



Clinical phenotypes of asthma should link up with disease mechanisms

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Purpose of review

Asthma is a common disease which presents in various clinical forms and levels of severity. The current 'one size fits all' approach to treatment is suboptimal. Using unbiased cluster analysis has identified several asthma phenotypes. Understanding the underlying mechanisms driving these clusters may lead to better patient-orientated medicines.

Recent findings

Clustering was initially performed on clinical features only, but the addition of biomarkers that characterize sputum and blood cellular profiles has enabled the prediction of responses to targeted therapies. Clusters of severe asthma include those on high-dose corticosteroid treatment associated with severe airflow obstruction and those with discordance between symptoms and sputum eosinophilia. Sputum eosinophilia can predict therapeutic responses to T-helper type 2 cytokine blockade. Further molecular phenotyping or endotyping of asthma will be necessary to determine new treatment strategies. Low T-helper type 2 expression may be predictive of poor therapeutic response to inhaled corticosteroids, but much less is known about this type of asthma.

Summary

Phenotype-driven treatment of asthma will be further boosted by the integration of genetic, transcriptomic and proteomic technologies to defining distinct severe asthma phenotypes and biomarkers of therapeutic responses. This will lead towards stratified medicine for asthma.

Keywords

neutrophilic asthma, sputum eosinophils, Th2 high

INTRODUCTION

There has been an ongoing debate that asthma should no longer be called asthma because this term groups 'diseases' caused by different triggers and pathophysiological mechanisms [1]. Hippocrates has been attributed as coining the Greek term 'αάζειν' (aazein) to denote breathing through the mouth or to pant, from which the term asthma is derived. Asthma has been defined clinically by the presence of an intermittent wheeze, dyspnoea and cough. This term may continue to serve clinicians well who are well aware of the varied presentation and natural history of the disease, such as the early onset versus the late onset of disease that may have different outcomes, and the varying severities of the disease which patients can present with. In addition, with the introduction of therapies such as corticosteroids and β -adrenergic compounds in the treatment of asthma, it is clear that there is a wide distribution of therapeutic responses to these pharmacological agents, which further indicates lack of uniformity. Coupled with this has been the recent advances made

in our understanding of the inflammatory response underlying asthma and in the associated pathophysiological mechanisms underlying this complex disease(s). The inflammatory response is now widely believed to be an essential part of the pathophysiology of asthma that it is now widely included in its definition. As described below, there is also a complexity of the inflammatory response in that it is likely that the different clinical phenotypes of asthma may be associated or 'caused' by different inflammatory mechanisms.

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Curr Opin Allergy Clin Immunol 2015, 15:56–62

DOI:10.1097/ACI.0000000000000134

KEY POINTS

- Unbiased clustering approaches have yielded several phenotypes of asthma characterized by age of onset of disease, lung function, bronchodilator response and asthma severity.
- Addition of inflammatory biomarkers such as sputum and blood eosinophils, nitric oxide levels in exhaled breath and serum periostin have provided more refined phenotypes with greater predictiveness of responses to targeted therapies.
- The next stage is the inclusion of transcriptomic, lipidomic and proteomic data which will yield even more refined phenotypes and endotypes that will be instrumental in the provision of stratified and personalized medicine.

Whereas potential phenotypes of asthma have been described by choosing particular characteristics of the asthma diathesis [2], the recent use of statistical approaches has allowed us to define phenotypes in an unbiased approach. With the realization that there is a group of patients with asthma called severe asthma whose disease do not seem to respond to currently available treatments [3[¶]], there has been great interest in phenotyping this particular group. It is in this group of patients that more efficacious treatments are needed. It is now becoming evident that severe asthma cannot be considered as one disease, but as a heterogeneous collection of several different phenotypes that could be determined by different pathway mechanisms. The idea that all asthma treatments are beneficial to all asthmatic patients is implicit in most current guidelines, but less applicable in the severe asthma patient. This review will focus the recent work in the past 4 years and will point out the path forward, the advantages of defining phenotypes of asthma and the importance of defining endotypes.

ASTHMA AS AN INFLAMMATORY DISEASE

The initial concept of eosinophils having an important role in this disease started with the description of eosinophils in the airways and lungs of patients who had died of asthma. The presence of blood eosinophilia in an asthmatic patient usually predicted a good therapeutic response to corticosteroid therapy [4]. There was a debate amongst scientists as to whether eosinophils were just a biomarker of asthma rather than an important effector cell in the pathogenesis of asthma, but recent research has in fact expanded the potential contribution of

eosinophils [5]. The issue regarding the role of other cells such as neutrophils, mast cells, B cells, and epithelial and airway smooth muscle cells in asthma was raised, and clearly all these cells are likely to contribute to asthma pathophysiology and inflammation. Clearly, the inflammatory response represents a complex interaction of these cells or could represent the heterogeneity of inflammatory cell involvement from patient to patient. Over the past 20 years, there has been focus on the T-helper (Th)2 activation pathway, which has led to the first biologic therapy being available for asthma treatment in the form of an anti-IgE antibody that binds to circulating IgE, a Th2 by-product. In the near future, biologics targeting other members of the Th2 cytokine family, namely interleukin (IL)-5 and IL-13, are likely to become available. However, other pathways such as the Th17 or T-regulatory (Treg) cells may also contribute to asthma pathophysiology, and whether these can underlie other different clinical phenotypes of asthma is an important question. In addition, such highly targeted therapeutic approaches will need to be used in conjunction with a biomarker that can predict responders to these therapies [6].

DEFINING PHENOTYPES BY CLINICAL CRITERIA

Statistical models that have been most commonly used for asthma phenotyping have included the hierarchical clustering that builds models based on distance connectivity and the k-means algorithm representing each cluster by a single mean vector [7–9]. The Severe Asthma Research Program (SARP) adult and paediatric cohorts (Fig. 1), the UK Leicester cohort, the European cohorts of Epidemiological Study on the Genetics and Environment of Asthma (EGEA2), the European Community Respiratory Health Survey (ECRHSII) [10] and cohorts from Korea and Japan [11[¶],12] report phenotypes that have common features with the identification of patients with little airflow obstruction and activity of disease, patients with early age of onset of disease with an atopic background and a more severe group of asthma patients associated with adult-onset disease and continued symptoms despite therapy. Age of onset of disease, lung function and atopic state featured highly in these clusters or phenotypes (Fig. 1). In the Outcomes and Treatment Regimens (TENOR) cluster analysis, five clusters distinguished by sex, atopic status and non-white race were reported in an adolescent and adult cohort and in a paediatric cohort; aspirin sensitivity was a distinguishing feature in the adolescent and adult cohort [13[¶]]. Clusters 4 and 5 of the adult SARP

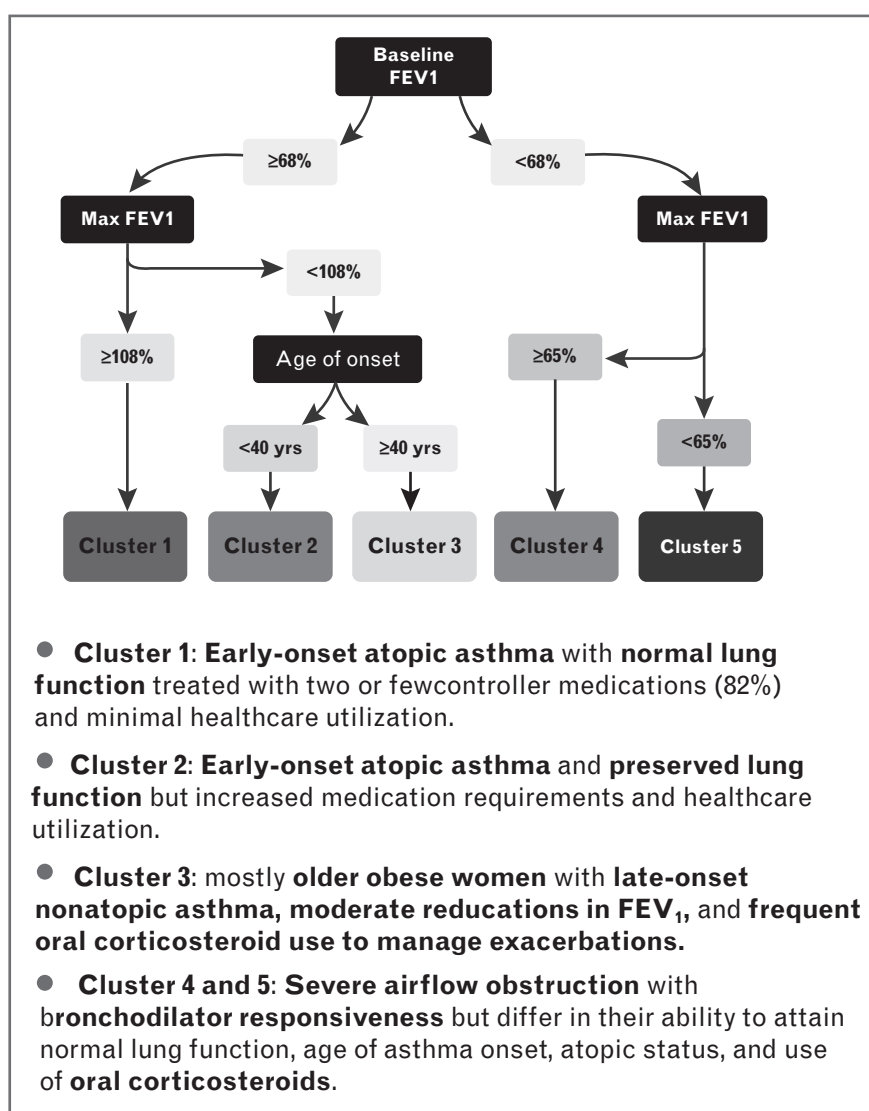


FIGURE 1. Upper panel: Tree analysis in the identification of adult asthma phenotypes using cluster analysis in the Severe Asthma Research Program (Moore *et al.* [7]). Using three variables (baseline FEV₁, maximal 'Max' FEV₁ after six to eight puffs of albuterol, and age of onset of asthma), patients can be assigned to the five clusters that range from milder asthma (cluster 1) to more severe disease (clusters 4 and 5). Lower panel: Description of the five clusters of the adult cohort. Reproduced from Moore *et al.* [7] with permission.

cohort described patients on high-dose inhaled corticosteroid (ICS) therapy, often taken together with oral corticosteroid treatment associated usually with severe airflow obstruction (Fig. 1). In the TENOR study [13[■]], a cluster of adolescents and adults was associated with aspirin sensitivity, in primarily white, female and atopic with late-onset asthma, and these patients were more likely than any patient in the other four clusters to experience exacerbations.

Clusters associated with obesity, defined in both the SARP and Leicester cohorts, have been described, in accordance with others [14,15[■]]. An obese uncontrolled asthma cluster and an obese well controlled asthma cluster have been recognized; these asthma

clusters differed from one another with respect to age of asthma onset, asthma symptoms and control, exhaled nitric oxide concentration and airway hyper-responsiveness, but were similar in terms of measures of lung function, airway eosinophilia and serum IgE [14]. A group of obese women with late-onset asthma and frequent symptoms with high healthcare use but with low sputum eosinophil counts has also been described [16].

PHENOTYPING/ENDOTYPING OF ASTHMA

Although phenotyping according to clinical and physiologic features may be useful to the clinician in terms of classifying patients, it does not provide

insight into mechanisms and neither does it help in terms of directing therapies. The addition of biomarkers into the phenotyping may provide an idea of pathophysiological mechanisms associated with a particular phenotype. Sputum eosinophil counts have been developed recently as a biomarker of asthma. Blood eosinophilia has long been known to indicate responsiveness to corticosteroid therapy [4] and increased sputum eosinophil has been associated with decreased asthma control and exacerbations in some asthmatic patients [17].

Cluster analyses that have included sputum eosinophilia and more recently concomitant sputum neutrophilia have been useful in defining corticosteroid-sensitive and insensitive groups. In the Leicester cohort of patients with refractory asthma, there was discordance between symptoms and the presence of sputum eosinophilia [9]. The cluster with minimal eosinophilic inflammation, as measured by sputum levels of eosinophils, was that of an early-onset, symptom-predominant group, with a high prevalence of obesity, and female sex. The cluster with an eosinophilic inflammation-predominant group had few symptoms, late-onset disease, and a greater proportion of men, with a high prevalence of rhinosinusitis, aspirin sensitivity and exacerbations. In the Amsterdam cohort of adult-onset asthma, the cluster associated with severe eosinophilic inflammation had persistent airflow obstruction with low symptom scores, whereas low sputum eosinophil scores was observed in obese women with frequent symptoms and high healthcare utilization [16].

Cluster analysis of the SARP cohort that included sputum eosinophil and neutrophil counts described severity-dependent phenotypes ranging from mild-to-moderate allergic asthma with eosinophil-predominant sputum inflammation, to moderate-to-severe asthma with neutrophil-predominant or mixed granulocytic inflammation, indicating the potential importance of neutrophils in the more severe asthma patients [18[¶]]. However, it must be pointed out that the definition of increased sputum neutrophils was taken to be greater than 40%, which is a relatively low cut-off point. Indeed, eosinophil and neutrophil sputum numbers show wide variability in severe asthma with patients demonstrating none to very high levels of either cell [19,20]. Using 76% neutrophils as the cut-off point for defining neutrophilic asthma, Schleich *et al.* [21] reported only 18% of patients with asthma having a 'neutrophilic' type of asthma. Neutrophils form part of the cells recovered in sputum from normal individuals, and what constitutes an abnormal number of neutrophils is still unclear. Using the percentage of neutrophils in

induced sputum may not be the best biomarker for neutrophilic asthma.

TRANSCRIPTOMIC ANALYSES IN PHENOTYPING

The definition of mechanistic phenotypes has been termed endotyping [3[¶],4] and is most useful at defining targets for the development of new therapies and treatments for well defined phenotypes or endotypes of asthma. However, in order for an endotype to be described, the biomarker in question needs to be shown to contribute to the pathophysiology of the disease. One of the potential approaches is to use transcriptomic data to provide some support for biomarker involvement in phenotypic characterization.

Woodruff *et al.* divided mild-to-moderate asthmatic patients into Th2-high and Th2-low groups, according to the degree of expression of the IL-13-inducible genes, periostin, chloride channel regulator 1 and serpin peptidase inhibitor [22], and showed that the Th2-high asthmatic patients had greater degree of bronchial hyper-responsiveness, higher serum IgE levels, greater blood and airway eosinophilia, sub-epithelial fibrosis and airway mucin gene expression [23], and responded well to ICS. Those with a low Th2 signature, on the contrary, showed little or no response to ICS treatment. Th2-gene signatures can also be obtained by performing reverse transcriptase (RT)-PCR of sputum cells and are used to denote Th2-high patients with asthma [24[¶]]. Whereas all the eosinophilic patients defined by having a sputum eosinophilia of at least 2% had high Th2 expression, only 54% of those with high Th2 expression had raised eosinophil counts [24[¶]].

The use of gene expression profiling of induced sputum and unsupervised hierarchical clustering of these expression profiles has led to the description of three phenotypes: chronic airflow obstruction and less well controlled asthma, increased exhaled nitric oxide and sputum eosinophils; airflow obstruction and higher sputum neutrophils and higher sputum macrophages and lower eosinophils and neutrophils, and lung function in the normal range [25]. Over-expressed genes in the IL-1 and tumour necrosis factor (TNF)- α /nuclear factor (NF)- κ B pathways correlated with clinical parameters and neutrophilic airway inflammation. An unbiased omics approach to gene and protein profiling would be preferable to defining the various inflammatory clusters derived from sputum analysis since there are many factors that could determine each cluster. Using whole-genome gene expression microarray, a six-gene expression biomarker signature derived

from induced sputum that did not contain Th2-associated genes was shown to discriminate the eosinophil from the non-eosinophil inflammatory phenotypes and also in predicting clinical responsiveness to ICS therapy [26^{***}].

PHENOTYPE-DRIVEN THERAPIES

A major proportion of mild-to-moderate asthma patients either do not respond to or respond poorly to a middle dose of ICS in terms of improvement in forced expiratory volume in 1 second (FEV₁) [27]. Increased sputum neutrophilia may indicate corticosteroid (CS) insensitivity [20], and smoking and obese asthmatic patients are more likely to develop CS insensitivity [28,29]. The basis for neutrophilic asthma remains unclear and undefined. Bacterial colonization in the airways of patients with severe asthma [30,31] and defective phagocytosis of bacteria or apoptotic cells by macrophages [32,33] may contribute to sputum neutrophilia. Oral corticosteroid therapy can also cause tissue neutrophilia to some degree, together with Th1 and Th17 activation factors [34,35].

In the SARP cohort, the group of patients with severe asthma on systemic corticosteroids that could be considered to have CS insensitivity was more likely to report a diagnosis of recurrent bronchitis, to have an FEV₁ of less than 60% and a higher fractional exhaled nitric oxide [36^{*}]. Work on cells from patients with CS-dependent asthma has pointed out some of the mechanisms that may be involved in CS insensitivity, including modulation of p38 mitogen-activated protein kinase activity [37,38], inability to recruit glucocorticoid receptor-associated co-factors such as histone deacetylase 2 [39], reduced glucocorticoid receptor ligand binding [40] and increased expression of the glucocorticoid receptor-spliced variant glucocorticoid receptor-beta (GR-β) [41]. Further characterization of this phenotype is required.

Demonstration of efficacy of new therapies will depend in part on the precision by which patients can be phenotyped for specific therapies [42]. Biologics targeted against Th2 cytokines appear to provide best therapeutic responses in patients who show high blood or sputum eosinophilia. Omalizumab – a humanized monoclonal antibody to IgE – reduced the rate of severe exacerbations and emergency visits, together with an improvement in asthma quality-of-life scores, and high levels of exhaled nitric oxide levels and peripheral blood eosinophils were the biomarkers that tracked those with the greatest reduction in exacerbations [43]. Mepolizumab – an anti-IL5 antibody – was efficacious in reducing exacerbation rates in patients with

recurrent severe asthma exacerbations and eosinophilic inflammation [44]. In a supervised cluster analysis, three predictors of response in terms of a reduction in exacerbation rates were high blood eosinophils, low airway reversibility and high BMI [45]. Similarly, dupilumab – a human monoclonal antibody to IL4-Rα – was associated with fewer asthma exacerbations with improved lung function in patients with moderate-to-severe asthma who had elevated eosinophil levels either in blood or sputum [46]. A monoclonal antibody to IL-13, lebrikizumab, improved FEV₁ in moderately severe asthmatic adults – an effect that was observed in those with elevated levels of serum periostin – a biomarker of Th2 response – or with raised levels of nitric oxide in the exhaled breath [47].

Thus, sputum or blood eosinophils, serum periostin and levels of nitric oxide in exhaled breath could define a subset of patients that may respond well to Th2-targeted therapies. Use of the Th2 signature derived from airway epithelial cells could be used to choose patients who would respond to ICS therapy; exhaled breath levels of nitric oxide could be used as a surrogate marker for therapeutic responsiveness to corticosteroid therapy [48]. Serum periostin is a biomarker that could replace the use of epithelial cell expression of Th2 cytokines, and has been shown to correlate with airway eosinophilia [49]. On the contrary, high serum periostin levels may also denote those with chronic airflow obstruction [50] and a cluster of well controlled asthmatic patients with a rapid decline in FEV₁ [51^{*}]. More validated markers are needed for non-eosinophilic asthma. Raised levels of hydrogen sulphide in induced sputum have been proposed as a potential marker for neutrophilic asthma, associated with chronic airflow obstruction [52].

SYSTEMS BIOLOGY APPROACH FOR PHENOTYPING

Clustering of patients according to clinical features has classified asthmatic patients into clinically recognized groups, but has not advanced our knowledge of mechanisms or sub-phenotypes. Use of clinical, physiologic and genetic, transcriptomic, lipidomic, proteomic and imaging data will provide a more definitive, albeit more complex, phenotypic representation of the patients' disease, and will represent a step nearer to defining endotypes. Analysis and integration of these datasets will reveal the role of specific biologic processes involved in inflammation, immunity, cell cycle, apoptosis or metabolism underlying selective phenotypes, which represents true endotyping. Furthermore, epigenetic mechanisms may mediate the effect of

environmental factors such as road traffic pollution and cigarette smoking on the development and course of asthma [53]. For example, severe asthma is associated with specific changes non-coding RNA species in blood CD8+ T cells [54] and with changes in more than 1800 proteins linked to acute-phase response signalling, cell-to-cell signalling and tissue development in bronchial biopsies [55]. Mathematical modelling of these protein–protein interactions have been used to predict new drug targets in asthma [56]. The Innovative Medicines Initiative (IMI)-funded project on Unbiased Biomarkers of Respiratory Diseases (UBIOPRED) is using a systems biology approach to phenotype severe asthma and to find new therapeutic targets [57]. It is clear that the collection and analysis of clinical and physiologic parameters along with genetic, transcriptomic, lipidomic and proteomic analyses can be used to predict disease onset and therapy at a personalized level [58], and to sub-phenotype and indicate responsiveness to specific interventions [59]. The promise of the omics technology in defining phenotypes and endotypes is currently under intense investigation.

CONCLUSION

We are now moving towards endotyping. A number of important issues remain to be resolved, including the need to include biomarkers in future cluster analysis to aid endotyping, biomarkers that predict therapeutic responses, disease course and exacerbation rates and wider use of various ‘omics’ data in cluster analysis and the computational tools required to enable this approach [58]. The delivery of phenotypes and endotypes may yield ‘complex’ phenotypes depending on the granularity of the phenotype/endotype we would wish to achieve, but might prove to be more useful in predicting outcomes in response to highly targeted therapies. The challenge of delivering the benefits of stratified medicine to the patient remains high [60], but this is the roadmap to take by which the right medications will be delivered to the right patient at the right time.

Acknowledgements

None.

Financial support and sponsorship

The authors are funded through the Innovative Medicines Initiative in terms of the UBIOPRED project, which is using a systems biology approach to severe asthma. No funding was obtained for the writing of this manuscript.

Conflicts of interest

The authors declare no conflicts of interest in relation to the contents of this review.

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