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Biomarkers of Airway Type-2 Inflammation and Integrating Complex Phenotypes to Endotypes in Asthma

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Abstract
Purpose of Review Over the past decade, the most important advance in the field of asthma has been the widespread recognition that asthma is a heterogeneous disease driven by multiple molecular processes.
Recent Findings The most well-established molecular mechanism in asthma is increased airway type-2 inflammation, and consequently, non-invasive biomarkers of increased airway type-2 inflammation, such as blood eosinophil counts or blood periostin levels, have proven important in stratifying asthma patients in clinical trials of type-2 cytokine inhibitors. However, it remains ambiguous how well these non-invasive biomarkers represent airway measures of type-2 inflammation in asthma. As a result, the utility of these biomarkers to assist with asthma management or as research tools to better understand asthma pathogenesis remains unclear.
Summary This article reviews primary data assessing biomarkers of airway type-2 inflammation in asthma and describes how the use of biomarkers can advance a precision medicine approach to asthma treatment.

This article is part of the Topical Collection on *Asthma*

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Introduction

Asthma is a common and complex respiratory disorder that affects over 300 million individuals globally [1] and leads to over 14 million hospitalizations annually in the USA [2]. Current asthma guidelines emphasize categorizing asthma patients into mild, moderate, and severe sub-groups based upon an assessment of asthma symptoms [3, 4]. Correspondingly, treatment algorithms for asthma recommend escalating asthma medications in response to increasing asthma symptoms [3, 4]. This step-wise and symptom-based approach has been effective at decreasing asthma morbidity in some asthma patients [2, 5], but the relative impact of these guidelines at decreasing asthma hospitalizations or improving asthma control has been small [2, 6]. These symptom-based approaches originate from a simplified view of asthma and do not recognize asthma as a heterogeneous disease both at the clinical and molecular levels.

Two complementary approaches have been used to study the mechanisms that drive asthma. In the first approach, asthma patients are clustered into sub-groups based upon phenotypic characteristics, such as obesity, age, gender, lung function, atopy, and exacerbation history. For example, there is a subset of asthmatics characterized by older age, obesity, and female gender. This work has reinforced the concept that asthma is a disease that affects a diverse array of individuals and has provided a basis for researchers to associate complex phenotypic profiles with disease in asthma [7–10].

However, using phenotypic traits to understand the pathophysiology of asthma has limitations. The main limitation is that phenotypic characteristics can be caused by multiple biologic

processes. For example, identifying subjects with lower FEV1 (forced expiratory volume in 1 s) measurements does little to support a specific biologic cause since airflow obstruction can be caused by coinciding disease processes (i.e., smooth muscle contraction, mucus impaction, and sub-epithelial fibrosis). Consequently, identifying these patients does little to determine an appropriate treatment target.

The second, alternative approach focuses on identifying the molecular pathways that drive asthma with the objective of identifying disease "endotypes," defined as disease subtypes with distinct functional or pathobiological mechanisms [11, 12]. To date, the most well-established disease endotype in asthma is "Type-2 High" asthma or asthma that is driven by increased airway type-2 inflammation [12]. This approach to better understand the pathophysiology of asthma has relied on the development of biomarkers, defined as objectively measured characteristics that indicate a biological process. Because biomarkers quantify molecular pathways, they can then be used to understand how molecular abnormalities lead to disease phenotypes or significant clinical outcomes, such as asthma exacerbations. Currently, the most prominent biomarkers in the field of asthma have been indicators of airway type-2 inflammation: blood eosinophil cell counts [13•], airway type-2 gene expression profiles [14••], and blood periostin levels [15•].

The strength of this second approach is its potential to advance a "precision medicine" treatment paradigm in asthma. Specifically, treatments can be developed to target dysfunctional molecular pathways identified by biomarkers. This potential has led to a recent field-wide focus on biomarkers, including their development, applications, limitations, and validity.

This focus leads us to the main limitation of the endotype approach: its reliance on consistent biomarkers. The development of biomarkers requires large-scale collection and analysis of human bio-specimens, necessitating resource-rich facilities and often costly laboratory techniques. Moreover, biomarkers are solely surrogate measures of biological processes, and inaccurate biomarkers can lead to disease misclassification in both discovery and intervention studies.

The objective of this review is to further clarify the strengths and limitations of proposed biomarkers of airway type-2 inflammation and to describe how these biomarkers can be used to better understand asthma pathogenesis. A clearer, more nuanced understanding of how type-2 biomarkers can be used in asthma will enable a successful transition from a symptom-based treatment approach to a precision medicine treatment approach in asthma.

Airway Type-2 Inflammation and the Type-2 High Asthma Endotype

The type-2 high asthma endotype is named for specific pathobiology involving the type-2 cytokines, Interleukin (IL)-4, -5, and -13. Understanding the biology of the type-2 response is a critical first step for fully understanding how type-2 inflammation leads to disease pathology in asthma and has been explained in other reviews [12, 16]. Briefly, inappropriate activation of the airway epithelium, via viruses or inhaled antigens, leads to bronchial airway secretion of IL-25, IL-33, or thymic stromal lymphopoietin (TSLP) [12]. These cytokines then promote a distinct inflammatory cascade orchestrated by the prototypic type-2 cytokines IL-4, IL-5, and IL-13 leading to airway infiltration of eosinophils, basophils, mast cells, IgE-mediated B-cells, and T-helper type-2 cells (Th2-cells) [17]. The protective function of the type-2 response is to eliminate helminth parasites [18]. However, innate and adaptive type-2 immune responses also have other functions, including roles in tissue repair [19] and energy metabolism [20].

It is important to clarify that the identification of a type-2 high asthma endotype indirectly asserts that a subgroup of asthma patients lack evidence of type-2 inflammation in their airway [14••, 21•]. These findings disprove the previously held assumption that airway type-2 inflammation is ubiquitous in asthma. Specifically, roughly half of asthma patients demonstrate a type-2 high molecular endotype of asthma as shown by epithelial cell brushing collected during bronchoscopy [14••]. The epithelial cells of these "type-2 high" patients are molecularly characterized by increased gene expression of three genes known to be up-regulated in airway epithelial cells following IL-13 stimulation: periostin (POSTN), calcium-activated chloride channel-1 (CLCA1), and serpin peptidase inhibitor (SerpinB2) [14••]. Phenotypically, these type-2 high asthmatic patients have increased subepithelial fibrosis, mucus hypersecretion, and have disease responsive to inhaled corticosteroid treatment [14••]. More recently, a composite gene expression signature of CLCA1, periostin, and SerpinB2 in epithelial brushings was defined in order to demonstrate the continuous spectrum of type-2 inflammation in the airways of asthmatic patients. This "three gene metric" continuous scale of airway type-2 inflammation has been related to lung function and treatment response and has become the gold-standard measure of airway type-2 inflammation in asthma [22].

A barrier to widespread implementation of this metric is that it requires qPCR analysis of bio-specimens obtained during bronchoscopy. Bronchoscopy is invasive and expensive, making it impractical to apply to large populations. Consequently, alternative biomarkers are needed to differentiate asthma patients with type-2 high asthma from those with type-2 low asthma.

Biomarkers of Airway Type-2 Inflammation

While the concept of asthma endotypes is relatively new [11], asthma classification schemes have been in use for over 60 years [23]. Initially, asthma classification focused on sub-

grouping patients based upon clinical features such as age of onset, or the presence or absence of sinus disease [23], but sub-grouping quickly transitioned to the use of biomarkers such as blood IgE levels in an attempt to distinguish allergic or "extrinsic" asthma from non-allergic or "intrinsic" asthma [24, 25]. An improved understanding of asthma pathophysiology and the development of new biologic medications have led to the renewed interest to sub-classify subjects into type-2 high and type-2 low asthma endotypes. The objective of this section is to review the primary data for the widely used biomarkers of airway type-2 inflammation starting from older methods such as blood IgE levels and ending with more novel biomarkers such as blood dipeptidyl peptidase-4 levels (Table 1).

Immunoglobulin E Levels (IgE)

Blood measurements of total (bound and unbound) IgE are detected at higher levels in asthma patients compared to healthy control subjects, and measures of total blood IgE levels were one of the first biomarkers developed in asthma [24]. The initial goal of measuring blood IgE in asthma was to distinguish asthmatic disease that occurred secondary to atopy (extrinsic) vs. non-atopic (intrinsic) mechanisms.

B-lymphocytes exposed to the type-2 cytokines IL-4 and IL-13 undergo class switching to produce Immunoglobulin E isotype antibodies [26, 27]. The interaction between antigen and IgE leads to type-1 hypersensitivity reactions which include the degranulation of mast cells and release of vasoactive mediators such as histamine and leukotrienes [27].

Due to IgE's crucial role in mediating atopy, the anti-IgE medication omalizumab was developed as a therapy for severe asthma. The subsequent success of omalizumab at preventing asthma exacerbations [28, 29] has demonstrated that reactions orchestrated by IgE must cause at least some asthma exacerbations.

However, it is now accepted that total blood IgE levels decrease with age [30, 31], thereby limiting the utility of blood IgE levels as a biomarker of atopy in large asthma populations. These limitations were exemplified by the inability of total blood IgE levels to differentiate responders from non-responders to omalizumab therapy [32, 33]. Furthermore, multiple studies have demonstrated that blood IgE levels poorly predict airway measurements of type-2 inflammation [21•, 22, 34]. Thus, while IgE levels do demonstrate weak correlations to airflow obstruction and disease severity [35], the clinical utility of IgE levels for predicting treatment responses or quantifying biological process is suspect.

Table 1 Features of proposed biomarkers of type-2 inflammation in asthma

Biomarker	Relevance to type-2 biology	Clinical utility	Limitations
Blood IgE	B-cells exposed to IL-4 and IL-13 undergo class switching to produce IgE isotype antibodies.	- Can be used as a surrogate marker of atopy - The anti-IgE antibody omalizumab decease asthma exacerbation rates.	- IgE levels decrease with increased age regardless of type-2 status. - Blood IgE levels poorly predict response rates to omalizumab
Sputum eosinophils %	IL-5 and IL-13 recruit eosinophils into the airway lumen.	Sputum eosinophil % accurately predict response to inhaled corticosteroid treatment	Techniques to measure sputum eosinophil % are not standard and require advanced techniques
FENO	Airway epithelial cell exposure to IL-4, -5, and -13 induces production of nitric oxide.	FENO measurements are easy to perform and track closely with sputum and blood eosinophil measures	FeNO-guided treatment algorithms have failed to outperform symptom guided plans.
Blood eosinophils	IL-5 is a key mediator of eosinophil activation and prolongs eosinophil survival.	Anti-IL-5 medications are exclusively effective in subjects with elevated blood eosinophil cell counts.	Different physiologic conditions such as fasting, obesity, or adrenal activation provide additional mechanisms by which blood eosinophil counts could fluctuate.
Serum Periostin	A matrix protein secreted basolaterally by the airway epithelium in response to IL-13 stimulation	The anti IL-13 medication, Lebrikizumab, was only effective in the subgroup of asthma patients with high serum periostin levels.	-No clear difference in periostin levels between asthma and healthy subjects. -Follow up studies have not yet confirmed a role for periostin as a predictor of lebrikizumab response.
Blood DPP-4	Secreted basolaterally by the airway epithelium in response to IL-13 stimulation	The anti IL-13 Tralokinumab improved FEV1% predicted values in subjects with high blood DPP-4 measures.	No confirmatory studies have been conducted in asthma
Sputum gene expression	qPCR-based approaches have demonstrated that gene expression measures of the type-2 cytokines IL-4, IL-5, and IL-13 can be quantified from induced sputum RNA.	Enables direct continuous metrics of airway type-2 inflammation in large cohorts of asthma patients to better understand the relationship between airway type-2 inflammation and clinical features of disease.	Technically challenging and impractical for use in clinical practice.

Sputum Eosinophilia

Eosinophils possess the unique property to take up acidophilic dyes. The distinctive red stain results from intra-cellular granules absorbing the red dye of eosin, the compound that gives these cells their name. This property has enabled easy identification of eosinophils, and for over 30 years, it has been known that eosinophils are increased in the airway and peripheral blood of asthma patients compared to healthy controls [36].

While staining eosinophils is relatively simple, methods of quantifying airway eosinophilia are not straightforward. The most established technique requires asthma subjects to undergo a procedure called sputum induction, in which subjects are induced with hypertonic saline to expectorate a sputum biospecimen [37, 38]. The resulting biospecimen is homogenized with DTT, spun down in a cytocentrifuge, stained using Diff-Quik, and a cell differential is counted [37]. Cell percentages are then calculated as the percentage of the non-squamous cells in the sample. Samples with greater than 80 % squamous cells are regarded as being inadequate for sputum cell count differentials and are discarded [37]. This exclusion process generally results in about 10–15 % of samples being excluded from analysis [37].

Using these, or similar techniques, studies have shown that increased airway eosinophil levels correlate with increased disease severity and predict poor asthma control [39]. Furthermore, it has been shown that using sputum eosinophil levels to titrate asthma medications decreases asthma exacerbations as compared to management guided by standard guidelines [40]. Recent work has also demonstrated that induced sputum measures of eosinophilia are relatively stable over time, and asthma patients with persistent airway eosinophilia preferentially respond to inhaled corticosteroid treatment when compared to subjects persistently without airway eosinophilia [41].

Conversely, other studies have found conflicting results and some asthma patients without airway eosinophilia still demonstrate a response to inhaled corticosteroids [42]. This conflicting data could be secondary to the high degree of technical expertise required to perform sputum induction, and the deficiency of standardized methods to quantify sputum eosinophil counts for biospecimens of induced sputum [43]. Due to the difficult nature of measuring sputum eosinophilia and the relative lack of data demonstrating clear benefit, systematic reviews have recommended against measuring sputum eosinophil levels in clinical practice [44, 45].

Fractional Exhaled Nitric Oxide (FeNO)

Nitric oxide (NO) is produced through the conversion of the amino acid L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS) [46]. In stable healthy conditions, relatively small amounts of NO are produced by the airway epithelium [47]. However, following exposure to inflammatory cytokines, inducible nitric oxide synthase (iNOS) is upregulated in the airway epithelium thereby increasing airway production of NO [47].

Fractional exhaled nitric oxide (FeNO) is a non-invasive metric to measure NO production in asthma. High FeNO measures correlate with airway eosinophilia in asthma and therefore have been proposed as a non-invasive metric of increased airway type-2 inflammation in asthma [48•]. However, other inflammatory conditions unrelated to type-2 inflammation, such as acute infections or ambient air pollution also increase FeNO measurements. Obesity and age have also been proposed to influence FeNO measures, but these associations appear to be weak [49, 50].

In spite of these potential limitations, FeNO has performed surprisingly well at predicting airway eosinophilia [3448•] and responsiveness to type-2 treatments such as inhaled corticosteroids [22, 51] or anti IL-13 biologics therapies [15•]. This data would support the concept that FeNO is an effective non-invasive biomarker of airway type-2 inflammation.

However, less evidence exists to support the clinical utility of FeNO as a biomarker to guide asthma management. Six studies have been conducted assessing the use of FeNO to guide asthma management [45], and only one [51] demonstrated a benefit for an FeNO-guided treatment plan as compared to a conventional symptom-based approach. Thus, while FeNO appears to be an excellent biomarker of type-2 inflammation, meta-analysis [45] and systemic reviews [52] recommend against the use of FeNO in clinical practice.

Blood Eosinophils

Given the relative ease of measuring blood eosinophilia from complete blood counts, it is surprising that the utility of blood eosinophilia as a biomarker of type-2 inflammation has only recently been explored.

IL-5 is a key mediator of eosinophil activation and prolongs eosinophil survival [53]. Clinical trials testing IL-5 inhibition in asthma were initially negative, and it was not until anti IL-5 therapies were directed at eosinophilic asthma patients that the effectiveness of these therapies were appreciated [54••, 55••]. More recently, it has been shown that the anti-IL5 medication, mepolizumab, is an effective therapy in patients with blood eosinophil cell counts >150 cells/uL respond. Remarkably, at this relatively low “cut off” of 150 cells/uL, asthma patients demonstrated decreased asthma exacerbations [55••] and improved systemic corticosteroid tapering [56] with mepolizumab treatment when compared to patients on placebo.

It is important to note that the response rate to mepolizumab was directly related to blood eosinophil measures. Specifically, subjects with blood eosinophil counts greater than 500 cells/uL had a 74 % reduction in clinically significant asthma

exacerbations compared to placebo, while subjects with greater than 150 cells/uL (including subjects >500 cells/uL) only demonstrated a 45 % reduction compared to placebo [55••]. This data demonstrates that response rates to mepolizumab therapy are directed correlated to blood eosinophil cell counts and provides additional evidence that blood eosinophils are a biomarker of response to IL-5 therapy.

Despite these findings, blood eosinophil cell counts as markers of type-2 inflammation may have limitations that have not yet been appreciated. Relatively ancient work demonstrated that blood eosinophil counts fluctuate diurnally, and these findings were attributed to fluctuations in adrenal corticosteroid hormone secretion [57]. More recently, it has been demonstrated that eosinophils play essential roles in metabolism [20], and it is likely that some of the previously observed diurnal variation in eosinophil counts could be attributed to the nightly fast that occurs during sleep. These findings are supported by data demonstrating that obese asthmatics have alterations in eosinophil levels that are complex and unanticipated [58]. Thus, the interplay between obesity, food consumption, and blood eosinophilia is currently not fully understood. Because obesity (described in detail below) is a prominent characteristic of severe asthma, these subjects are more likely to receive type-2 biologic agents. Understanding the relationship between peripheral eosinophilia, obesity, and airway type-2 inflammation needs to be further explored in order to understand how blood eosinophil cell counts can be used to predict treatments responses in obese asthma.

Serum Periostin

Periostin is a matrix protein that is secreted basolaterally by the airway epithelium into the mesenchyme in response to IL-13 stimulation [59]. Because vascular structures run through the mesenchyme, proteins secreted basolaterally are excellent candidates for blood-based biomarkers of epithelial type-2 activation.

Periostin was validated as a type-2 biomarker in a randomized clinical trial comparing change in FEV1% predicted levels following treatment with Lebrikizumab, an anti-IL-13 antibody, in periostin-high and periostin-low asthma sub-groups. In subjects with high periostin measures, treatment with Lebrikizumab improved FEV1% predicted levels, with a 8.1 % relative increase compared to placebo treated subjects [15•]. Alternatively, subjects with low periostin measures only demonstrated a 1.6 % relative increase in FEV1% predicted levels following treatment with lebrikizumab as compared to placebo [15•]. Furthermore, the recruitment of periostin-low and periostin-high asthma subgroups in this study enabled the authors to demonstrate an interaction effect between increased periostin levels and response to lebrikizumab treatment. A follow up study subsequently demonstrated that blood periostin levels outperformed blood eosinophil cell counts and blood IgE measures and was marginally better than fractional exhaled nitric oxide measures for predicting airway eosinophilia [34].

The exciting results of these initial studies were tempered by recent work demonstrating that serum periostin levels are not elevated in asthma subjects compared to healthy control subjects and were not different between severe and non-severe asthma sub-groups [60]. The results of this more recent work could reflect the competing causes of systemic increases in periostin levels. Periostin plays a key role in bone-growth and osteoblast proliferation [61]. Consequently, other conditions, such as childhood growth, bone regeneration, or bone fractures, can increase serum periostin levels in the absence of airway type-2 inflammation.

In summary, serum periostin measurements initially created much excitement for a personalized medicine approach in asthma, but until additional data demonstrates a clear role for periostin as a biomarker of treatment response it remains unclear if it will make its way into clinical practice.

Dipeptidyl Peptidase-4 (DPP-4)

Like periostin, dipeptidyl peptidase-4 (DPP-4) production is induced by human bronchial epithelial cells by IL-13 and is secreted basolaterally [62]. A recent trial testing, the efficacy of Tralokinumab (another IL-13 neutralizing monoclonal antibody) at preventing asthma exacerbations investigated the performance of DPP-4 as a biomarker of IL-13 response [63].

The primary results of the trial were negative, namely tralokinumab did not decrease asthma exacerbation rates or improve FEV1% predicted measures compared to placebo [63]. However, pre-specified sub-group analysis demonstrated that asthma subjects with high (greater than the median) blood DPP-4 measurements had relatively robust improvements in FEV1 (10.8 %, 95 % CI 3.4–18.3) and a trend towards decreased annual asthma exacerbation rates (34 % reduction, 95 % CI −6 to 59, $p = 0.08$) in the treatment group compared to placebo. Furthermore, asthma symptoms scores on the Asthma Control Questionaire-6 (ACQ-6) and Asthma Quality of Life Questionnaire (AQLQ) were markedly improved in DPP-4 high patients treated with tralokinumab [63]. Because the DPP-4 high subgroup had a larger response to tralokinumab when compared to the periostin-high subgroup, DPP-4 could be superior to periostin as a blood based biomarker of airway type-2 activation. Further studies are needed to validate the role of DPP-4 as a biomarker in asthma, but the initial data is encouraging.

Sputum Gene Expression Profiling

As described above, gene expression profiling in airway epithelial cells is considered the gold standard approach to quantifying airway type-2 inflammation in asthma. However, bronchoscopy-based methods are not practical in large clinical

cohorts or in severe asthma patients. To this end, induced sputum gene profiling is an intriguing alternative approach, as induced sputum samples are relatively cheap and easy to obtain.

Induced sputum samples are comprised of a mixture of airway inflammatory cells and recent work has demonstrated that gene profiling in sputum is feasible. Specifically, methods have now been developed to extract RNA from cells residing in the induced sputum matrix [21•], and qPCR-based approaches have demonstrated that gene expression measures of the type-2 cytokines IL-4, IL-5, and IL-13 can be quantified from induced sputum RNA [21•]. Using a scaled and averaged metric of IL-4,-5, and -13 named the “Th2 gene mean”, a continuous read out of airway type-2 inflammation can then be generated in asthma patients. This work is an enabling technology that can allow researchers to identify the type-2 high endotype in larger populations. For example, continuous metrics of airway type-2 inflammation can now be obtained in large cohorts of asthma subjects to determine how patient-oriented outcomes (i.e., asthma exacerbation rates, asthma severity, or drug response rates) relate to measures of airway type-2 inflammation.

While qPCR-based techniques in sputum are too technical to be of substantial use clinically, they do offer an opportunity to better understand how type-2 inflammation drives disease severity in asthma. Furthermore, these approaches can lead to a better understanding of how clinical phenotypes integrate with metrics of airway type-2 inflammation.

Unbiased- and transcriptomics-based approaches have also been used to identify airway transcription profiles in asthma [64–66]. While this work is beyond the scope of this review, this approach has the potential to identify novel, non-type-2 inflammatory mechanisms in asthma.

Relationship Between Biomarkers of Airway Type-2 Inflammation and Complex Clinical Phenotypes

Cluster analysis-based approaches have identified multiple clinical phenotypes of asthmatic disease [7, 8, 10, 67, 68]. Unfortunately, many of these complex clinical phenotypes have not been reproducible. However, certain clinical features, such as increased body mass and older age, are clearly associated with increased asthma severity. One interesting question is how these clinical features relate to measures or biomarkers of airway type-2 inflammation. For example, multiple studies have suggested that obesity is associated with lower measures of airway type-2 inflammation. Below, I will review the data behind how obesity and age associate with measures of airway type-2 inflammation.

Obesity-Associated Asthma

Obesity is associated with increased asthma prevalence [69] and worse asthma severity in both type-2-low and type-2-high endotypes [7, 70], making it plausible that the effect of obesity on asthma severity is independent of airway type-2 inflammation.

First, research studies employing clustering algorithms have consistently identified a type-2-low, female-predominant, obese clinical phenotype of asthma [7–9, 71]. Second, obese asthma is also present at a higher frequency in more severe phenotypes of type-2-high asthma. Specifically, clinical trials using blood eosinophil cell counts or blood periostin levels to identify type-2-high asthma patients disproportionately identify overweight and obese subjects [13•, 15•]. Furthermore, clustering approaches in more severe asthma have shown that obese asthma occurs in severe sub-groups of both type-2-low and type-2-high asthma endotypes [7]. These findings suggest that obesity affects asthma severity independent of airway type-2 inflammation.

Recent work supports this hypothesis. Specifically, it was recently shown that systemic interleukin (IL)-6 inflammation occurs in a subset of asthma patients who are characterized by obesity and severe disease [72••]. A key finding of this work was that increases in plasma IL-6 occurred in some but not all obese patients, and it was only the obese patient group with high IL-6 levels that had more severe asthma. Thus, the presence or absence of low-grade systemic inflammation, as represented by IL-6 level, explains the heterogeneity of asthma severity amongst obese patients. In the same study, measures of IL-6 and metabolic dysfunction did not correlate to biomarkers of airway type-2 inflammation [72••], which makes it unlikely that obesity causes severe asthma through a type-2 inflammatory mechanism. Consequently, while a sub-group of obese asthmatics demonstrate evidence of increased airway type-2 inflammation and will likely benefit from type-2 inhibition [13•], another sub-group of obese asthma will require treatment with alternative non type-2 therapies.

Aging and Asthma

Advanced age increases the risk of type-2 diabetes [73], cardiovascular disease [74], and cerebral vascular accidents [75]. Recently, advanced age has also been implicated as a key risk factor for the development of severe asthma [7, 76]. Asthmatics in the most severe cluster of a study by Moore [7] were on average 20 years older when compared to asthmatics in the less severe clusters. Zein et al. demonstrated that age is more important than asthma duration as a predictor of severe asthma prevalence [76].

It is generally accepted that asthma in older patients is characterized by decreased atopy, and this decreased atopy is why older asthma patients are less responsive to conventional asthma therapies that target type-2 inflammation. However, the concept that older asthma is a disease of less atopy was largely based upon the finding that blood IgE measures are generally lower in older compared to younger asthma patients

[77, 78]. As highlighted above, IgE measurements decrease uniformly with age [30, 31]. Therefore, it is plausible that we have misinterpreted the pathophysiology of asthma in older patients, and IgE measurements in older patients are not related to their asthma or airway type-2 inflammation.

Conclusion

Biomarker development for type-2 inflammation represents a significant advance in altering the paradigm of asthma management: moving from the traditional approach of using symptoms and non-specific clinical characteristics to guide care, towards a more nuanced approach based on identifying dysfunctional molecular pathways. This approach will enable a precision medicine approach to asthma treatment, as well as help researchers understand how clinical phenotypes of disease are related to biologic pathways. While biomarkers of airway type-2 inflammation currently have a very limited role in asthma management in the clinical setting, it is likely that biomarker-based asthma guidelines will eventually supplant symptom-based guidelines.

Compliance with Ethics Standards

Conflict of Interest Drs. Peters, Nguyen, and Dunican declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012;12:204.
2. Moorman JE, Akinbami LJ, Bailey CM, Zahran HS, King ME, Johnson CA, et al. National surveillance of asthma: United States, 2001-2010. Vital Health Stat Ser 3 Anal Epidemiol Stud US Dept Health Hum Serv Public Health Serv Natl Cent Health Stat. 2012;35:1–58.
3. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007;120(5 Suppl):S94–138.
4. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43(2):343–73.
5. Cloutier MM, Hall CB, Wakefield DB, Bailit H. Use of asthma guidelines by primary care providers to reduce hospitalizations and emergency department visits in poor, minority, urban children. J Pediatr. 2005;146(5):591–7.
6. Higashi A, Zhu S, Stafford RS, Alexander GC. National trends in ambulatory asthma treatment, 1997-2009. J Gen Intern Med. 2011;26(12):1465–70.
7. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315–23.
8. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178(3):218–24.
9. Sutherland ER, Goleva E, King TS, Lehman E, Stevens AD, Jackson LP, et al. Cluster analysis of obesity and asthma phenotypes. PLoS One. 2012;7(5):e36631.
10. Newby C, Heaney LG, Menzies-Gow A, Niven RM, Mansur A, Bucknall C, et al. Statistical cluster analysis of the British Thoracic Society Severe refractory Asthma Registry: clinical outcomes and phenotype stability. PLoS One. 2014;9(7):e102987.
11. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet Lond Engl. 2008;372(9643):1107–19.
12. Fahy JV. Type 2 inflammation in asthma—present in most, absent in many. Nat Rev. 2015;15(1):57–65.
13.• Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med. 2013;368(26):2455–66. **In this double-blind, placebo-controlled trial, dupilumab, a monoclonal antibody against interleukin 4 receptor, is associated with a reduced frequency of exacerbations when LABAs and inhaled glucocorticoids were withdrawn, with improved lung function and reduced levels of Th2-associated inflammatory markers in patients with moderate-severe asthma and blood or sputum eosinophilia**.
14.•• Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med. 2009;180(5):388–95. **The first study to propose that asthma can be broadly divided into Th2-high and Th2-low molecular phenotypes of asthma. It challenged the concept that asthma is exclusively a Type 2 driven inflammatory disease by showing that a gene signature for Type 2-inflammation was present in only half of patients with asthma**.
15.• Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med. 2011;365(12):1088–98. **In this double-blind, placebo-controlled trial, lebrikizumab, a monoclonal antibody to interleukin-13, was associated with a greater improvement in lung function in patients with high pretreatment levels of serum periostin than in patients with low periostin levels**.
16. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol. 2015;16(1):45–56.
17. Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med. 2012;18(5):684–92.
18. Urban JF, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, et al. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. Immunity. 1998;8(2):255–64.
19. Gause WC, Wynn TA, Allen JE. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nat Rev Immunol. 2013;13(8):607–14.
20. Wu D, Molofsky AB, Liang H-E, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively

activated macrophages associated with glucose homeostasis. Science. 2011;332(6026):243–7.

21.• Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. J Allergy Clin Immunol. 2014;133(2):388–94. **This group demonstrated that gene expression of the type-2 cytokines IL-4, IL-5, and IL-13 can be quantified from induced sputum RNA, using qPCR based techniques. This technology allows researchers to endotype large cohorts of asthma subjects as Th2-high and Th2-low and examine the relationships between patient outcomes and measures of type 2 inflammation**.

22. Bhakta NR, Solberg OD, Nguyen CP, Nguyen CN, Arron JR, Fahy JV, et al. A qPCR-based metric of Th2 airway inflammation in asthma. Clin Transl Allergy. 2013;3(1):24–7022 – 3–24.

23. Rackemann FM. A working classification of asthma. Am J Med. 1947;3(5):601–6.

24. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med. 1989;320(5):271–7.

25. Ostergaard PA. Non-IgE-mediated asthma in children. Acta Paediatr Scand. 1985;74(5):713–9.

26. Pène J, Rousset F, Brière F, Chrétien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci U S A. 1988;85(18):6880–4.

27. Platts-Mills T a E. The Role of Immunoglobulin E in Allergy and Asthma. Am J Respir Crit Care Med. 2001;164(supplement_1):S1–5.

28. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol. 2001;108(2):184–90.

29. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. N Engl J Med. 2011;364(11):1005–15.

30. Mediaty A, Neuber K. Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis. Immun Ageing A. 2005;2:9.

31. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 1994;24(6):530–9.

32. Bousquet J, Wenzel S, Holgate S, Lumry W, Freeman P, Fox H. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. Chest. 2004;125(4):1378–86.

33. Bousquet J, Rabe K, Humbert M, Chung KF, Berger W, Fox H, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. Respir Med. 2007;101(7):1483–92.

34. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol. 2012;130(3):647–54.e10.

35. Naqvi M, Choudhry S, Tsai H-J, Thyne S, Navarro D, Nazario S, et al. Association between IgE levels and asthma severity among African American, Mexican, and Puerto Rican patients with asthma. J Allergy Clin Immunol. 2007;120(1):137–43.

36. Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. N Engl J Med. 1990;323(15):1033–9.

37. Gershman NH, Wong HH, Liu JT, Mahlmeister MJ, Fahy JV. Comparison of two methods of collecting induced sputum in asthmatic subjects. Eur Respir J. 1996;9(12):2448–53.

38. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax. 1992;47(1):25–9.

39. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. Factors associated with persistent airflow limitation in severe asthma. Am J Respir Crit Care Med. 2001;164(5):744–8.

40. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet Lond Engl. 2002;360(9347):1715–21.

41. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med. 2012;185(6):612–9.

42. Godon P, Boulet LP, Malo JL, Cartier A, Lemière C. Assessment and evaluation of symptomatic steroid-naive asthmatics without sputum eosinophilia and their response to inhaled corticosteroids. Eur Respir J. 2002;20(6):1364–9.

43. Arron JR, Izuhara K. Asthma biomarkers: what constitutes a “gold standard”? Thorax. 2015;70(2):105–7.

44. Peters SP. Counterpoint: Is measuring sputum eosinophils useful in the management of severe asthma? No, not for the vast majority of patients. Chest. 2011;139(6):1273–5. discussion 1275–8.

45. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, et al. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax. 2012;67(3):199–208.

46. Moncada S, Palmer RM, Higgs EA. Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. Biochem Pharmacol. 1989;38(11):1709–15.

47. Meurs H, Maarsingh H, Zaagsma J. Arginase and asthma: novel insights into nitric oxide homeostasis and airway hyperresponsiveness. Trends Pharmacol Sci. 2003;24(9):450–5.

48.• Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. Lancet Respir Med. 2015;3(4):290–300. **This systematic review and meta-analysis addresses the diagnostic accuracy of markers of airway eosinophilia (FeNO, blood eosinophils and total IgE) against a reference standard of induced sputum, bronchoalveolar lavage, or endobronchial biopsy. It shows that their use as a single surrogate marker for airway eosinophilia in patients with asthma will lead to a substantial number of false positives or false negatives**.

49. Jacinto T, Alving K, Correia R, Costa-Pereira A, Fonseca J. Setting reference values for exhaled nitric oxide: a systematic review. Clin Respir J. 2013;7(2):113–20.

50. Singleton MD, Sanderson WT, Mannino DM. Body mass index, asthma and exhaled nitric oxide in U.S. adults, 2007-2010. J Asthma Off J Assoc Care Asthma. 2014;51(7):756–61.

51. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med. 2005;352(21):2163–73.

52. Petsky HL, Cates CJ, Li A, Kynaston JA, Turner C, Chang AB. Tailored interventions based on exhaled nitric oxide versus clinical symptoms for asthma in children and adults. Cochrane Database Syst Rev. 2009;4:CD006340.

53. Kouro T, Takatsu K. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. Int Immunol. 2009;21(12):1303–9.

54.•• Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet Lond Engl. 2012;380(9842):651–9. **In this double-blind, placebo-controlled trial, mepolizumab, a monoclonal antibody against interleukin 5, is associated with a reduced frequency of exacerbations in patients with a history of recurrent severe asthma exacerbations, and signs of eosinophilic inflammation (blood eosinophilia, sputum eosinophilia or high FeNO)**.

55.•• Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014;371(13):1198–207. **In this double-blind, placebo-controlled trial, mepolizumab was shown to be effective at reducing frequency of exacerbations and improve systemic corticosteroid tapering in patients with exacerbation-prone asthma, and a blood eosinophil count of ≥150 cell per microliter**.
56. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. N Engl J Med. 2014;371(13):1189–97.
57. Acland JD, Gould AH. Normal variation in the count of circulating eosinophils in man. J Physiol. 1956;133(2):456–66.
58. Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. Am J Respir Crit Care Med. 2013;188(6):657–63.
59. Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. Proc Natl Acad Sci U S A. 2010;107(32):14170–5.
60. Johansson MW, Evans MD, Crisafi GM, Holweg CTJ, Matthews JG, Jarjour NN. Serum periostin is associated with type 2 immunity in severe asthma. J Allergy Clin Immunol. 2016;137(6):1904–7.e2.
61. Zhu S, Barbe MF, Liu C, Hadjiargyrou M, Popoff SN, Rani S, et al. Periostin-like-factor in osteogenesis. J Cell Physiol. 2009;218(3): 584–92.
62. Shiobara T, Chibana K, Watanabe T, Arai R, Horigane Y, Nakamura Y, et al. Dipeptidyl peptidase-4 is highly expressed in bronchial epithelial cells of untreated asthma and it increases cell proliferation along with fibronectin production in airway constitutive cells. Respir Res. 2016;17:28.
63. Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015;3(9):692–701.
64. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. J Allergy Clin Immunol. 2014;133(4):997–1007.
65. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. J Allergy Clin Immunol. 2011;127(1):153–60. 160.e1–9.
66. Yan X, Chu J-H, Gomez J, Koenigs M, Holm C, He X, et al. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. Am J Respir Crit Care Med. 2015;191(10):1116–25.
67. Kupczyk M, Dahlen B, Sterk PJ, Nizankowska-Mogilnicka E, Papi A, Bel EH, et al. Stability of phenotypes defined by physiological variables and biomarkers in adults with asthma. Allergy. 2014;69(9):1198–204.
68. Boudier A, Curjuric I, Basagaña X, Hazgui H, Anto JM, Bousquet J, et al. Ten-year follow-up of cluster-based asthma phenotypes in adults. A pooled analysis of three cohorts. Am J Respir Crit Care Med. 2013;188(5):550–60.
69. Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am J Respir Crit Care Med. 2007;175(7):661–6.
70. Akerman MJH, Calacanis CM, Madsen MK. Relationship between asthma severity and obesity. J Asthma Off J Assoc Care Asthma. 2004;41(5):521–6.
71. Holguin F, Comhair SAA, Hazen SL, Powers RW, Khatri SS, Bleecker ER, et al. An association between L-arginine/asymmetric dimethyl arginine balance, obesity, and the age of asthma onset phenotype. Am J Respir Crit Care Med. 2013;187(2):153–9.
72.•• Peters MC, McGrath KW, Hawkins GA, Hastie AT, Levy BD, Israel E, et al. Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. Lancet Respir Med [Internet]. 2016 Jun [cited 2016 Jun 8]; Available from: http://linkinghub.elsevier.com/retrieve/pii/S2213260016300480. **Cross-sectional study showing that systemic IL-6 inflammation and clinical features of metabolic dysfunction, occur most commonly in a subset of obese asthma patients but also in a small subset of non-obese patients, and are associated with more severe asthma. This study provides a strong rationale for clinical trials of IL-6 inhibitors or treatments that reduce metabolic dysfunction in a subset of patients with severe asthma and high plasma IL-6**.
73. Halter JB, Musi N, Horne FM, Crandall JP, Goldberg A, Harkless L, et al. Diabetes and cardiovascular disease in older adults: current status and future directions. Diabetes. 2014;63(8):2578–89.
74. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res. 2012;110(8):1097–108.
75. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics—2015 update a report from the American Heart Association. Circulation. 2015;131(4):e29–322.
76. Zein JG, Dweik RA, Comhair SA, Bleecker ER, Moore WC, Peters SP, et al. Asthma is more severe in older adults. PLoS One. 2015;10(7):e0133490.
77. Stoy PJ, Roitman-Johnson B, Walsh G, Gleich GJ, Mendell N, Yunis E, et al. Aging and serum immunoglobulin E levels, immediate skin tests, RAST. J Allergy Clin Immunol. 1981;68(6):421–6.
78. Shaaban R, Zureik M, Soussan D, Neukirch C, Heinrich J, Sunyer J, et al. Rhinitis and onset of asthma: a longitudinal population-based study. Lancet. 2008;372(9643):1049–57.