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EXPERT REVIEWS

Molecular phenotyping and biomarker development: are we on our way towards targeted therapy for severe asthma?

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Although different phenotypes of severe asthma can be identified, all are characterized by common symptoms. Due to their heterogeneity, they exhibit differences in pathogenesis, etiology and clinical responses to therapeutic approaches. The identification of distinct molecular phenotypes to define severe asthmatic patients will allow us to better understand the pathophysiology of the disease and thus to more precisely target the treatment for each patient. To achieve this goal, a systematic search for new, reliable and stable biomarkers specific for each phenotype is essential. This review focuses on the current known molecular phenotypes of severe asthma and highlights the need for biomarkers that could (either alone or in combination) be predictive of the treatment outcome.

KEYWORDS: severe asthma • molecular phenotypes • biomarkers • personalized medicine • target therapy • monoclonal antibodies • T helper 2

AQ1

Bronchial asthma is a highly prevalent chronic respiratory disease. It may be heterogeneous in etiology, pathogenesis, clinical manifestations and outcomes. Comorbidities, pathogenic aspects, symptom severity, natural history, treatment responsiveness and extrinsic and/or intrinsic factors are primarily responsible for this variability. According to the Global Initiative for Asthma Guidelines, asthma severity is classifiable based on different levels of intensity, with an estimated 5–10% of patients suffering from severe asthma.[1] As recently described by the American Thorax Society/European Respiratory Society Taskforce, *"severe asthma requires treatment with high dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids) to prevent it from becoming uncontrolled or which remains uncontrolled despite this therapy."*[2] This context demonstrates the true refractory nature of asthma.[3]

Despite the fact that pathogenic mechanisms of severe asthma have been detailed over the years, only a single clinical approach remains available for most patients. Over the last few decades, technological advances have achieved relevant progress, thereby providing significant improvements in the understanding and management of severe asthma.[4,5] Data from genomics, proteomics, transcriptomics, metabolomics and epigenetics studies demonstrated distinct functional and pathological mechanisms related to specific biomarkers and different treatment responses for the severe asthma phenotypes. This clinical entity was introduced into clinical practice several years ago and became increasingly known, accepted and shared.[6] As mentioned above, key elements are needed to cluster and better characterize patients to identify the phenotypes. Recently, it has been established that a single clinical characteristic is not adequate to define a phenotype if it is not integrated with several other factors. This integration of genetics, biology and clinical aspects should lead to better

AQ2

identified phenotypes matched to biomarkers and provide prognostic and potential therapeutic information, thereby resulting in an improved understanding of asthma pathogenesis and an increased ability to effectively treat complex heterogeneous diseases such as severe asthma. A successful identification of reliable and specific biomarkers for severe asthma should be the basis for the introduction of biomarker-related personalized medicine, where clinicians could be oriented to a specific therapeutic approach and severe asthmatics could receive the most effective drug or biological agent on the basis of their single profile.[7–9] In this review, the authors will focus on severe asthma and provide an overview of the evolving biomarkers, molecular phenotypes and target-related therapies, paying special attention to lymphocyte Th2 and non-Th2 pathways.

Biomarkers of severe asthma: an overview

In clinical practice, an ideal biomarker is a physical trait or a laboratory measurement that is reliable and reproducible and can help identify the best therapeutic approach, track changes in disease activity, confirm a diagnosis or predict a response. It should also be easy to collect and evaluate, noninvasive and inexpensive.[10,11] For example, in asthma and airway disease management, biomarkers can be assessed in the sputum,[12–15] bronchoalveolar lavage fluid (BALF),[16,17] bronchial epithelial brushings,[16,18] bronchial biopsies,[19,20] exhaled air via fractional exhaled nitric oxide (FeNO) evaluation,[13,21–24] exhaled breath condensate (EBC) analysis,[25–28] peripheral blood [12,13,29] and urine.[30,31] Diagnostic procedures such as bronchoalveolar lavage, bronchoscopy or bronchial biopsy are sometimes additionally used to evaluate airway inflammation and remodeling and to provide detailed pathological information on the patient's condition. Nonetheless, these are invasive techniques that may be risky and are not easily applicable in severe asthma patients. Compared to bronchoalveolar lavage and endoscopy with bronchial specimen collection, spontaneous or induced sputum cell counts have been the method of choice to investigate the inflammatory pattern of bronchial tissues for a long time.[32] Subjects with a concurrent increase in eosinophils (≥2%) and neutrophils (≥40%) usually have the clinical characteristics of severe asthma.[33] Several studies have demonstrated the high reproducibility of sputum eosinophil and neutrophil counts and proposed them as an index for the therapeutic adjustment and prediction of exacerbation or steroid responsiveness. [34,35] Blood eosinophilia was recently confirmed to be the best surrogate marker for the identification of sputum eosinophilia in moderate-to-severe asthma patients; this analysis was more specific and accurate then FeNO levels and serum periostin.[13] Over time, sputum and the other above-mentioned samples have become substrates for the implementation of new clinical research techniques and trials. The aim of previous studies was essentially to investigate cytokines (primarily Th1/Th2), chemokines, growth factors, metabolites, proteins and gene expression with a recognized or hypothetical pathogenic role in severe asthma. To date, the most relevant cells and mediators considered to be promising biomarkers are those belonging to the type 2 immune

response pathway; these biomarkers are known to be inherent to severe allergic asthma, which is the most common form of severe asthma.[2] The existence of two different functional subsets of human CD4+ T cells (termed Th1 and Th2) that produce distinct cytokine panels was demonstrated in mice in 1986 by Mossman *et al.* and later in human T cells.[36,37] During allergic sensitization and the immune response to allergic stimuli, the respiratory epithelium produces cytokines and chemokines such as thymic stromal lymphopoietin, IL-25 and IL-33. These molecules enhance the Th2 immune response by activating pulmonary dendritic cells and inducing the maturation of CD4+ progenitor cells towards a CD4+ Th2 functional cellular profile.[38] Activated Th2 cells produce specific cytokines (i.e., IL-4, IL-5, IL-9 and IL-13) that favor and enhance the Th2 response. IL-5 is the primary specific trigger for eosinophils. It drives the hematopoietic process from their bone marrow progenitors (CD34+IL-5Rα+) to their release, activation and tissue recruitment.[39] IL-9, which was previously classified as an exclusive Th2 cytokine, is also secreted by another novel subtype of CD4 + T cells termed Th9 cells. IL-9 induces T cell activation and mast cell proliferation and differentiation.[40] IL-4 and IL-13 are crucial for type 2 inflammation. These cytokines share a common receptor subchain (IL-4Rα) that is present in both type 1 (IL-4 exclusive) and type 2 (IL-4 and IL-13) receptors. IL-4 and IL-13 synergistically promote the allergen-specific synthesis of immunoglobulin E (IgE) from B lymphocytes. The secreted IgE binds to its receptor FcεRI on mast cells and basophils, resulting in its opsonization. Allergen-IgE complexes trigger the release of a cascade of preformed pro-inflammatory mediators (cytokines, interleukins, prostaglandins and leukotrienes) that contribute to inflammatory cell recruitment.[41] Moreover, IL-4/IL-13 together play a key role in airway remodeling and inflammation, causing increased NO production, airway hyperresponsiveness, goblet cell hyperplasia, mucus hypersecretion, the induction of epithelial inducible nitric oxide synthase (only IL-13) and periostin expression in bronchial epithelial cells and lung fibroblasts.[42] The measurement of FeNO is a simple, quantitative, noninvasive, reproducible and safe method to assess airway Th2 and eosinophilic inflammation and to monitor the responsiveness of inhaled corticosteroid (ICS) therapy.[22,43] It has been standardized for clinical use in adults and children but is not considered to be an effective biomarker for severe asthma management.[2]

Periostin is a secreted matricellular protein that is involved in chronic inflammation in allergic diseases and tissue remodeling. [44,45] Thus, serum periostin is considered to be a promising marker of Th2 inflammation and airway eosinophilia despite the fact that it is not lung specific.[12,18,46] We recently studied airway remodeling in severe allergic asthma patients treated with omalizumab. We evaluated bronchial biopsies collected pretreatment and 12 months after anti-IgE therapy using morphometric and proteomic approaches.[19,20] The authors' data showed that Galectin-3 could serve as a predictive biomarker of airway remodeling improvement.

Th2 cytokines also trigger the production of eotaxins, which are strong chemoattractant proteins. Eotaxin-1 is involved in
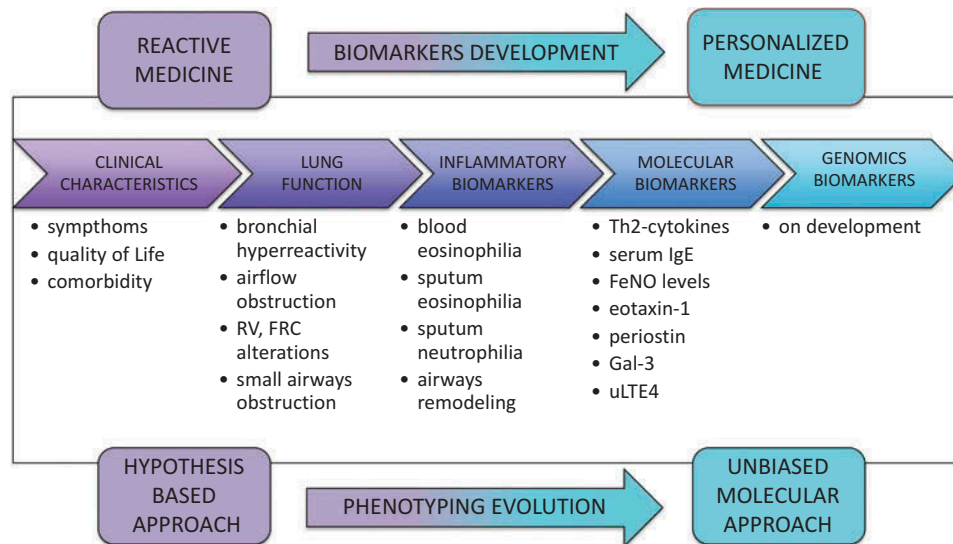
Figure 1. Biomarker discovery and phenotyping in severe asthma: actuality and perspectives. FeNO: Fractional exhaled nitric oxide; FRC: Functional residual capacity; Gal-3: Galectin-3; IgE: Immunoglobulin E; RV: Residual volume; uLTE4: Urinary leukotriene E4.

eosinophil recruitment and activation and has been detected in the blood, sputum, BALF and EBC. Recently, Wu *et al.* proposed eotaxin-1 as a novel potential biomarker for the assessment of asthma severity and control status.[47]

Finally, various markers of airway nitrosative/oxidative stress and inflammation (leukotrienes and lipoxines) have been found to be elevated in EBC from severe asthmatics compared with healthy subjects.[27,48]

Leukotrienes are released from various cells, including eosinophils, neutrophils and mast cells, and can also be detected in the urine. Urinary leukotriene E4 is used as a systemic marker of LT production, and high levels in asthmatic patients seem to be linked to a better response to leukotriene antagonist administration [30] (Figure 1).

Despite ongoing treatment with high doses of ICS, some severe asthmatics present a Th1 inflammatory signature characterized by the production of (IFN)-γ and reduced Th2 and IL-17 responses.[49] However, Th17 cells (CD4+IL-17+ expressing T cells) have been recognized to play a key role in the promotion of neutrophil selective migration to the site of airway inflammation.[50]

Severe asthma molecular phenotyping

As mentioned above, severe asthma patients are heterogeneous in terms of their severity, natural history and treatment responsiveness; this heterogeneity is partially related to the underlying mechanisms. Nonetheless, they are characterized by common features that allow clinicians to group them into so-called "phenotypes." [6] In this context, phenotype means *the observable properties of an organism that are produced by the interaction of the genotype and the environment.*" [51] Each phenotype exhibits a subtype of a condition that is defined by distinct functional

or pathophysiological mechanisms. These features or pathways are defined as "endotypes." [6,52] By applying the clinically oriented phenotype definition to traditional medicine, we can say that a *"phenotype"* is the result of a specific interaction between a patient's genes and the environment, and many different approaches have been proposed to characterize these subgroups in severe asthma. Clinical, biological and physiological characteristics have been used to classify the different forms of asthma.[53] Severe asthma phenotypes are currently identifiable by two main strategies: a hypothesis-based approach and an unbiased approach. Severe asthma phenotyping began decades ago using a biased approach. Patients were classified into broad categories based upon a single variable, including disease severity, symptomatic triggers, age at onset, patterns of inflammation, exacerbations and airflow obstruction.[54–56] Using this approach, overlaps between different groups were common. In contrast, the statistically unbiased approach attempted to cluster patients to avoid the clinical biases. The Severe Asthma Research Program (SARP) study identified five asthma clusters using an unsupervised hierarchical analysis of the predominant clinical features. Each of the clusters contained subjects who met the ATS definition of severe asthma.[57] Another SARP study was performed on 161 asthmatic children. The authors identified four phenotypic clusters and placed children with severe asthma into each cluster.[58] Recently, Schatz *et al.* performed a *post hoc* cluster analysis on a large number of severe or difficult-to-treat asthmatic children, adolescents and adults belonging to "The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens" study cohort. Cluster-defining variables were chosen, including sex, race, atopy, age at onset and others. Five clusters were identified in each age stratum, and the association between clusters and asthma-related health outcomes were
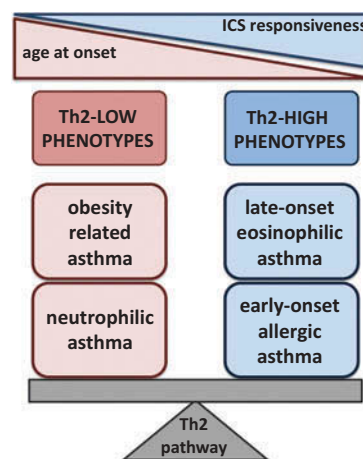
searched. The results confirmed the existence of distinct pheno-types that were related to outcomes in adults and adolescents but not in children.[59] It became progressively clear that the above-mentioned clinical clusters were not adequate to fully explain and clarify patients' phenotypes. Thus, studies of molecular pheno-typing analysis were developed to investigate the molecular path-ways underlying the clinical phenotypes. In a randomized control trial, Woodruff *et al.* analyzed airway epithelial brushings from 42 mild-to-moderate nonsmoking asthmatic patients and nonsmok-ing healthy subjects using microarray and PCR analyzes. The authors also investigated airway epithelium gene expression to explore its possible dysfunctional mechanism in asthma and reported that the *chloride channel, calcium-activated, family member 1 (CLCA1), periostin, and serine peptidase inhibitor, clade B (ovalbumin), and member 2 (serpinB2)* genes were upregulated in the asthma patients. In *in vitro* studies, the authors found that this enhanced gene expression was stimulated by IL-13.[60] Based on the hypothesis that IL-13 could be used to identify subsets of asthma patients, the gene expression of cells isolated from epithelial brushing were re-evaluated by taking the high or low expression of IL-13-inducible genes into consideration. The experimental results suggested the classification of asthmatic patients into cohorts in two subgroups according to the degree of Th2-driven inflammation. IL-5 and IL-13 expression, airway hyperresponsiveness, ICS responsiveness, serum IgE and blood and airway eosinophilia were significantly increased in the "Th2-high" group versus the "Th2-low" group/healthy controls.[18] The authors analyzed induced sputum cells from 37 asthmatic patients and 15 healthy control subjects to better profile the epithelial cell gene expression following IL-13 exposure. The genes evaluated included periostin, *LCA1* and *SERPINB2*, Th2 genes and other genes involved in the Th2 response. Periostin and *CLCA1* (but not SerpinB2) exhibited significantly increased expression in the sputum-obtained cells from asthmatic subjects. Induced sputum proved to be an easy-to-collect biological sample method for molecular evaluation. IL-4, IL-5 and IL-13 tran-scripts could be easily detected in sputum cells from asthmatic patients, and their expression levels were proposed for use to classify asthma into Th2-high and Th2-low endotypes.[14] Thus, the concept of "severe asthma molecular phenotypes" was defined.

Th2-high phenotypes, their biomarkers and targeted therapy

Historically, asthma has been always considered a Th2 disease that is linked to atopy, allergy and eosinophilic inflammation, and therefore, is responsive to corticosteroids (CSs). The major-ity of patients fit this common context, but asthmatics are a heterogeneous group that range from allergic asthma to aspirin-induced asthma to exercise-induced asthma (EIA) [61] (Figure 2). EIA is usually found in individuals who suffer from bronchoconstriction following strenuous exercise, fre-quently under cold and dry conditions. In this phenotype, increased levels of mast cells and their mediators can be observed in addition to the Th2 component, although the underlying

Figure 2. The primary defined Th2-high and Th2-low severe asthma phenotypes. ICS: Inhaled corticosteroid.

inflammatory response is not fully understood and no specific biomarker has been described.[62] These subjects are often responsive to anti-IL-9 therapy and cysteinyl leukotriene modi-fiers, but they usually have milder asthma and therefore will not be considered in this review.[63]

Overall, as the complexity of the immuno-inflammatory background increases, additional immune pathways are likely to be engaged beyond the original Th2 core, leading to greater severity and the reduction of ICS responsiveness.[64] Some biomarkers have been associated with Th2 inflammation, but many of them still need to be understood. Moreover, targeted Th2 pathway inhibition in non-phenotyped patients is less frequently effective, and therefore, new biomarkers need to be found to offer a tailored therapy to the patient (Figure 3).

Early-onset allergic asthma

This group accounts for approximately 40–50% of all severe asthmatics and includes subjects who developed their disease during childhood (often school age). These patients are char-acterized by an atopic condition and Th2 inflammation. Early-onset allergic asthma can present with mild-to-severe forms, and it is not clear whether severe asthma is the result of a progression from a milder form or instead arises as severe during childhood. [58] These patients are polysensitized, have high total IgE levels, a frequent family history of atopy and a clearly identified symptom exposure. Genome-wide association studies have evi-denced a genetic component linked to early-onset asthma, and the genes associated with this phenotype are epithelial-related rather than allergy-related.[65] Generally, CS therapy can mod-ulate Th2 cytokines and associated inflammation, but its activity is nonspecific. Thus, treatment with molecules that selectively target components of the Th2 pathway will be more appropri-ate.[56] High FeNO levels, sputum eosinophils and increased airway periostin have been proposed as biomarkers for Th2-associated asthma and could be used to identify patients respon-sive to IL-4/IL-13-targeted therapy. Periostin identifies a
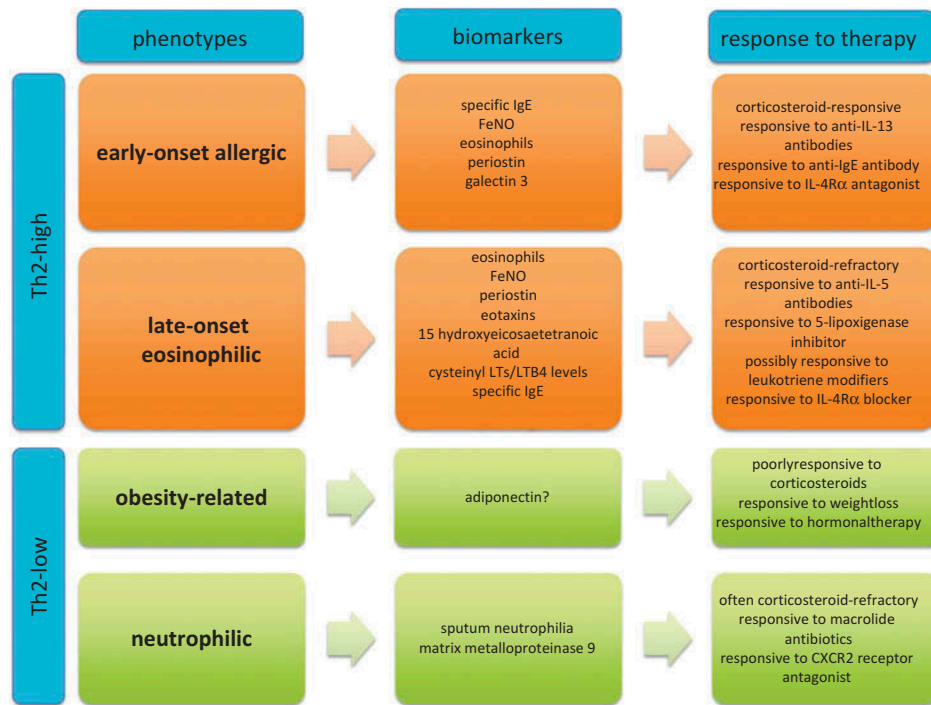
Figure 3. Currently known aspects of asthma phenotypes. CXCR2: CXC chemokine receptor 2; FeNO: Fractional exhaled nitric oxide; Ig: Immunoglobulin; IL4Rα: Interleukin 4 receptor alpha; LT: Leukotriene.

subgroup of moderate-to-severe asthmatics with lung eosinophilia who respond to therapy with a monoclonal antibody directed against IL-13 (lebrikizumab), whereas IgE levels, atopy and blood eosinophils do not allow the prediction of a favorable clinical response. Only subjects with a high serum concentration of periostin showed an improvement in lung function parameters, and to a lesser extent exacerbations, following lebrikizumab therapy.[66] Piper *et al.* recently evaluated another anti-IL-13 antibody (tralokinumab). In this study, only patients with sputum IL-13 levels above 10 ng/ml showed a good response, with a significant improvement in the asthma control questionnaire score and forced expiratory volume in 1 s.[67] The presence of high IgE levels makes this group an ideal target for anti-IgE treatment; however, the available data evidenced a modest efficacy of omalizumab in severe asthma patients with high IgE.[68,69] Recently, Hanania *et al.* described a retrospective analysis of a trial with omalizumab using the Th2 biomarkers periostin, FeNO and blood eosinophils. The authors split the asthmatics into two groups: subjects with high and low biomarker values. Subjects with high levels of these biomarkers responded better to omalizumab therapy than subjects with lower levels. Moreover, patients on placebo therapy in the Th2-high group had more frequent exacerbations than patients without Th2-high-related inflammation.[22] Recently, the authors' group demonstrated that the expression of Galectin-3 in bronchial biopsies of allergic severe asthma patients could also be used to predict the modulation of airway remodeling following omalizumab treatment.[20] In a 12-week study, Corren

et al. failed to demonstrate significant efficacy of an antibody to IL-4Rα in severe asthma patients, although a modest benefit was seen in the most symptomatic subjects.[70] Recently, the analysis of IL-4Rα polymorphisms allowed the identification of participants with a more favorable IL-4Rα antagonist therapy response.[71,72] A monoclonal antibody to IL-5 reduced blood eosinophils but did not show significant efficacy in these patients, suggesting that other inflammatory components may have a more important role than eosinophils. Additional data would certainly improve our knowledge concerning the pathological mechanisms involved in this phenotype and enhance our tools for the prediction of the degree of the treatment response.

Late-onset highly eosinophilic asthma

Several studies with patients characterized by late-onset disease have observed persistent blood and lung eosinophilia despite treatment with inhaled and oral CSs. This phenotype represents a more heterogeneous and less well-defined group of subjects and includes approximately 25% of severe asthma patients.[56] Adult eosinophilic asthma is severe at the time of onset and is sometimes associated with chronic sinusitis and nasal polyps without a clear atopy history. Moreover, this group also includes patients with aspirin-exacerbated respiratory disease (AERD). AERD refers to patients with non-IgE-mediated responses to aspirin and other cyclooxygenase-1 inhibitors; the cysteinyl leukotriene pathway in these patients is upregulated by Th2 cytokines. Eosinophilia is not specific for this phenotype, suggesting that other biomarkers may be

more relevant. Many patients are characterized by a strong Th2 skew, with increased FeNO, periostin, eotaxins, 15-lipoxygenase-1 and 15-hydroxyeicosaetetranoic acid.[16,73] Moreover, an increase in urinary cysteinyleukotrienes can be observed. Generally, eosinophils undergo apoptosis in the presence of CSs because Th2 inflammation is usually CS-sensitive.[74] In this phenotype, a persistent eosinophilia is indicative of CS refractoriness, and high systemic doses are able to overcome this CS inefficacy.[75] The lack of a significant response to CS treatment in this phenotype implies that the Th2 process differs from the process associated with the early-onset phenotype and is probably more complex. However, late-onset persistent eosinophilic asthma is associated with a better response to Th2-targeted therapy than early-onset allergic asthma, and the presence of eosinophilic inflammation detected in blood (or preferably sputum) identifies patients responsive to selective anti-eosinophilic therapies, including anti-IL-5.[76] IL-5 is a quite selective cytokine that is involved in the recruitment, maturation and activation of eosinophils, and therefore, is an optimal target for treatment. In these patients, the monoclonal antibody to IL-5 (mepolizumab) decreased the exacerbation rate, allowed systemic CS withdrawal and improved lung function.[77,78] Another anti-IL-5 monoclonal antibody (reslizumab) improved airway function compared with the placebo.[79] Moreover, beneficial effects on lung function in these individuals could also be obtained with 5-lipoxygenase inhibitors and leukotriene modifiers.[80] Finally, Wenzel *et al.* found that the IL-4Rα blocker pitrakinra was significantly better at reducing the exacerbation rate compared with the placebo in asthma patients with baseline blood eosinophils >350 mm^3. [81] Pitrakinra is a recombinant protein with a double inhibitory effect on IL-4 and IL-13. It acts as a competitive antagonist to prevent cell activation by binding to the α subunit of the IL-4 receptor, which is shared by both cytokines. Moreover, dupilumab (a human monoclonal antibody to IL-4Rα) seems to be associated with fewer asthma exacerbations and improved lung function in moderate-to-severe asthma patients with elevated eosinophil levels.[82]

Th2-low phenotypes, their biomarkers and targeted therapy

Th2-low asthma is generally identified by the absence of a clear Th2 aspect because several other factors (i.e., Th1 and Th17 immunity, neutrophil inflammation, mast cell activation, smooth muscle hyperreactivity, infection and oxidative stress) seem to play more important roles. Th2-low asthma probably represents a relevant percentage of all severe asthmatic patients, and the lack of efficacy of Th2-targeted treatments, including CSs supports the existence of asthma phenotypes not characterized by Th2 immunity. However, because the phenotypes included in this group are not well defined, detailed studies are needed to enable the definition of new phenotypes, specific biomarkers and clinical/pathological features.[83,84]

Obesity-related asthma

These patients are most commonly women with severe symptoms despite a moderately preserved lung function. The patients typically have a high body mass index, onset of disease in the fifth decade or later and less frequent history of allergic diseases. [85] The obesity condition is associated with a generalized inflammatory state with increased TNF-α, IL-6 and leptin expression, but confirmatory data are needed to identify potentially useful specific biomarkers.[86] Murine models of obesity demonstrated that adiponectin played a protective role and was inversely correlated with lung inflammation; however, in humans these data are discordant.[87–89] Obesity-associated asthma is usually less responsive to inhaled CS therapy than allergic asthma.[18] Whether the lack of response to CSs is due to additional immune pathways is not fully understood. Dixon *et al.* observed improvements in symptoms, quality of life and bronchial hyperresponsiveness in a group of nonallergic, obese, late-onset subjects after weight loss was achieved following bariatric surgery [90]; the same result was reported by Lombardi *et al.*[91]

Neutrophilic asthma

Several authors have detected increased neutrophil counts in the sputum, BALF and biopsy tissues from adult-onset, severely obstructed patients that were associated with a high dose of CS therapy and sometimes with high past/current tobacco use. Neutrophilia can coexist with eosinophilia. However, the definition of the specific role of neutrophils in asthma pathogenesis is difficult because neutrophilic inflammation can also be the result of concomitant diseases or can be derived from the CS inhibitory effect on the apoptosis of these cells.[92] Gene expression analysis of cells from sputum found increased expression of the TNF-α pathway and IL-1 concentrations in patients with neutrophilic asthma, although anti-TNF-α therapy did not show significant clinical efficacy in these subjects.[93,94] Enhanced expression of α-defensins and neutrophil proteases was also detected in the peripheral blood of these patients.[95] Moreover, the presence of neutrophils was associated with increased levels of matrix metalloproteinase 9 in the BALF and tissues, and these high concentrations were not reduced by CS treatment. Whether Th17 lymphocytes are involved in the pathological process is not clear because the majority of the data are derived from experimental models and studies in humans have not associated Th17 pathways with neutrophil asthma inflammation.[96] Currently, there are few neutrophil asthma-targeting drugs. Macrolide antibiotics have an anti-neutrophilic activity, and recently a CXC chemokine receptor 2 antagonist was shown to decrease sputum neutrophils in severe asthmatics.[97,98]

Other possible phenotypes

It is very likely that new distinct phenotypes will be added in the future to those described here based on current knowledge.[99] Further studies are necessary to better characterize severe asthma patients. Many authors have agreed with the existence of a pauci-granulocytic phenotype characterized by the absence of an

observable inflammatory process and a general lack of both sputum eosinophils and neutrophils. In this case, the underlying pathological mechanisms are poorly understood and add further difficulty to the selection of appropriate therapeutic approaches. Furthermore, smoking-associated asthma may also be a distinct phenotype that overlaps with chronic obstructive pulmonary disease.[100]

Conclusions

The so-called "personalized medicine" is expected to be more advantageous and possibly more cost-effective than the current diagnostic and clinical approach. A number of important aspects are still under investigation, including the identification of biomarkers that can be used to predict patient progression and therapeutic responses.[101,102] A more detailed investigation into biomarkers may help clinicians determine the best treatment based on the phenotype and the expected response. "Omics" approaches (genomics, proteomics and metabolomics) are expected to be of great help in identifying biomarkers and phenotypes. Currently, several drugs acting at different levels in the cascade of reactions involved in Th2 pathogenesis are under investigation, whereas there has been less progress for the so-called non-Th2 asthma (the obesity-related, neutrophilic and paucigranulocytic phenotypes).[103]

The identification of new asthma phenotypes is essential to make the best use of the new monoclonal drugs still under study and in the experimental phases. The possibility of introducing these biologicals in therapy allows us to integrate high-dosage therapy that often fails to control the symptoms of patients with severe asthma. The cooperation between academia, research and the pharmaceutical industry could provide new resources and tools to seek new phenotypes of severe asthma and related biomarkers for characterization.[104]

Expert commentary

The advances in the field of biomedical research are changing the world and contributing to the expansion and improvement of our knowledge, with important, crucial consequences for the management of complex and difficult to treat diseases such as severe asthma. Along the pathway of hard sciences, we progressively abandoned traditional reactive medicine, mass targeted therapy and descriptive studies. According to the concept of "heterogeneity of severe asthma," we focused our resources on phenotyping and investigating molecular pathways that led to more stratified medicine. Many biological drugs for Th2 pathway targets and specific biomarkers for severe asthma phenotypes and endotypes are now available in clinical practice. However, the state of the art in biomarker discovery and phenotyping still does not allow us to plan a personalized therapeutic approach and to predict the pathological evolution of severe asthma patients.

Five-year view

The recent advances in immunology, genetics and "omics" sciences have allowed us to better understand complex diseases such as severe asthma, which still represents a diagnostic and therapeutic challenge for clinicians. Research has focused on an improved and refined definition of the phenotypes and the identification of biomarkers that will allow a more accurate diagnosis and targeted prescription. According to recent opinions, it is important to move from generalized and standardized medicine, where every severe asthma patient is treated only on the basis of his/her symptoms, to personalized medicine where each subject will receive the best targeted therapy.[105] Severe asthma patients should be referred to highly specialized Asthma Units whenever possible to optimize both patient outcomes and the cost-effectiveness of the new biological agents and to improve our current knowledge of asthma mechanisms. This integrated approach by experienced clinicians and researchers would surely help to identify the definitive pathway to personalized medicine for asthma.

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Key issues

- The heterogeneity of severe asthma limits its characterization and makes the selection of appropriate therapeutic approaches difficult.
- The response to an anti-inflammatory drug depends on the presence of a specific type of airway inflammation.
- A biomarker can be indicative of a specific inflammatory process.
- A biomarker can be useful for the prediction of the degree of treatment response.
- New biomarkers need to be identified to allow clinicians to choose the most appropriate targeted therapy for each patient.
- In many cases, the characterization of patients with severe asthma with a single biomarker is not sufficient to identify the specific phenotype.
- Understanding the immune pathways beyond severe asthma inflammation is moving the concept of clinical phenotypes towards the concept of molecular phenotypes.
- Panels of different, noninvasive, stable biomarkers should be studied for application in daily clinical practice.
- Severe asthma patients should be referred to specialized units for optimal disease management.

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