

Phenotypes and Endotypes of Uncontrolled Severe Asthma: New Treatments

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■ Abstract

Severe asthma is a heterogeneous disease that affects only 5%-10% of asthmatic patients, although it accounts for a significant percentage of the consumption of health care resources. Severe asthma is characterized by the need for treatment with high doses of inhaled corticosteroids and includes several clinical and pathophysiological phenotypes. To a large extent, this heterogeneity restricts characterization of the disease and, in most cases, hinders the selection of appropriate treatment. In recent years, therefore, emphasis has been placed on improving our understanding of the various phenotypes of severe asthma and the identification of biomarkers for each of these phenotypes. Likewise, the concept of the endotype has been gaining acceptance with regard to the various subtypes of the disease, which are classified according to their unique functional or pathophysiological mechanism. This review discusses the most relevant aspects of the clinical and inflammatory phenotypes of severe asthma, including severe childhood asthma and the various endotypes of severe asthma. The main therapeutic options available for patients with uncontrolled severe asthma will also be reviewed.

Key words: Severe asthma. Biomarkers. Endotype. Phenotype. Treatment.

■ Resumen

El asma grave es una enfermedad heterogénea que constituye entre un 5-10% de los sujetos asmáticos, pero representan un porcentaje significativo del consumo de los recursos sanitarios. El asma grave se caracteriza por precisar tratamiento con altas dosis de corticosteroides, e incluye diversos fenotipos tanto clínicos como fisiopatológicos. Esta heterogeneidad dificulta en gran medida la caracterización de la enfermedad, y en la mayoría de los casos también la elección del tratamiento adecuado. Por esta razón, en los últimos años se ha hecho hincapié en la mejora en el conocimiento de los distintos fenotipos del asma grave, y en la identificación de biomarcadores para cada uno de ellos. Así mismo, también ha tomado fuerza el concepto de endotipo en referencia a los distintos subtipos de la enfermedad divididos en base a su mecanismo funcional o fisiopatológico único. En esta revisión serán discutidos los aspectos más relevantes de los fenotipos clínicos e inflamatorios del asma grave, incluyendo el asma grave infantil y los diferentes endotipos del asma grave serán discutidos en este capítulo. Así mismo, se revisan las principales opciones terapéuticas disponibles en este grupo de pacientes serán revisadas.

Palabras clave: Asma grave. Biomarcadores. Endotipo. Fenotipo. Tratamiento.

1. Introduction

Severe asthma is a heterogeneous disease characterized by the need for treatment with high doses of inhaled corticosteroids. It affects 5%-10% of asthmatic patients, although it accounts for a significant percentage of the consumption of health care resources [1]. Severe asthma comprises various clinical and pathophysiological phenotypes. Improved characterization of the disease could contribute to improved selection of appropriate treatment and a greater understanding of its pathophysiology and natural history. It could also help link genotypes with their phenotypic manifestations [2] and their natural history and prognosis. A greater understanding of the phenotypes of severe asthma could enable identification of biomarkers for each phenotype.

In recent years, the concept of the endotype has become increasingly important in the study of asthma. The term endotype is used to describe "a subtype of a disease defined by a unique or distinctive functional or pathophysiological mechanism" [3]. Therefore, the endotype represents a different classification of the phenotype, given that it defines an etiology or pathogenic mechanism.

The present review examines the most relevant aspects of the phenotypes and endotypes of severe asthma in adults and children and discusses new therapeutic approaches.

2. Phenotypes of Uncontrolled Severe Asthma

In general, asthma is described based on its various phenotypes, which are defined as the expression of an individual's observable characteristics that result from the interaction between the individual's genes (genotype) and the environment. These characteristics may be clinical, physiological, morphological, and biochemical.

However, to simplify classification, phenotypes may be divided into 2 main categories: clinical phenotypes and inflammatory phenotypes [4] (Table 1).

2.1. Clinical Phenotypes

The 3 most relevant clinical phenotypes are the following [5]:

- (a) Frequent severe exacerbations with periods of relative stability between exacerbations (asthma with frequent severe exacerbations)
- (b) Irreversible airway obstruction (asthma with fixed airflow obstruction)
- (c) Disease requiring systemic corticosteroids for its routine control (corticosteroid-dependent asthma)

2.1.1 Asthma with frequent severe exacerbations

More than 40% of patients with severe asthma experience severe exacerbations [5], which are the component most associated with the costs of severe asthma [6].

The best predictive factor for exacerbation in both children and adults is a previous history, suggesting that patients with

Table 1. Phenotypes of Uncontrolled Severe Asthma

Clinical Phenotypes

- Asthma with frequent severe exacerbations
- Asthma with fixed airflow obstruction
- Corticosteroid-dependent asthma

Inflammatory Phenotypes

- Persistent severe eosinophilic asthma
- Noneosinophilic severe asthma with increased neutrophils
- Severe paucigranulocytic asthma

Clinical Phenotypes of Childhood Asthma

- According to age range:
 - Wheezing in preschool children (0-5 years)
 - School-aged children (6-11 years)
 - Adolescents (12 to 17 years)
- According to treatment response
- According to the course of the disease
 - Rare but severe exacerbations
 - Brittle asthma
 - Severe asthma with sensitization to fungi
- Inflammatory phenotypes
 - In stable asthma
 - In acute asthma

frequent exacerbations are susceptible to exacerbations as a result of environmental and genetic factors [7].

In addition to a history of exacerbation, various risk factors for frequent exacerbations have been identified in patients with severe asthma. These are classified as potentially modifiable and nonmodifiable.

Potentially modifiable: exposure to cigarette smoke, exposure to allergens, obesity, gastroesophageal reflux, sinusitis, psychological factors, and occupational factors [8].

In the United States, the most frequent exacerbations were identified in nonwhite patients with a low socioeconomic status and high body mass index who were smokers and had associated psychological factors [9]. Occupational factors and pollution also appear to be associated with exacerbations. The factors most frequently associated with near-fatal asthma exacerbations were undertreatment, lack of compliance with treatment, and psychological factors such as anxiety and depression [10].

Nonmodifiable: viral infections, race/ethnicity, premenstrual asthma, and genetic factors.

Asthma exacerbations appear to have a seasonal component, with fewer exacerbations observed in the summer months. This seasonality may be due to the fact that viruses are the main trigger for exacerbations in both children and adults [9]. Recent studies indicate that the epithelial cells of patients with asthma are more suitable for the replication of rhinovirus than those of healthy individuals. This replication may be favored by an underlying inflammatory allergic process [11].

A subgroup of women who are predisposed to very severe exacerbations during menstruation has been identified, although the mechanism that produces the exacerbations is not clear [4].

Finally, with regard to the genetic factors involved in this phenotype, most studies link polymorphisms of the *IL4* gene and its receptor (*IL-4R α*) with severe exacerbations and,

more recently, with polymorphisms of the chitinase and *IL10* genes [12].

Asthma exacerbations are also characterized by a series of pathological changes, which vary depending on the type of exacerbation. Sudden-onset exacerbations are characterized by the presence of low-grade inflammation, neutrophilic inflammation (when it occurs), and constriction of bronchial smooth muscle, which is often related to bronchospasm in the absence of inflammation. Slow-onset exacerbations, on the other hand, are characterized by the presence of eosinophilic inflammation, mast cell degranulation, CD8⁺ lymphocytes, and abundant mucus plugs. The presence of CD8⁺ lymphocytes suggests a viral component, although various studies suggest that viral infections often lead to neutrophilia and, to a lesser extent, eosinophilia in exacerbations [9,13]. The presence of mucus plugs is due to poor fragmentation and proteolysis of glycoproteins in mucus, which favors increased accumulation and viscosity of mucus during exacerbations [14].

2.1.2 Asthma with fixed airflow obstruction

Although asthma is considered a disease with reversible airflow obstruction, one subgroup of patients develops irreversible persistent and progressive obstruction. The most widely used definition of fixed obstruction is a reduced postbronchodilator ratio of forced expiratory volume in the first second (FEV₁) to forced vital capacity (FVC) in a patient receiving treatment with high doses of inhaled corticosteroids (fluticasone 1000 µg/d or equivalent) during a stable phase of the disease for at least 4 weeks [15]. Prevalence varies between studies, ranging from 23% [16] to 60% [17] of patients diagnosed with severe asthma. Fixed obstruction is probably related to bronchial inflammation, which worsens after exacerbations, and airway remodelling [18]. A number of patients who develop asthma in adulthood can present a faster reduction in lung function; this is particularly true in males with nonallergic asthma [5,16]. In childhood-onset asthma, individuals who remain symptomatic in adolescence are the population that will most likely develop fixed obstruction. Patients with irreversible obstruction generally have a poorer prognosis than those with reversible obstruction. A number of studies have shown a genetic predisposition (variations in the *ADAM33* gene [19]) and no clear association with α -1-antitrypsin deficiency.

Several factors suggest a risk for developing irreversible obstruction. The onset of bronchial symptoms in childhood and smoking are the most widely recognized, although other factors can also contribute. These include female gender, development of nonatopic asthma in adulthood after an infection (*Chlamydia pneumoniae*, *Mycoplasma pneumoniae*), and sensitization to *Aspergillus fumigatus*, especially in patients with associated bronchiectasis [15].

Continuous exposure to an allergen in the workplace may lead to a more pronounced decrease in FEV₁ and a poorer prognosis. In some cases, if diagnosis is delayed, lung function does not normalize despite having stopped workplace exposure. Associations have been found between adult-onset severe asthma and exposure to high-molecular-weight agents (OR, 3.7; 95%CI, 1.3-11) and low-molecular-weight agents

(OR, 4.4; 95%CI, 1.9-10) [20]. Therefore, early diagnosis and cessation of exposure to the allergen are essential for preventing greater deterioration of lung function.

2.1.3 Corticosteroid-dependent asthma

In corticosteroid-dependent asthma, symptoms cannot be controlled, despite high doses of inhaled corticosteroids, and patients require daily doses of oral corticosteroids, which have numerous side effects. Reducing the dose of oral corticosteroids can often lead to clinical worsening and exacerbations [21].

This situation could be a consequence of resistance to the effects of corticosteroids; therefore, it is important to determine indicators of resistance to corticosteroids in this group. Corticosteroid-resistant asthma has been defined as disease in which FEV₁ improves by <15% after 14 days of treatment with oral prednisone (40 mg/kg/day) and by >15% after inhalation of salbutamol [21]. The indicators of response to corticosteroids include eosinophils in induced sputum, fraction of inhaled nitric oxide (FE_{NO}) levels, eosinophils in blood, and the results of asthma control tests, whose usefulness varies in adults and children [22].

2.1.3.1 Mechanisms of resistance or reduced response to corticosteroids

Several mechanisms have been proposed to explain the phenomenon of resistance to corticosteroids. Analysis of cell cultures from patients with therapy-resistant asthma have revealed reduced response to corticosteroids, namely, a lower number of receptors and decreased affinity for the drugs [23,24]. Using molecular biology techniques, defects have been observed in the nuclear translocation of these receptors, which interfere with the mechanisms of interaction between glucocorticoids and their response elements at the DNA level, namely, the glucocorticoid response elements [25]. A predominance of glucocorticoid receptor β (GCR- β) over GCR- α , which acts under normal conditions, has been found in inflammatory and respiratory epithelial cells [26,27]. GCR- β lacks the ability to bind with glucocorticoids and could act as a functional antagonist.

In corticosteroid-resistant asthma, alterations are produced in the acetylation/deacetylation of histones in various lysine residues (in particular, histone H4). This alteration leads to an increase in the expression of certain proinflammatory transcription factors such as the AP-1 protein activator and the nuclear factor κ B (NF- κ B) [28]. Oxidative stress affects the airway of many patients with severe asthma and patients with chronic obstructive pulmonary disease (COPD), contributes significantly to the inflammatory state of the airway, and alters histone acetylation mechanisms, which may contribute to insensitivity to corticosteroids [29]. Cigarette smoke stimulates these oxidative mechanisms and could be the source of the poor response to medication in some asthmatic smokers [30].

Enzyme disorders have also been described in the regulation of GCR in patients with corticosteroid-resistant asthma. The enzymes affected include protein kinases, such as mitogen-activated protein kinase, which are important for the activation of T lymphocytes [31], and MKP-1, whose phosphatase activity has been observed in the alveolar

macrophages of obese patients with high body mass indices [32]. Other enzymes, such as phosphoinositol-3-kinase can also play a role in the development of corticosteroid-resistant asthma [33].

In a recent study, Bhavsar et al [34] found metabolic disorders in the membrane phospholipids of alveolar macrophages in patients with severe corticosteroid-resistant asthma, namely, reduced lipoxin A₄ and increased leukotriene B₄. These metabolic disorders led to a proinflammatory state in the airway.

Knowing which of our patients are more likely to have a reduced response will allow us to establish early high-intensity therapeutic strategies in order to control the disease. Genetic studies have found polymorphisms at the level of NF- κ B, interleukin (IL) receptors, and other transcription factors that help identify these patients [35]. Biomarkers to classify the asthma phenotype and predict the degree of treatment response would be very useful. ten Brinke et al [36] found a direct relationship between the degree of eosinophilic inflammation in the airway and the positive response to corticosteroids. In addition to the determination of FE_{NO}, Teng et al [37] consider hydrogen peroxide levels in exhaled breath condensate to be a promising biomarker of the response to treatment and control of asthma.

Table 2 summarizes the mechanisms that are potentially involved in resistance to corticosteroids.

Table 2. Mechanisms of Resistance or Reduced Response to Corticosteroids

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- Reduction in the number of receptors
 - Reduced steroid receptor affinity
 - Alterations in the nuclear translocation of receptor-steroid complex
 - Predominance of steroid receptor β
 - Histone acetylation alterations
 - Increased airway oxidative stress
 - Protein-kinase enzyme alterations
 - Membrane phospholipid metabolism disorders
-

2.2. Inflammatory Phenotypes

Several phenotypes of severe asthma have been described, and each is associated with specific clinical and pathophysiological characteristics [38]. The distinction between phenotypes based on inflammatory markers has been improved by the introduction and use of noninvasive techniques for measuring airway inflammation [18]. These techniques facilitate the collection of numerous samples when compared to invasive methods such as bronchoalveolar lavage and bronchial biopsy [39]. Noninvasive methods allow for the analysis of cells and mediators in induced sputum, peripheral blood, urine, exhaled gases (FE_{NO}, exhaled breath condensate), and volatile organic components using electronic noses (e-nose) [39].

Induced sputum is a reproducible and validated technique for the assessment of airway inflammation in severe asthma that makes it possible to analyze cells and mediators in the supernatant [40]. Measurement of FE_{NO} is reproducible,

safe, and well tolerated, although the cutoff that indicates increased inflammation is different for each FE_{NO} analyzer [41]. Interpretation of the results of exhaled breath condensate measurement remains limited [40], and the analysis of volatile organic components using e-nose shows promising results, although it is still under assessment [42].

Three cellular phenotypes have been described.

2.2.1 Persistent severe eosinophilic asthma

Persistent severe eosinophilic asthma is characterized by eosinophilia in bronchial biopsies and induced sputum despite high doses of inhaled or systemic corticosteroids [5]. Studies suggest that between half and two-thirds of patients with severe asthma have persistent eosinophilia in the main airway [21]. The presence of eosinophils is characterized by more symptoms, lower FEV₁ values, and more severe exacerbations [43] than the noneosinophilic subtype. It is also associated with a higher incidence of sinus disease, greater involvement of the peripheral airway and remodelling, fixed obstruction of the airway, and a good response to treatment with the monoclonal antibody anti-IL-5 [5,44].

Within the phenotype of persistent severe eosinophilic asthma, we can find specific differences according to whether onset is early (<12 years) or late (>12 years). Persistent eosinophilia is more prevalent in early-onset asthma, regardless of the dose of corticosteroids used [45], and in the presence of higher levels of cysteinyl leukotrienes [21]. However, the levels of a number of mediators in tissue and in disorders such as subepithelial basement membrane thickening are similar at any age of onset [45].

In this phenotype, it is unclear whether pulmonary eosinophilia is caused by type 2 helper T cell (T_H2) inflammation. Although allergic asthma is more common in early-onset severe asthma, patients with allergic asthma have persistent eosinophilia less frequently than those with late-onset severe asthma. Likewise, patients with severe asthma in the ENFUMOSA study had lower rates of atopy than those with milder asthma, although they did have a higher percentage of hypersensitivity to aspirin [46]. More studies are required to establish an association between persistent eosinophilia and T_H2-dependent inflammation.

2.2.2 Noneosinophilic severe asthma with increased neutrophils

In this group, eosinophils are either absent from the airway or suppressed by treatment despite the presence of several symptoms, with inflammation of the airway characterized by an increased percentage of neutrophils [5]. Occasionally, neutrophils and eosinophils are present concomitantly in tissue [21]. These increased neutrophil counts have been detected in sputum, bronchoalveolar lavage fluid, and biopsy tissue from patients with severe asthma receiving high doses of corticosteroids. Neutrophilic inflammation can be due to a concomitant disease (eg, bronchiolitis obliterans), residual inflammation resulting from the reduced eosinophil count after treatment with corticosteroids, and the inhibitory effect of corticosteroids on the apoptosis of neutrophils [21].

The presence of neutrophils is associated with increased

levels of matrix metalloproteinase 9 (MMP-9, eg, lipocalin) in bronchoalveolar lavage fluid and in tissues and with reduced lung function. In severe asthma, corticosteroids do not reduce expression of MMP-9 in vivo or in vitro [21].

2.2.3 Severe paucigranulocytic asthma

Severe paucigranulocytic asthma does not involve inflammation by the classical cell types in the bronchial biopsy [21]. The pathogenic mechanism is poorly understood, since inflammation may be located in the distal airway, which is inaccessible for biopsy. It may also be due to a bronchiolitis-type disease. In severe paucigranulocytic asthma, no thickening of the subepithelial basement membrane or signs of classic inflammation are observed. Other inflammation pathways and other cell types could also be activated [45,47].

3. Phenotypes of Severe Asthma in Children

Various classifications of the phenotypes of severe asthma in children have been proposed based on clinical and/or pathological findings. We can use the patient's age to classify clinical phenotypes [48]. Other authors have proposed establishing subphenotypes based mainly on the type of inflammatory cells in induced sputum or on a combination of factors (eg, pattern of symptoms, sputum cellularity, and airway functioning) [48-50].

3.1 Clinical Phenotypes of Childhood Asthma

Childhood covers the period from birth to 14 or 18 years of age, depending on the center. Such a broad age range means that symptoms, pathophysiology, diagnostic tools, and therapeutic arsenal differ considerably.

3.1.1 Clinical phenotypes according to age range

Wheezing in preschool children (birth to 5 years): Few data are available on asthma in preschoolers. The typical pattern consists of good baseline control and exacerbations caused by viral infections, which may occasionally be severe. However, it is not uncommon for preschoolers to experience chronic symptoms, often with early-onset atopy [48].

The average age of symptom onset for mild-to-moderate asthma in children is 60 months, although most children with severe asthma have symptoms in the first 24 months of life. Children with severe asthma also have a high prevalence of atopic dermatitis and positive prick test results with aeroallergens, which can be used as an indicator of severe asthma in small children. Only preschoolers with wheezing who present sensitization to aeroallergens experience asthma symptoms and airflow obstruction between the ages of 6 and 13 years [51].

Preschoolers with wheezing show considerable phenotypic heterogeneity in terms of the inflammatory pattern. This heterogeneity leads to different responses to treatment. Neither eosinophilia in the airway nor thickening of the reticular basement membrane is identified in children aged 12 to 24

months. Other preschoolers, however, have neutrophilic inflammation patterns and increased IL-8 levels, perhaps owing to viral respiratory infections [52].

School-aged children (6-11 years): Children who experience persistent wheezing from preschool age will present airflow obstruction at 9 years that will last until adolescence. In this age group, severe asthma is characterized by air trapping and increased residual volume and total lung capacity. In addition, children who have bronchial hyperreactivity by age 9 are 3 times more likely to present airway remodeling in adulthood [53].

As for histopathology, children with severe asthma have greater amounts of smooth muscle in the airway and a denser vascular network. However, thickening of the basement membrane is similar to that of patients with mild-to-moderate asthma.

Immunologically, children with severe asthma are characterized by a higher level of expression of CD4⁺ T lymphocytes, a greater number of activated eosinophils, and lower levels of IFN- γ and IL-5. The inflammatory response is expressed as higher levels of FE_{NO}. Eosinophils are common in the airway, and when neutrophils are detected, they coexist with the eosinophils [54].

Adolescents (12 to 17 years): Patients who continue with asthma into adolescence often have factors associated with atopy, namely, airflow obstruction and severely a positive response to bronchodilators and methacholine.

The baseline FEV₁ of severely asthmatic adolescents is lower than the theoretical value for their age group and does not reverse after bronchodilation, suggesting that airway remodeling patterns are established before adolescence [55].

3.1.2 Clinical phenotypes according to treatment response

In contrast to asthma in adulthood, no consensus has been reached on how to measure the response to corticosteroids in childhood. First, there is no agreement on the dosage, administration route, or duration of treatment with corticosteroids. Moreover, measurement of the therapeutic response using spirometry is of limited value in pediatric asthma [49]. Therefore, other parameters have been suggested, such as assessment using the Asthma Control Test questionnaire [56], bronchodilator response, reductions in FE_{NO}, and reductions in eosinophilia in sputum [57].

For patients whose disease is refractory to corticosteroids or who require high doses of corticosteroids for maintenance treatment, alternative therapeutic options include omalizumab, methotrexate, ciclosporin, and intravenous immunoglobulin [58-60].

3.1.3 Clinical phenotypes according to disease course

Depending on the degree and number of asthma exacerbations, 3 characteristic patterns of childhood asthma can be identified [49].

Rare but severe exacerbations: Patients who experience rare but severe exacerbations have stable baseline asthma, which is sometimes optimally controlled, although exacerbations are difficult to treat and are often due to viral infections. They can

also occur after massive exposure to an allergen. In children, no evidence of eosinophilic airway inflammation between attacks has been found. The measures to be taken in these cases include initiating basic treatment with low-dose inhaled corticosteroids, optimizing lung function and baseline control, and minimizing exposure to allergens [61].

Brittle asthma: Brittle asthma has received little attention in the pediatric population. Type 1 consists of chaotic and extended oscillations in peak flow, while type 2, which is less common and harder to treat, presents as a single severe reduction in peak flow in patients with good baseline control [62].

Severe asthma with sensitization to fungi: Patients with this pattern have severe asthma and a positive skin test or specific immunoglobulin (Ig) E to airborne fungi. In addition, a diagnosis of allergic bronchopulmonary aspergillosis should have been ruled out. Patients have responded to treatment with itraconazole in isolated cases [63].

3.2 Inflammatory Phenotypes

Phenotypic classification of childhood asthma based on the inflammatory pattern is problematic and can easily vary over time. Moreover, given that children with eosinophilic asthma can have bacterial bronchitis or viral infection of the lower airway, inflammation can progress from an eosinophilic pattern to one of mixed cellularity. It can also progress to a neutrophilic phenotype, thus preventing its inclusion in a specific phenotype; however, this does not involve a change in the baseline pathophysiology of the disease [64]. Neutrophilic inflammation is usually associated with early wheezing.

In patients with mild asthma and in patients with severe asthma, the primary impairment occurs in the distal airways, where the number of eosinophils is increased.

Inflammatory subphenotypes are classified according to the predominant cells in sputum as eosinophilic (>3% eosinophils), neutrophilic (>61% neutrophils), and paucigranulocytic (both cells are within normal values) [50].

3.2.1 Inflammatory phenotypes of stable asthma

The 2 main phenotypes of airway inflammation in children with stable asthma are paucigranulocytic asthma and eosinophilic asthma. Neutrophilic asthma is very rare. The eosinophilic subphenotype is the most severe and occurs in atopic patients with more deteriorated lung function and greater bronchial hyperreactivity than those of patients with the paucigranulocytic subphenotype [65].

3.2.2 Inflammatory phenotypes of acute asthma

Asthma exacerbations in children mostly present an eosinophilic inflammatory pattern. Once again, the disease is more severe in patients with this pattern. Given that IL-5 promotes differentiation and proliferation of eosinophils, the most recent clinical trials focus on treatment with anti-IL-5 [66].

Although the classification of childhood asthma into clinical subphenotypes has major limitations, it is essential for a proper understanding of the disease and helps to improve treatment strategies [67].

4. The Search for New Phenotypes of Severe Asthma

In many cases, characterization of patients with severe asthma based on a single biomarker or clinical feature is insufficient to accurately describe the various phenotypes, because no correlation is found between biological and clinical markers [20]. Therefore, it is possible that each factor provides independent information.

New approaches, such as multivariate statistical cluster analysis, can objectively identify homogeneous subgroups of patients (clusters) based on a considerable amount of data, thereby leading to a hypothesis that is not biased by the a priori approaches of the researcher [66]. Several cluster-type analyses have been performed in the context of the American Severe Asthma Research Program (SARP) and in other studies [69,70]. The first study to use this approach observed that various aspects of the disease (airway obstruction, bronchial hyperreactivity, and eosinophilic inflammation) contribute independently to the disease [71].

Two recent studies identified subgroups or clusters of refractory asthma. The study by Moore et al [69] (SARP cohort) differentiates between 3 clusters: 1 in which a correlation is found between eosinophilic airway inflammation and symptoms (early-onset atopic asthma) and 2 with considerable discrepancy between expression of symptoms and airway inflammation (obese women with symptomatic asthma and late-onset asthma with a considerable inflammatory component) [5,69]. Four clusters have been identified in children [70] and are classified mainly according to the presence of atopy, age at onset, and airflow restriction.

In an effort to define severe asthma more accurately, a European consortium is working on the project "Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome (U-BIOPRED)". This project uses an innovative diagnostic algorithm based on data from invasive tests (bronchial biopsies) and noninvasive tests (peripheral blood, exhaled air, sputum) and clinical symptoms to correctly identify patients with severe asthma [5]. This new approach could contribute to more detailed phenotyping, which will in turn facilitate better diagnosis and treatment.

5. Endotypes: A New Asthma Classification

For many years, it has been debated whether asthma is a single disease with a variable clinical presentation or different diseases whose common link is the presence of variable airflow obstruction [72]. Asthma is often typically described in terms of the characteristic phenotypes, without these being necessarily related to the underlying mechanism. The hypothesis of the endotype, however, proposes a different type of classification, in which asthma syndrome is divided into different entities with specific biological causal mechanisms called asthma endotypes. Thus, an asthma endotype can encompass various phenotypes, and a specific phenotype may be present in several endotypes. The characteristics of these endotypes have been

recorded in the PRACTALL consensus (PRACTical ALLergy), which was developed by experts from the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy Asthma and Immunology [72].

In order to differentiate between the various endotypes, 7 different parameters have been identified as clinically relevant. Six endotypes that meet at least 5 of the 7 suggested parameters (clinical characteristics, biomarkers, lung functionalism, genetics, histopathology, epidemiology, and response to treatment) have been proposed. Comorbidities were not selected as defining criteria of the endotype, given that they could influence the phenotype but not the endotype. Severe asthma is included in various endotypes depending on the base mechanism. Wenzel [3] recently proposed another classification of severe asthma endotypes. These endotypes and those of the PRACTALL consensus [72] are shown in Table 3.

6. Therapeutic Alternatives and New Treatments

One of the current challenges in asthma research consists of developing new treatments aimed at patients with more severe disease. A number of options have become available for the treatment of airway inflammation, either through synthesis of new steroid molecules with fewer side effects or by using drugs that enable reduced dosing of the corticosteroids necessary to control the disease. Other therapeutic innovations attempt to interfere with or block the inflammatory cascade in the airway at various levels, either at the onset of the allergic inflammatory response by binding to IgE or by acting on various proinflammatory cytokines and chemokines. Numerous molecules and drugs have been researched for asthma therapy; however, very few are currently used or will be used in clinical practice. A few of these are briefly described below. Most of these molecules are in more or less advanced phases of research and have generated mixed results in their attempt to find a place within the therapeutic arsenal.

6.1 Immunomodulatory Drugs

Immunomodulatory drugs inhibit the immune response and

show some degree of anti-inflammatory activity. In patients with severe disease receiving long-term corticosteroid therapy, the use of immunomodulators may be an option to reduce the dosage of corticosteroids and their side effects.

6.1.1 Methotrexate

Methotrexate is the most studied immunomodulatory drug in patients with asthma [74]. Various investigations have been performed on corticosteroid-dependent asthmatic patients receiving oral methotrexate weekly at doses ranging from 7.5 mg to 30 mg. Although these studies are fairly heterogeneous in terms of their design and length of treatment [75-77], longer studies (some lasting several years) reveal a corticosteroid-sparing effect, even achieving withdrawal in certain cases. Therefore, methotrexate requires prolonged administration to be effective.

Three meta-analyses [78-80] encompassing 11 studies have confirmed a small but significant reduction in the doses of corticosteroids taken by patients who continue treatment with this drug.

Methotrexate can lead to abnormal liver test results, gastrointestinal discomfort, and stomatitis. Nevertheless, these side effects are reversible and abate after the drug is withdrawn. Although there are no parameters that can predict which patients will respond to treatment, the risk-benefit balance of methotrexate is superior to that of oral corticosteroids in daily doses greater than 10 mg administered on a continuous basis. Therefore, methotrexate could be considered a treatment of choice for patients with asthma [74].

6.1.2 Ciclosporin

Ciclosporin inhibits activation of the T lymphocytes involved in the pathogenesis of asthma. A meta-analysis [81] of 3 studies reported a modest reduction in doses of systemic corticosteroids in patients with severe asthma. Nevertheless, its dose-dependent renal toxicity and its deleterious effect on hypertension limit its use. Future studies with analogs of this drug, such as tacrolimus and pimecrolimus, both of which have better safety profiles, could prove useful in this type of asthma.

Current scientific evidence is insufficient to endorse treatment with gold salts [82], azathioprine [83], and

Table 3. Endotypes of Severe Asthma

PRACTALL Consensus ^a	Wenzel ^b
<ul style="list-style-type: none"> • Aspirin-sensitive asthma • Allergic bronchopulmonary mycosis • Allergic asthma (adults) • Preschoolers with wheezing and positive asthma predictive indices • Severe late-onset hypereosinophilic asthma • Asthma in cross-country skiers 	<ul style="list-style-type: none"> • Early-onset allergic asthma • Persistent eosinophilic asthma • Allergic bronchopulmonary mycosis • Obese female • Neutrophilic asthma

^aReference [72]

^bReference [3]

chloroquine [84], all of which have a questionable risk-benefit ratio.

6.1.3 Macrolide antibiotics

Asthma treatment guidelines do not recommend antibiotics for the treatment of asthma if a bacterial infection is not present. However, the use of antibiotics for asthma exacerbations is relatively common in emergency departments, especially for children [85].

The role of microorganisms such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* remains a subject of debate, both in exacerbations and in the chronicity of bronchial asthma [86]. As a result, it is logical that antibiotics, especially macrolides, have gained relevance in the treatment of asthma. However, a Cochrane review performed in 2005 did not find sufficient evidence to recommend the use of macrolides as a therapeutic option in patients with asthma [87]. A subsequent study by Simpson et al [88] concluded that clarithromycin seems to play a beneficial role as an anti-inflammatory agent in infectious and predominantly neutrophilic asthma. The role of antibiotics within the therapeutic arsenal for severe asthma remains to be defined, although future research may consolidate this role, especially in predominantly neutrophilic asthma.

6.2 Dissociated Corticosteroids

The usefulness of corticosteroids is limited by the inevitable side effects of prolonged high-dose treatment. The so-called dissociated or selective corticosteroids, such as AL-438 and ZK 216348, which interact with proinflammatory transcription factors in the nucleus but do not bind to GREs and other hormonal receptors, may constitute an important advance [89].

6.3 Long-acting Bronchodilators

6.3.1 Long-acting β_2 -adrenergic agents

β_2 -Adrenergic agents such as salmeterol and formoterol are clearly useful in severe asthma. Indacaterol, which recently came onto the market in Spain, has a rapid action and is longer-lasting, allowing for administration every 24 hours [90]. This drug is currently approved in Spain only for the treatment of COPD. Other molecules in this new group of drugs with ultralong action, such as carmoterol, vilanterol, and milveterol, are in more or less advanced phases of research [91].

6.3.2 Tiotropium bromide

Although asthma guidelines do not currently recommend the use of tiotropium bromide [92,93], recent controlled trials suggest that the addition of this agent is effective in asthma patients [94] and improves pulmonary function in patients with poorly controlled severe asthma [95].

6.4 Phosphodiesterase 4 Inhibitors: Roflumilast and Cilomilast

Phosphodiesterase 4 acts by hydrolyzing and inactivating cyclic adenosine monophosphate. The inhibitors of this

enzyme exert control on cell activation and may be powerful anti-inflammatory agents in bronchial asthma. Cilomilast and roflumilast show a certain degree of efficacy in the control of asthma, although roflumilast offers more compelling results [96]. Its side effects include nausea, diarrhea, and headache. Oral roflumilast is approved in Spain, although exclusively for use in COPD.

6.5 Biological Treatments

Various molecules have been tested in the treatment of corticosteroid-dependent bronchial asthma. Most are in more or less advanced phases of research. Currently, only omalizumab is approved for use.

6.5.1 Anti-IgE: omalizumab

This humanized monoclonal antibody binds to free circulating IgE, thus preventing its binding to mast cells, with a subsequent reduction in the release of mediators of the allergic reaction. A meta-analysis has confirmed its usefulness as a corticosteroid sparer and in reducing the frequency of asthma exacerbations [97]. As mentioned above, omalizumab is currently the only biological product approved for the treatment of asthma. It is well profiled and is used to treat the most severely ill patients.

6.5.2 Anti-IL-5: mepolizumab

If IL-5 is essential for the action of eosinophils in the inflammatory response, blocking this cytokine should prove useful in severe asthma. IL-5 acts primarily in the peripheral blood and to a lesser extent in the airways [98]. This observation may explain the contradictory results of various clinical trials, although it seems to be able to reduce the number of exacerbations and control airway remodelling [99].

6.5.3 IL-4 and IL-13 inhibitors: altrakincept and pitrakinra

Altrakincept is a soluble IL-4 receptor that captures the cytokine and prevents its binding to surface receptors and subsequent cell activation. It is a humanized recombinant protein that is administered using nebulization. Although efficacy and safety results are encouraging, altrakincept is in the experimental phase [100]. Ongoing clinical trials with humanized recombinants of soluble IL-13 receptors are under way in patients with severe asthma.

Pitrakinra is a recombinant protein with a double inhibitory effect on IL-4 and IL-13. It acts by binding to subunit α of the IL-4 receptor, which is shared by both cytokines and acts as a competitive antagonist to prevent cell activation. The results of various clinical trials on severe asthma indicate its efficacy in reducing bronchial hyperreactivity and in the late phase of the allergic reaction when administered by nebulization [101]. The effectiveness of pitrakinra in atopic dermatitis is also being studied.

6.5.4 Inhibitors of tumor necrosis factor anti-tumor necrosis factor: infliximab, etanercept, and golimumab

Infliximab is a chimeric anti-tumor necrosis factor (TNF) α

monoclonal antibody. Intravenous administration in patients with moderate asthma appears to reduce the frequency of exacerbations and the variability of peak flow values [102].

Etanercept is a soluble TNF- α receptor. A placebo-controlled study in patients with mild-to-moderate asthma showed decreased bronchial hyperreactivity and improved quality of life [103]. This slight beneficial effect has not been reported for severe asthma.

Golimumab is a humanized anti-TNF- α monoclonal antibody. Available data indicate that it is not effective for severe asthma, and serious concerns about its safety have been voiced [104].

6.5.5 IL-2 inhibitors: daclizumab

Daclizumab is a humanized monoclonal antibody that acts against the α subunit of IL-2 receptors in T lymphocytes by competitively antagonizing the binding of the cytokine to its receptor, thus inhibiting its biological functions. Daclizumab has been used to control kidney transplant rejection and in the treatment of multiple sclerosis. Busse et al [105] reported a positive effect on respiratory function and the symptoms of patients with corticosteroid-resistant asthma.

6.5.6 Inhibition of chemokines

Eotaxin has a chemotactic effect on eosinophils. In patients with mild and stable asthma, administration of modified oligonucleotides (TPI ASM8), which have an inhibitory effect on its CCR3 receptors, leads to a reduction in the immediate response and in sputum eosinophilia after bronchial provocation with allergens [106].

6.6 Bronchial Thermoplasty

Bronchial thermoplasty, a new technique to improve control of moderate-to-severe asthma [107,108], has been approved by a number of regulatory agencies, such as the United States Food and Drug Administration, for routine clinical use. It delivers thermal energy to the large airways during bronchoscopy to decrease the amount of bronchial smooth muscle. This intervention has been shown to reduce the frequency of asthma exacerbations and improve asthma control and quality of life over a 3-year period, with no significant complications up to 5 years [109]. It could be considered an additional option in the treatment of selected patients requiring oral and/or high doses of inhaled corticosteroids to control asthma. However, bronchial thermoplasty should be performed in specialized centers on patients who understand the potential benefits and side effects of this technique. The response to treatment varies from one patient to another. Consequently, additional studies are needed to establish accurate phenotyping of positive responders, durability of the effect, and long-term safety [110].

7. Conclusions

Asthma is a heterogeneous disease. Ongoing clinical trials are searching for new therapeutic targets and attempting to profile the phenotype of patients who will benefit from specific treatment. New biological markers need to be found so that we can make the most appropriate choice of therapy for patients

who present little or insufficient response to available options. All efforts should be aimed at offering on-demand therapy that is tailored to the patient. In other words, the patient should not be forced into rigid management schemes, which, in some cases, such as difficult-to-control severe asthma, may not be useful.

Conflicts of Interest

P Barranco has received speaker's honoraria from MSD, Novartis, and Chiesi. S Quirce has been on advisory boards and has received speaker's honoraria from AstraZeneca, GlaxoSmithKline, MSD, Novartis, Almirall, Altana, Chiesi, and Pfizer. C Pérez-Francés has received speaker's honoraria from Almirall and GSK. E Gómez-Torrijos has received speaker's honoraria from MSD, GSK, and Alk-Abelló. JM Olaguibel has received speaker's honoraria from Astra-Zeneca, Chiesi, GlaxoSmithKline, and MSD and has participated in research supported by MSD. J Delgado has received speaker's honoraria from GlaxoSmithKline, MSD, Novartis, Chiesi, Almirall, and Pfizer and has coordinated studies promoted by Esteve and Pfizer.

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