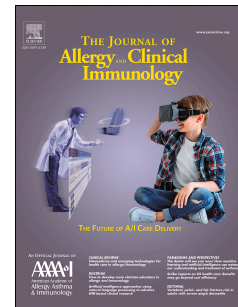


Journal Pre-proof

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PII: S0091-6749(21)02663-4

DOI: <https://doi.org/10.1016/j.jaci.2021.11.021>

Reference: YMAI 15389

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 5 October 2021

Revised Date: 25 November 2021

Accepted Date: 30 November 2021

Please cite this article as: Custovic A, Siddiqui S, Saglani S, Considering biomarkers in asthma disease severity, *Journal of Allergy and Clinical Immunology* (2022), doi: <https://doi.org/10.1016/j.jaci.2021.11.021>.

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Considering biomarkers in asthma disease severity

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Conflict of interest statement: Dr. Custovic reports personal fees from Novartis, personal
fees from Thermo Fisher Scientific, personal fees from Philips, personal fees from
Stallergenes Greer, personal fees from AstraZeneca, personal fees from GSK, outside the
submitted work. All other authors have nothing to disclose.

ABSTRACT

Amongst patients with asthma, reliance on the type/dose of prescribed medication and symptom control does not adequately capture those at risk of adverse outcomes, and we need biomarkers for risk and treatment stratification which are consistently accurate, readily quantifiable and reproducible. The majority of patients with severe asthma, regardless of age, have predominant type-2 (T2) inflammation mediated disease, making airway/blood eosinophils, FeNO, periostin and/or allergic sensitization potentially important biomarkers for severe disease. In both adult and pediatric asthma, there is scope to improve prediction of severe attacks by using a composite T2 biomarkers of blood eosinophils and FeNO. Technological advances in component-resolved diagnostics (CRD) microarray technologies coupled with the development of interpretation software offer a possibility to use CRD as biomarkers of asthma severity amongst sensitized asthmatics. Genetic predisposition and polygenic risk scores of relevant traits (e.g., lung function, host immune responses, biomarkers of exposure from the indoor and outdoor environment, infection and microbial dysbiosis) may also contribute to prediction algorithms. We challenge the idea that asthma can be accurately defined in an individual patient by a discrete and static “endotype” (e.g., T2-high asthma). As we traverse the new era of molecular endotyping in asthma, we need to understand how relevant mechanisms impact patient outcomes, and in parallel develop new tools and approaches to stratify therapies and define individual patient trajectories.

Keywords: severe asthma; T2 asthma; eosinophils; FeNO; allergic sensitization; personalized medicine; data-driven methodologies; immune responses; microbial dysbiosis

ABBREVIATIONS

T2 inflammation:	Type-2 inflammation
FeNO:	Fractional exhaled nitric oxide
CRD:	Component-resolved diagnostics
ICS:	Inhaled corticosteroids
PSA:	Problematic severe asthma

50	DA:	Difficult asthma
51	STRA:	Severe therapy-resistant asthma
52	CRP:	C-reactive protein
53	IgE:	Immunoglobulin E
54	U-BIOPRED:	Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
55	ABPA:	Allergic bronchopulmonary aspergillosis
56	CDHR3:	Cadherin Related Family Member 3
57	RV:	Rhinovirus
58	COPD:	Chronic obstructive pulmonary disease
59	VOC:	Volatile organic compounds

60

The approach to asthma diagnosis and management is changing as a result of the better understanding about the heterogeneity of mechanisms, driven in part by the expansion of high dimensional multi-omics phenotyping approaches, coupled with developments in data integration and analysis techniques. However, although the description of different asthma endotypes has long been posited(1, 2), the framework for implementation of asthma endotyping is still primarily a hypothetical construct, and not a single asthma endotype has been identified with absolute certainty(3).

Differentiating clinical severity from disease mechanisms to identify patients at risk

The ATS/ERS Task Force defines severe asthma as “asthma which requires treatment with high-dose inhaled corticosteroids (ICS) plus a second controller (and/or systemic corticosteroids) to prevent it from becoming ‘uncontrolled’, or which remains ‘uncontrolled’ despite this therapy”(4). Severe asthma is not a unique endotype *per se*, but an extreme end of the severity spectrum of different asthma endotypes(5, 6). There may be different reasons for poor control among patients on maximum treatment, ranging from the wrong diagnosis(7), to non-adherence with prescribed treatments(8), and genuine resistance to current asthma therapies(9).

In pediatric severe asthma, Bush *et al* proposed to use the term “Problematic severe asthma” (PSA) for children with perceived lack of response to high doses of medications(10). Once other diagnoses are excluded, patients with PSA may have either difficult asthma (DA, characterized by modifiable and actionable factors such as poor adherence(11), adverse environmental exposures and psychosocial factors(12)), “asthma plus” (asthma with comorbidities), or severe therapy-resistant asthma (STRA), comprising patients who do not respond to standard therapies, such as systemic corticosteroids administered either as intra-muscular injections or oral direct observed therapy, and need different therapeutic approaches, including biologics(13, 14). However, there is a considerable within-group heterogeneity in each of these categories, and their differentiation may be challenging. Nonetheless, this is useful in both research and clinical context and can be translated into adult severe asthma(15), and similar definitions differentiating severe adult asthma from difficult asthma, have been proposed for adult management(16).

While most current definitions of severe asthma include the requirement for a patient to be on maximum treatment, the UK National Review of Asthma Deaths reported that many patients who die from asthma would be considered as having mild or moderate disease based upon their treatment requirements(17). This contradiction demonstrates that reliance on the type/dose of

prescribed medication coupled with symptom control does not adequately capture patients at risk(7, 18), and we need biomarkers which are consistently accurate, readily quantifiable and reproducible, and not confounded by other host factors, or the presence of comorbidities (Figure 1).

Type-2 inflammation, eosinophils and FeNO

Severe childhood asthma is associated with airway hyperresponsiveness, diminished lung function, obstructed airways, air trapping, elevated airway eosinophils and high FeNO(13). However, for each of these, there is a considerable overlap with patients with mild/moderate disease. In adult severe asthma, a registry study from eleven countries has shown that >83% of patients had an eosinophilic phenotype which was associated with worse lung function(19). It is therefore apparent that the majority of patients with severe asthma, regardless of age, have predominant type-2 (T2) mediated disease, making airway/blood eosinophils and/or FeNO important biomarkers for severe disease at all ages (Table1).

Although data for a relationship between airway and blood eosinophils in childhood severe asthma is variable, the relative ease of monitoring blood eosinophils in the clinic, compared to the practical challenges of sputum induction and processing, has made blood a much more attractive and feasible biomarker to assess T2 asthma(20). A caveat for childhood disease is variation according to age, and the need to identify age-specific cut-offs for both prediction of attacks, but also to determine initiation of biologics(20). Another important consideration for childhood disease is the long-term outcome. Longitudinal analysis of well characterized children with severe asthma showed only 30% of children still met criteria for severe asthma 3 years after initial assessment, with an odds ratio of 2.75 in favor of resolution of severe asthma in those with blood eosinophils >436 cells/ μ l at baseline assessment(21). Of note, clinical symptom resolution does not equate to improved lung function, thus highlighting the long-term impact of childhood-onset disease on adult outcomes(22).

Recent severe acute asthma attack and overuse of reliever medication have been identified as risk factors for asthma death(17). To date, our ability to predict asthma attacks has been limited to knowledge that recent attack will predict future attack, but the objective biomarkers to predict risk of a future severe exacerbation were lacking. In adult asthma, clinical T2 biomarkers such as blood eosinophils and FeNO have been shown to be associated with higher risk of exacerbations, with a >3.5-fold risk of exacerbations observed with a FeNO $\geq$ 50 ppb and blood eosinophils level $\geq$ 300 cells/ μ L relative to patients with low FeNO and

eosinophil levels(23). Real-world data of blood eosinophil counts taken on at least three occasions from >148,000 asthma patients aged >13 years suggests elevated blood eosinophils, whether intermittent or persistent, signify increased risk of exacerbations(24). A prototype risk scale, incorporating FeNO and blood eosinophils, has been developed using data from patients aged >12 years who were included in clinical trials of biologics, to predict annual asthma attack rate(25). A separate pooled analysis of placebo data from seven phase II/III randomised trials of biologics for severe asthma has also shown greatest annualised asthma exacerbation rates were in those with blood eosinophils ≥ 300 cells/ μ L and FeNO>50ppb(26). The combined use of blood eosinophils and FeNO had greater prognostic value than either biomarker alone. These data suggest there is scope to improve prediction of severe attacks by using a composite biomarker of blood eosinophils and FeNO in patients with T2 asthma, but a prospective validation is needed before implementation into clinical care.

In addition, recent data has identified that in adult patients with severe eosinophilic asthma established on anti-IL-5 therapy, a FeNO<20ppb, was likely to be associated with a non-eosinophilic exacerbation, bacterial infection and elevated CRP levels(27). These observations highlight the potential of clinical biomarkers of the T2-phenotype to guide clinical practice by stratifying prospective risk (and hence severity) and guide phenotyping and management of exacerbation events. It is, however, of note that in a recent trial in adult severe asthma patients which evaluated a composite T2 biomarker algorithm of blood eosinophil level, FeNO and serum periostin for corticosteroid titration, the biomarker-based corticosteroid adjustment did not result in a greater proportion of patients reducing corticosteroid dose *versus* control(28). However, a significant proportion of patients failed to follow their treatment advisories to down-titrate corticosteroids. These studies highlight the need for new molecular stratification tools combined with behavioral interventions in patients who remain poorly controlled and the critical reappraisal of the stepwise approach to asthma management.

Allergic sensitization

A strong association between severe asthma and allergic sensitization has been observed in school-age children and adolescents. The co-occurrence of sensitization and viral infection increases the risk of hospital admissions with asthma attacks, both in children(29) and in adults(30), and sensitization is associated with a differential response to ICS(31). However, a study of children with severe asthma reported no difference in the proportion of sensitized children between STRA and DA (with almost all children having evidence of sensitization),

but has shown that children with STRA had significantly greater size of skin tests responses compared to those with DA(32). This suggests that there may be distinct types of sensitization which differ in their association with asthma severity(33), some being more pathologic than others(34).

A machine learning analysis, which took into account the type of allergen, and the timing of onset and remission of IgE responses from infancy to school-age, suggested existence of four distinct classes of sensitization, and identified one of these (“Multiple early”) as a strong associate of severe asthma (including impaired lung function and high risk of severe exacerbations)(35). However, at the time of writing of this article, individual children could be assigned to these classes only through complex modelling of longitudinal data collected over years/decades. What is urgently needed are biomarkers to differentiate in individual sensitized patients, at a single time point of clinical consultation, whether sensitization is an important driver of asthma presence/severity, or a chance finding of little relevance to the disease process (i.e., to distinguish between “pathologic” and “benign” sensitization)(34). Quantification of sensitization through standard diagnostic tests (i.e., using IgE titre and the size of skin test wheal, rather than arbitrary cut-offs) can increase the specificity, both in terms of diagnostic accuracy and the capacity to predict the persistence of symptoms(36-39). However, the problem of a significant number of false-positive test results remains(39).

We can now assess sensitization in much greater detail using component-resolved diagnostics (CRD), which measures sIgE to multiple allergenic molecules, and assessment of sensitization by CRD may be more informative than standard sensitization tests in asthma. A recent novel approach to the analysis of data obtained through commercially available CRD chips (which measures sIgE to >100 of allergenic molecules simultaneously) moved this closer to clinic(40, 41). In a marked contrast to food allergy, in which sensitization to individual components is important, in asthma it was the pattern of interaction between allergen component-specific IgEs rather than the presence of sIgE to any individual allergenic molecule(s) that predicted diagnosis(40). Furthermore, a study in the U-BIOPRED severe asthma cohort has shown that network of interactions between IgEs to multiple proteins are predictors of asthma severity among sensitized school-age children and adults with asthma(41). Severe asthma CRD network was characterized by higher connectivity among components, but the individual connections were weaker. In contrast, mild/moderate CRD asthma network had fewer connections between individual components, but the connections were stronger. Furthermore, the connectivity between components with no structural

homology characterized severe asthmatics, whilst in those with mild/moderate asthma there was a tendency of sIgE to structurally homologous components to connect(41).

Another disease area that may benefit from CRD is allergic bronchopulmonary aspergillosis (ABPA). Hypersensitivity to thermotolerant, filamentous fungi, represents a distinct eosinophilic phenotype of airways disease, often manifesting in severe eosinophilic asthma in the presence or absence of bronchiectasis(42). A recent meta-analysis identified that a combination of rAsp antigens (f1, f2, f3, f4 and f6) is likely to be helpful in the diagnosis of ABPA in both asthma and cystic fibrosis(43).

These studies offer clues that among sensitized individuals, different mechanisms contribute to asthma presence, persistence and severity(40, 41, 44). Importantly, these findings suggest that it may be possible to develop CRD-based screening biomarkers to help diagnose asthma(40), prognostic biomarker to assess its severity among sensitized asthmatics(41) and ascertain future risk of asthma among sensitized children(44), and predictive biomarker to identify promising subgroup(s) in randomized trials and/or predict treatment response(45). While the same CRD technology platforms (microarray assays) may be used when addressing different asthma domains (screening, prognostic and predictive), the interpretation algorithms will almost certainly differ. Therefore, if these findings are to be translated into clinically useful biomarkers, technological advances in CRD microarray technologies will have to be coupled with the development of interpretation software to provide real value for clinicians and patients. The important caveat is that such biomarkers will be of greatest value among sensitized patients, and that the choice of test will depend on the clinical question (e.g., we envisage that CRD microarrays may be useful to differentiate asthmatics from non-asthmatics and STRA from DA using predictive algorithms, while CRD using conventional methods such as ImmunoCAP may be more useful for assessing sensitization to specific proteins of interest, such as *Aspergillus* allergens for diagnosing ABPA).

Genetic susceptibility

While there is a substantial overlap in genetic associates between mild and severe asthma, there are several polymorphisms associated only with moderate-to-severe disease, including variants in/near to exonic regions of *GATA3*, *MUC5AC* and *KIAA1109*(46). The association with *MUC5AC* is intriguing, as adult severe asthma patients are characterized by increased mucous plugs scored systematically on airway imaging, that appear to be associated with the degree of airflow obstruction, T2 inflammation, airway *MUC5AC:MUC5B* gene expression

and are in part refractory to systemic corticosteroids(27). Eosinophil granule proteins were shown to stiffen thiolated mucous hydrogels *in vitro* via oxidant induced mechanisms, raising the possibility that novel airway imaging biomarkers and/or biomarkers of mucous rheology(47), may be useful for identifying severe asthma patients and potentially stratifying novel mucolytic therapies. The high population frequency of the *MUC5AC* variant minor allele associated with moderate-severe asthma, may also render recruit by genotype studies as a feasible enrichment option for future clinical trials(48).

In childhood asthma, a variant in Cadherin Related Family Member 3 (*CDHR3*) was identified as associate of a phenotype of severe asthma characterized by the early-onset and recurrent severe exacerbations(49). *In silico* and mechanistic studies suggested that *CDHR3* may be a receptor for Rhinovirus C (RV-C)(50). Whether this information can be used in a clinical setting to identify patients who are susceptible to RV-C-induced asthma exacerbations and for treatment stratification remains unclear.

Ethnicity has also been associated with severe asthma, and ethnic variation in response to intramuscular triamcinolone in children with STRA(51) may be related to genetic susceptibility.

Finally, abnormal lung function is a prototypical feature of severe asthma in both children and adults. Polygenic risk scores of adult lung function and COPD(52) may identify children at risk of developing impaired lung structure and function in later life and tractable mechanisms (e.g., genes associated with airway cilia development and TGF- β signalling via SMAD3) of adult lung disease at the intersection of asthma and COPD.

Host immune responses, infection and microbial dysbiosis

The host innate immune responses to viruses may be important determinants of asthma severity and exacerbations. Children with STRA have impaired interferon induction by rhinovirus, and low interferon- β and interferon- λ induction was demonstrated in bronchial epithelial cells from children with STRA cultured *ex vivo*(53). Low interferon induction and high pro-inflammatory cytokine responses by peripheral blood mononuclear cells after the stimulation with RV-16 are associated with early-onset severe asthma and early-life allergic sensitization(54). However, these findings are yet to be translated to patient care through development of a clinical biomarker to identify patients at risk of RV-induced exacerbation, or those who may benefit from anti-viral treatments such as inhaled interferon- β .

Another important area involves the role of infection and microbial dysbiosis in severe asthma. Several studies have demonstrated that the airway microbiome is altered in moderate-severe asthma, with an enrichment of proteobacteria such as *Haemophilus* in airway samples, inverse associations between proteobacteria and tissue eosinophils and positive correlations between proteobacteria and type-17 epithelial gene expression(55). These observations may indicate that suppression of T2 inflammation promotes a mucosal environment that selectively enriches proteobacteria and dysbiosis, a hypothesis that requires confirmation in prospective studies.

A recent study in severe pre-school wheeze has suggested that in addition to sensitization and peripheral eosinophilia, bacteriology in induced sputum may be useful biomarkers to stratify treatment in future interventions studies to test if the information on airway microbiota may be used for targeted antibiotic therapy(56). Furthermore, it is possible that sputum biomarkers reflective of specific pathogens or indeed microbiome derived from quantitative PCR, may be appropriate enrichment tools for future microbiome targeted interventions.

Targeting T2-mediated severe disease

A specific exacerbation-prone phenotype exists within severe pediatric(5) and adult asthma and is exemplified by the remarkable success of T2 monoclonal therapies. A range of targeted inhibitors directed at both downstream and upstream (anti-alarmin) T2 cytokines are now licensed, have undergone, or are undergoing pivotal phase III clinical trials in severe eosinophilic exacerbation-prone asthma(57, 58). These elegant ‘human knockout’ clinical trials have shed new insights into T2 inflammation biology and raise several pertinent questions for future biomarker research. For example, ablating eosinophils with anti-IL-5/IL-5R directed treatment (several thousand patients across pivotal phase III trials and additional real world evidence studies), does not appear to have any significant adverse effects (such as enhanced parasitic infection or increased risk of malignancy) in adults(59). Further studies evaluating eosinophils and their heterogeneity following depletion in tissue are now required to fully understand the impact of these therapies. Additionally, whilst targeting T2 immune pathways (IL-5, IL-5R, IL-4R α , TSLP) broadly reduces exacerbations by 50% or more in exacerbation-prone severe asthmatics with elevated T2 biomarkers (blood eosinophils $\pm$ FeNO)(57), striking differences in clinical impact are seen between the biologics. For example, targeting the IL-4R α appears to deliver the greatest impact on lung function and nasal polyp-related outcomes(60) perhaps due to the impact of IL-13 inhibition on airway smooth muscle tone and mucous hyper secretion, as well as broader impacts of inhibition on

other resident T2 cells such as ILC2 cells. Targeting TSLP appears to reduce exacerbations even in patients without elevated T2 biomarkers. Furthermore, the impact of T2 therapies on asthma control and symptoms is highly variable across the biologics. Finally, none of the T2 monoclonals appear to be disease modifying, for example the cessation of anti-IgE in patients with severe asthma was associated with relapsing disease in patients with elevated T2 biomarkers(61). These observations highlight the important unmet need to develop asthma therapies that not only switch off pertinent T2 inflammation pathways, but also modify the host response within structural cells resident in airway tissue, e.g., ongoing airway remodeling, mucus hyper secretion and smooth muscle dysfunction, and are therefore capable of longer-term disease modification. Bio-stratification tools that enable these objectives remain a significant unmet need in severe asthma.

Beyond T2 Asthma?

There are currently no accepted biomarkers for non-T2 asthma, with the exception of the absence of T2 activity. Whilst it is tempting to speculate that T-cell phenotypes and their cognate cytokines and chemokines beyond the classical T2 repertoire may be important contributors to severe asthma, this has not been borne out by targeted intervention trials, and in some cases phenotypic expression may arise as a consequence of corticosteroid treatment(62). For example, clinical trials targeting the neutrophil chemokine CXCR2 (stratified by blood neutrophil level)(63), and non-stratified trials of anti-IL-17(64), anti-IL-23 and anti-TNF α (65), have failed to identify significant impact on asthma control and/or exacerbations. Whether these trials would be positive if the correct patients were enriched using endotypic biomarkers remains to be established. For example, it is possible that extracellular pro-neutrophilic matrikines(66) that are reflective of *in situ* matrix turnover consequent to neutrophilic inflammation may be more suitable biomarkers for strategies targeting neutrophilic airway inflammation such as CXCR2 inhibition. In addition, multi-omics biomarkers, reported in UBIOPRED from sputum transcriptional profiling(67), or transcriptional profiles derived from sorted innate immune cells in sputum(68), may provide more robust enrichment tools for future stratification of non-T2 inflammatory pathways.

Frequent exacerbations are a hallmark feature very specific to pre-school children with severe wheezing/asthma(69). Although a distinct subtype characterized by aero-allergen sensitization, blood eosinophilia (≥ 300 cells/ μ l) and associated corticosteroid responsiveness is recognized in this age group(31), in a similar manner to adult non-T2 asthma, there is another subtype characterized by a predominance of lower airway bacterial infection,

rhinovirus detection and neutrophilia(56). Of note, both subtypes are associated with a bacterial dysbiosis, the atopic, eosinophilic endotype with a predominance of *Moraxella catarrhalis*, while the neutrophilic endotype associated with *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus*(56). The challenge is to prospectively utilize biomarkers for each subtype (blood eosinophils, induced sputum, nasopharyngeal swabs) to stratify therapy and determine clinical utility in reducing exacerbations.

Asthma severity and exacerbations may be context-dependent

Asthma severity and exacerbations are context-dependent among individual patients. When discussing asthma exacerbations, the focus is usually on virus infections. However, while virus infections are undoubtedly important, they are not the only trigger of asthma attacks. Meta-analyses indicate that constituents of the outdoor environment, for example ozone and nitrogen exposure, increase the risk of asthma exacerbations in a dose-dependent manner(70). The indoor environment may be equally important in predicating asthma risk, for example – volatile organic compounds have been shown to be associated with increased asthma symptoms(71). Other environmental exposures may be associated with more instantaneous risk. For example, during the Melbourne epidemic thunderstorm asthma event which occurred in November 2016, over a 30-hour period there was a staggering 7-fold increase in respiratory-related presentations to hospitals, 476 excess asthma-related admissions, and 10 deaths(72). The gust front from thunderstorm and very high airborne ryegrass pollen concentrations were the key contributing environmental factors(73). This offers several important learning points, among them the fact that most patients who were hospitalized with severe asthma attack did not have prior diagnosis of asthma(73), and that allergen-specific immunotherapy appeared to offer protection(74). Patients who suffered from asthma attacks and died during this episode clearly had a very different endotype compared to those who present with exacerbations due to virus infections. We suggest that they suffered from a condition with a clear pathophysiological mechanism (IgE-driven disease), for which there are available biomarkers (sIgE or skin test to relevant allergens) and mechanism-based treatment (allergen-specific immunotherapy), i.e. that this is the currently the only clear asthma endotype(75). Whilst commonly perceived as a mild disease, the Melbourne episode clearly demonstrated the context-dependency of asthma severity, exacerbation and death for individual patients. Furthermore, the association of asthma exacerbations and poor symptom control with both outdoor and indoor pollutants/VOCs(70, 71) provides evidence to support the role of tailored socio-environmental interventions to reduce asthma risk.

Moving forward

We suggest that concomitantly with disease-modifying drugs, the companion diagnostics which includes biomarkers of specific molecular mechanisms of different endotypes have to be developed to facilitate treatment stratification and improve design of clinical trials. This should be extended to currently available biologics to minimize uncertainties when choosing a biologic medication for patients with severe asthma and maximize treatment response. However, the mechanisms giving rise to symptoms in an individual patient may change over time, and the response to biologics may differ over time in the same patient (Figure 2). For example, a patient may develop virus-induced exacerbation due to impaired antiviral responses, but may also have allergen-induced asthma attacks, or their combination, and their relative importance may change over time. The effectiveness of treatments may therefore also change over time as more than one mechanism is likely operating in most patients.

Going forward, we will have to reform the taxonomy of severe asthma from traditional symptom/medication-based criteria towards a mechanism-based framework. A significant challenge as we traverse this new era of molecular endotyping in asthma, is understanding how relevant mechanisms impact patient outcomes, as well as developing new tools and approaches to stratify therapies and define individual patient trajectories. The idea that asthma can be accurately defined in an individual patient by a discrete and static endotype (e.g., T2-high asthma) should be challenged, as new approaches such as single cell sequencing highlight the plasticity of dysregulated airway biology, suggesting that a more plastic viewpoint of 'mechanistic trajectories' may be more appropriate.

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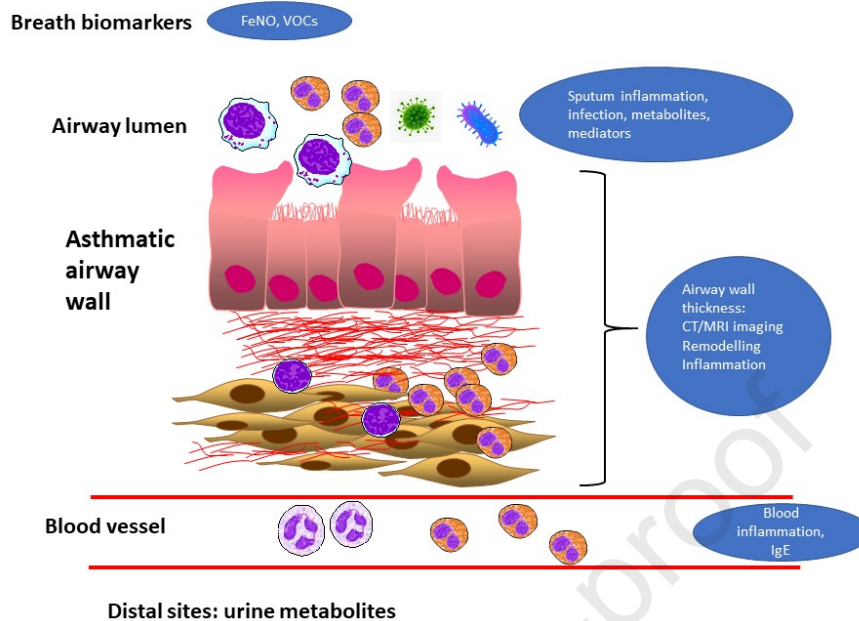
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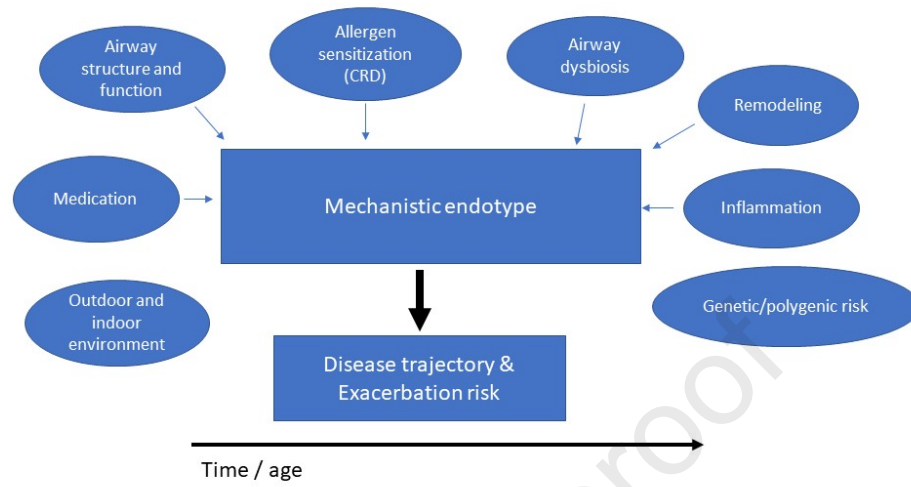
Figure 1. Biomarkers reflecting the pathophysiology of asthma; from the airway lumen, wall and more distal sites including the circulation and urine

Figure 2. Components contributing to a mechanistic endotype of severe asthma, which determines disease trajectory and exacerbation risk, and may change over time

LEGEND FOR TABLE:

Table 1. Currently used biomarkers to support the diagnosis and management of asthma





Biomarker	Abnormal level and age-group	Clinical use / indication
Eosinophils - sputum	$\geq 3\%$	Children: evidence of severe therapy resistant asthma; not used for any biological therapies Adults: evidence from meta-analyses suggests that sputum biomarkers can be used to titrate steroid therapy in asthma with a view to reducing exacerbations ¹
Eosinophils - blood	≥ 150 cells/ μ l (adults – ERS/ATS ²) ≥ 300 cells/ μ l (adults – GINA ³) ≥ 300 cells/ μ l (children 6-16 years – NICE ⁴) ≥ 300 cells/ μ l (children aged 1-5 years ⁵)	Start anti-IL5 biological therapies Increased response to daily inhaled corticosteroids
FeNO	≥ 25 ppb in children ⁶ and adults ⁷	Supportive of asthma diagnosis, Levels > 45ppb may be suggestive of ICS non-adherence in adult severe asthma, warranting FeNO suppression testing. Enriched response to anti-IL-4Ra in adults. ⁸
FeNO plus blood eosinophils	FeNO ≥ 50 ppb & eosinophils ≥ 300 cells/ μ l	Predictive of asthma exacerbation ⁹
IgE	30-1500 IU/mL (adults and children)	Start anti-IgE antibody therapy
Infection - sputum	Bacterial infection with neutrophilia and frequent exacerbation	Consider prophylactic antibiotics