonists

TARC: thymus and activation-regulated chemokine

TCM: Traditional Chinese medicine

TGF: transforming growth factors

TLRs: Toll-like receptors

TNF: tumor necrosis factor

VCAM-1: vascular cell adhesion molecule 1

VEGF: vascular endothelial growth factor

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The clinical specificities developed in this book, particularly from those reported in the pediatric population to those reported in complex shapes at ACOS patients, emphasize the importance of identifying not only biomarkers but also critical aspects regarding the variability in pharmacogenomics responsible for the individual response to the different drugs on the therapeutic plan. The contribution of several well-known specialists with their profound knowledge inherent to this issue into different age groups and socio geographical contexts has resulted in this interesting book with relevant key contents in asthma.

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