Chapter

Childhood and Adult Asthma: Phenotype- and Endotype-Based Biomarkers

Joy N. Eze and Samuel N. Uwaezuoke

Abstract

The concept of asthma has changed from that of a single disease entity to that of a heterogeneous disease comprising several phenotypes linked to specific endotypes. Recently, significant progress has been made in disease classification into phenotypes and biologically distinct variants (endotypes). Classification of patients into endotypes has led to precision medicine in which specific biomarkers and appropriate individualized treatments have now been identified. Despite the ongoing classification of disease endotypes, the presence or absence of a T-helper 2 (Th2) molecular signature has resulted in the association of asthma endotypes with phenotypes so as to establish responders and non-responders to inhaled corticosteroid therapy. More importantly, biologic therapies predicated on disease endotypes may in future constitute a paradigm shift from the traditional pharmacologic treatments and lead to better prognosis in moderate-to-severe forms of the disease (in which they are presently used). This book chapter aims to discuss the current concepts on asthma classification and biomarker-based diagnosis.

Keywords: asthma, biomarkers, endotypes, heterogeneous disease, phenotypes

1. Introduction

Asthma represents one of the major childhood noncommunicable respiratory diseases worldwide [1]. Asthma is now seen as a complex heterogeneous disease with variable natural history, severity, comorbidities, and therapeutic response. The disease is thus defined in several ways. For instance, asthma is defined as an airway disorder with underlying chronic inflammation characterized by hyper-responsive airway, which results in nonspecific symptoms like recurrent wheezing, breathlessness, nocturnal or early morning cough, and chest tightness. The symptoms tend to change over time and intensity, in conjunction with variable airflow limitation [2]. The disease also represents a syndrome with several phenotypes (the observable physical characteristics from the gene–environment interactions) and endotypes [3]. Research within the last decade has sought to better understand the heterogeneous nature of asthma. Disease heterogeneity particularly manifests in the clinical features, as well as the type and degree of airway inflammation and remodeling. Thus, there is now a paradigm shift in the concept of asthma as a single disease entity to that of a complex cluster of disease phenotypes [4]. Various subtypes of inflammation and complex immunoregulatory pathways and the factors responsible for their failure have now been documented.

2. Asthma phenotypes and endotypes: A snapshot

An endotype is a subtype of a disease recognized by a characteristic pathophysiologic mechanism, whereas a disease phenotype refers to any identifiable characteristic without any evidence of a mechanism [5]. Recent advances in asthma management have tried to group patients by a plethora of possible phenotypic features including age of onset, presence of atopy, airway inflammation and severity of airway obstruction, and the need for drugs. On the basis of the diverse cellular and molecular mechanisms, several phenotypes are currently recognized [6]. Using sputum cytological examination, there is now a classification of the major inflammatory phenotypes into eosinophilic, neutrophilic, mixed-complex inflammation, and pauci-granulocytic phenotypes [7]. Other recognizable phenotypes include early-onset mild allergic asthma, late-onset asthma associated with obesity, and severe nonatopic asthma with frequent exacerbations [8]. Experts from the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma, and Immunology produced the PRACTALL (Practical Allergy) consensus report which proposed the use of parameters such as clinical features, biomarkers, pulmonary physiology, genetics, histopathology, therapeutic response, and epidemiology for characterizing disease endotypes [9]. The consensus of opinion was that each endotype should meet at least five of these seven criteria [9].

3. Phenotype- and endotype-based biomarkers

Biomarkers are unique parameters linked to disease endotypes which are estimated for the evaluation of any biologic or pathogenic processes, including responses to therapeutic interventions [5]. Their use has made it possible for novel diagnostic tools and targeted therapies to be developed.

3.1 Phenotype-based inflammatory biomarkers

Several biomarkers are now veritable sources of information with respect to disease phenotypes and therapeutic responses. The major examples are described as follows:

3.1.1 Inflammatory cells

Marked blood eosinophilia has been linked to a severe form of late-onset asthma. In fact, blood or sputum eosinophilia is an indicator of Th2-type inflammation in the lungs, while sputum eosinophilia is associated with exacerbations [10, 11] and airway remodeling in asthma [12, 13]. The actions of T-helper 2 (Th2) cells are believed to trigger the stimulation of eosinophilic infiltration into the airways. Eosinophils are made to evolve from an inactive state to a state of increased hyper-responsiveness by priming agents such as these cytokines, interleukin (IL)-3, IL-4, IL-5, and IL-13 [8], and granulocyte-monocyte colony-stimulating factor (GM-CSF) [14]. IL-4 and IL-13 upregulate vascular adhesion molecules and facilitate the migration of eosinophils into tissue sites of inflammation, while IL-5 facilitates differentiation, survival, and chemotaxis of eosinophils [8, 13, 14].

3.1.2 Proteins

While Th2 cytokines can be assayed from bronchial washings, the approach may not be practicable. Proteins emanating from the bronchus which are linked to Th2 airway inflammation are used as surrogate markers for disease phenotype and endotype. Three genes upregulated by Th2 cytokines (IL-13) have been identified, namely,

POSTN, which encodes periostin; CLCA1, which encodes calcium-activated chloride channel regulator 1; and SERPINB2, which encodes serpin peptidase inhibitor, clade B (ovalbumin), member 2 (serpinB2, also known as plasminogen activator inhibitor-2) [13]. Increased levels of these proteins correlate with higher amount of bronchial tissue IL-13 and IL-5 messenger RNA and elevated number of eosinophils and mast cells. Blood levels of periostin have been studied as a surrogate marker for airway eosinophilia and as a method for predicting response to pharmacologic IL-13 blockade with lebrikizumab and anti-IL-13 antibody. The findings of these proteins in subjects with high Th2 also correlate with better response to ICS therapy than Th2-low group. Thus, identification of these proteins is predictive of corticosteroid-responsive asthma.

3.1.3 Epithelial microRNAs

There is a high differential expression of microRNAs (miRNAs) in the airway epithelium of subjects with asthma as compared with healthy controls [15]. MicroRNAs have been identified as regulators of key biologic processes in epithelial cells such as cell proliferation, cell differentiation, and cell death [16, 17]. Woodruff et al. conducted in vitro experimentation with bronchial epithelial cells and observed that IL-13 had obvious effects on bronchial epithelial miRNA expression and that several of these changes underscored the differences between asthma and health that were seen in humans [13]. Subsequent work focused on constant in vivo and in vitro suppression of four members of the miR-34/449 family (miR-34c-5p, miR- 34c-5p, miR-449a, and miR-449b-5p) in asthma and by IL-13, respectively. These data lend credence to the possible biological role of the miR-34/449 family in airway epithelial cells. It is uncertain whether the potential regulation of miRNAs, or others by IL-13, can be an indicator of a high-Th2 asthma endotype. However, miRNAs possess a relatively distinct characteristic which may qualify them as potential biomarkers. In other words, miRNAs can assume forms in extracellular fluids which are resistant to breakdown by RNases and thus can be estimated in sputum, bronchoalveolar lavage fluid, and blood using PCR, microarrays, and sequencing methods [13]. The following proteins, miR-181a, miR- 146a, and miR-146b, are expressed in spleen CD41 T lymphocytes and probably function as proinflammatory agents in an animal model of asthma [18, 19]. Specifically, there was downregulation of miR-375 in IL-13 transgenic mice and its repression in human bronchial (and esophageal) epithelial cells by IL-13 [20]. In addition, miRNA let-7 possesses a complex but proinflammatory activity in an animal model of the disease [21].

3.1.4 Exhaled nitric oxide (FeNO)

There is a moderate correlation between exhaled nitric oxide and bronchial or blood eosinophilia in asthmatics. The enzyme nitric oxide (NO) synthase that produces NO is under direct regulation of IL-13, which is a Th2 cytokine. Elevated FeNO level reflects increased IL-13 activity [22] and indicates the presence of Th2 phenotype. The FeNO is a consistent predictor of a potential steroid responsiveness more than other indices (spirometry, airway hyper-responsiveness to methacholine, bronchodilator response, peak flow variation, etc.) [23].

3.2 Asthma endotypes and associated biomarkers

3.2.1 Allergic asthma

This is a form of persistent asthma which commences in the pediatric-age period. Sensitization to allergens and allergic rhinitis are prominent features. Inhalation of a specific allergen is a stimulus for the acute constriction of bronchial

smooth muscles and subsequent infiltration of inflammatory cells, usually followed by a late asthmatic presentation [9]. This condition is believed to be sustained by a Th2-dominant inflammation. Airway eosinophilia is a common feature, and the disease comprises a wide spectrum of disease severities and therapeutic responses. The explorations of IL-4/IL-13 pathway modifiers and the effectiveness of omalizumab in severe allergic asthma underscore the role of IgE and Th2 cells/cytokines in this endotype. Children with asthma predictive indices (API) are susceptible to developing asthma and may or may not include the classic"allergic asthma endotype." The API include presentation with recurrent wheezing episodes (more than three episodes in the first 3 years of life) and at least one of the three major criteria (personal atopic dermatitis, parental asthma, or sensitization to an aeroallergen) or two of the three minor criteria (peripheral eosinophils >4%, wheezing unrelated to the common cold, or sensitization to a food allergen) [24, 25]. Patients who fulfilled these criteria at 3 years of age are clearly at increased risk of manifesting with active asthma symptoms at 6 years of age [9, 25].

3.2.2 Allergic bronchopulmonary mycosis (ABPM)

This condition develops in adults with asthma or in adult/pediatric patients with cystic fibrosis [26]. It is characterized by hypersensitivity reaction to airway colonization by molds, especially *Aspergillus fumigatus* [9, 26]. The main histological feature of ABPM is allergic (eosinophilic) mucin-harboring hyphae in the bronchi, as the induction of the formation of eosinophilic extracellular DNA cell death (ETosis) by viable fungi remains vital [26]. Clinically, ABPM is characterized by episodic bronchial obstruction and mucoid impaction, peripheral blood eosinophilia, elevated serum IgE levels, IgE and IgG antibodies specific for fungi, and typical radiographic findings [9, 26]. A mixed picture of neutrophilic and eosinophilic airway inflammation has also been described [9]. This endotype is characterized by severe bronchial asthma with recurrent exacerbations and progressive lung damage but may respond to systemic glucocorticoids, antifungal agents, and the anti-IgE monoclonal antibody (mAb), omalizumab [9, 26]. Patients develop bronchiectasis and fixed airflow obstruction over time. Early-onset ABPM may be a sequela of the allergic asthma endotype or cystic fibrosis [9].

3.2.3 Aspirin-sensitive asthma (ASA)

It almost always appears in adulthood and has a distinct clinical presentation, presenting after the intake of a nonsteroidal anti-inflammatory drug (NSAID) [9, 27]. Severe and prolonged airway obstruction is characteristically associated with chronic/severe rhinosinusitis and nasal polyps (aspirin-exacerbated respiratory disease), peripheral blood eosinophilia, and raised urinary leukotrienes at baseline and post-aspirin challenge. Pathophysiologically, ASA has been linked to increased elaboration of cysteinyl leukotriene and increased expression of leukotriene C4 synthase. Cysteinyl leukotriene receptor antagonists and leukotriene C4 synthesis inhibitors ameliorate ASA symptoms although these medications do not protect the patient from NSAID adverse effects [28].

A subgroup of individuals with late-onset asthma in adulthood fulfills the criteria for a distinctive asthma endotype. They constitute about 20% of patients grouped as having refractory asthma and exhibit a typical pattern of severe exacerbations which are circumvented by systemic corticosteroid but not ICS, as well as hyper-eosinophilia in the blood (>1000/mm^3) and sputum (>10%) [29]. These patients also have a lower prevalence of atopy than the "allergic asthma" endotype [9]. Moreover, the degrees of bronchodilator responsiveness and nonspecific airway hyper-responsiveness may be

less than those in the "allergic asthma" endotype. Studies have suggested that anti-IL-5 therapy may also be effective in this endotype [30, 31].

3.2.4 Cross-country skiers' asthma

It is defined as episodes of asthma symptoms and/or wheeze closely associated with strenuous skiing-related exercise and concomitant airway hyper-responsiveness. An extremely cold, dry climate promotes the evolution of this type of asthma in comparison with warmer, more humid conditions [32, 33]. Cross-country skiers' asthma is rarely associated with allergic sensitization but is characterized by airway inflammation dominated by elevated numbers of lymphocytes, macrophages, and neutrophils but rarely eosinophils. Lymphoid aggregates in the form of bronchus-associated lymphoid tissue in the mucosa, as well as evidence of airway remodeling with thickening of the reticuloepithelial membrane can be identified in bronchoscopic studies.

Amateur endurance runners had an elevated number of bronchial epithelial cells and apoptosis of bronchial cells in induced sputum evolving through repeated half-marathon races, in addition to increased serum levels of CC16 and raised supernatant interleukin (IL)-8 levels in induced sputum [34]. Furthermore, urinary levels of CC16 are increased following exercise [35, 36]. Increased expression as measured by polymerase chain reaction (PCR) of the gel-forming mucin, MUC5AC, in induced sputum and levels of supernatant cysteinyl leukotrienes and higher ratio of cysteinyl leukotrienes to prostaglandins have been reported. This endotype is resistant to ICS therapy, but its symptoms often improve with a drop in intensity of training.

Phenotype	Eosinophilic asthma
	Endotypes: allergic asthma (adult)*, aspirin-sensitive asthma, severe late-onset hyper-eosinophilic asthma*, ABPM*
Phenotype	Exacerbation-prone asthma
	Endotypes: allergic asthma (adult)*, aspirin-sensitive asthma*, late-onset hyper-eosinophilic asthma, API-positive preschool wheezers*, ABPM*, viral-exacerbated asthma, premenstrual asthma
Phenotype	Obesity-related asthma
	Endotypes: airflow obstruction caused by obesity, severe steroid-dependent asthma, severe late-onset hyper-eosinophilic asthma*
Phenotype	Exercise-induced asthma
	Endotypes: cross-country skiers' asthma, other forms of elite-athlete asthma, allergic asthma, API-positive preschool wheezers*
Phenotype	Adult-onset asthma
	Endotypes: aspirin-sensitive asthma*, infection-induced asthma, severe late-onset hyper-eosinophilic asthma*
Phenotype	Fixed airflow limitation
	Endotypes: noneosinophilic (neutrophilic) asthma
Phenotype	Poorly steroid-responsive asthma
	Endotypes: noneosinophilic (neutrophilic) asthma, steroid-insensitive eosinophilic asthma, airflow obstruction caused by obesity

Culled from [9].
**NB: asthma phenotypes can be present in more than 1 endotypes and endotypes can contain more than 1 phenotype.*

Table 1.
Proposed relationship between asthma phenotypes and endotypes.

Endotypes	Biomarkers
Asthma predictive index preschool wheezers	>4% eosinophil in blood (minor), aeroallergen-specific IgE
Allergic asthma (adults)	Positive SPT, elevated IgE/elevated FeNO
Severe late-onset hyper-eosinophilic asthma	Peripheral blood eosinophilia
Allergic bronchopulmonary mycosis (ABPM)	Blood eosinophilia, markedly elevated IgE and specific IgE
Aspirin-sensitive asthma	Blood eosinophilia, increased urinary LTEs
Cross-country skiers' asthma	FeNO normal, normal blood eosinophil count, increased urinary LTEs

SPT, skin prick test; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; LTEs, leukotrienes.

Table 2.
Biomarkers associated with some endotypes.

Obviously, the pathophysiologic mechanisms underlying the various asthma phenotypes and endotypes are diverse. Thus, the biomarkers of these phenotypes and endotypes are different but may be interwoven since phenotypes may be linked with more than one endotype and vice versa. **Table 1** shows the possible relationship between asthma phenotypes and endotypes, while **Table 2** shows some of the biomarkers associated with disease endotypes.

4. Conclusion

The pathogenic concept of asthma in childhood and adulthood is changing. Its current concept is that of a heterogeneous and genetically complex disease with several phenotypes presenting with distinct clinical features which are linked to endotypes with different underlying mechanisms and characteristic therapeutic responses. More importantly, the categorization of endotypes in childhood asthma is still evolving as disease classification has now been able to associate phenotypes with endotypes based on airway and serum biomarkers. Better still, there is a potential nexus between disease phenotypes and endotypes or biomarkers, as well as some potential personalized therapeutic options. In the future, endotypes may be used together with specific biomarkers to predict responses to targeted treatments.

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