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Recent insights in the role of biomarkers in severe asthma management

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Contemporary asthma management requires a proactive and individualized approach, combining precision diagnosis and personalized treatment. The introduction of biologic therapies for severe asthma to everyday clinical practice, increases the need for specific patient selection, prediction of outcomes and monitoring of these costly and long-lasting therapies. Several biomarkers have been used in asthma in disease identification, prediction of asthma severity and prognosis, and response to treatment. Novel advances in the area of personalized medicine regarding disease phenotyping and endotyping, encompass the development and application of reliable biomarkers, accurately quantified using robust and reproducible methods. The availability of powerful omics technologies, together with integrated and network-based genome data analysis, and microbiota changes quantified in serum, body fluids and exhaled air, will lead to a better classification of distinct phenotypes or endotypes. Herein, in this review we discuss on currently used and novel biomarkers for the diagnosis and treatment of asthma.

KEYWORDS

biomarkers, severe asthma, T2 asthma, non-T2 airway inflammation, omics, microbiome & dysbiosis

Introduction

In the past decades, asthma has been increasingly recognized as a heterogeneous disease, with many diverse pathogenetic mechanisms and variable responses to standard therapy (1). Targeting of the underlying inflammatory pathways is the current therapeutic approach in asthma, especially for patients with severe or difficult-to-control disease (2).

Particularly in severe asthma, diverse inflammatory pathways may be activated in different patient subsets, leading to the emergence of distinguished clinical characteristics or phenotypes (3). Based on cluster analysis of several large asthmatic cohorts, we have identified four major clinical phenotypes in adult patients with severe asthma, considering relevant observable characteristics such as age at onset, potential triggers, type of inflammation, lung function impairment, and response to treatment: (a) the early-onset, atopic asthma phenotype, presenting partially variable airflow obstruction,

frequent symptoms and relative response to corticosteroid treatment, (b) the late-onset, non-atopic, eosinophilic phenotype, with fixed airway obstruction, corticosteroid resistance and frequent asthma-related comorbidities (c) the late-onset, non-allergic, non-eosinophilic, obesity-related asthma phenotype, usually female predominant, with increased symptom burden and resistance to corticosteroids despite relatively normal lung function, and (d) the late-onset, non-atopic, neutrophilic phenotype, with corticosteroid resistance and severe lung function impairment (4–6). However, considerable overlapping between asthma phenotypes is often observed, and this phenomenon may be attributed to the different variables assessed in various studies and ethnic, geographical and other methodological issues.

Moreover, based on the presence or absence of Type 2 (T2) inflammation, asthma is now frequently categorized into T2 high (T2 asthma) and T2 low (non-T2 asthma) (7). The characterization of the distinct disease endotypes, that is the underlying pathophysiologic mechanisms, has currently become the central therapeutic strategy in asthma management, as it enables clinicians to better diagnosing, stratifying and monitoring of their patients (8).

Consequently, the clinical and pathophysiological heterogeneity of asthma makes it extremely suitable for precision medicine (9). Biological markers (biomarkers), defined as measurable indicators of a biological state or disease with clear cutoff values (10), are considered valuable clinical tools for diagnosing, predicting, and monitoring asthma, with the aim to reduce its burden and to minimize future risk (11).

During the last decades, as the contribution of atopy, eosinophilic-driven inflammation and airway epithelial dysfunction have been recognized in the pathogenesis of severe asthma, significant progress has been made in the identification of valid asthma biomarkers (12, 13). Hence, serum immunoglobulin E (IgE), sputum and blood eosinophils, and the fraction of nitric oxide in exhaled air (FeNO), have all been used as potential biomarkers, suggestive of the underlying activation of these respective pathogenetic pathways (14–17). In this review, we aimed to discuss on the most important existing and emerging biomarkers with the greater clinical applicability in asthma (Figure 1).

Biomarkers in T2 asthma

At present, T2 asthma comprises the best defined asthma subtype, regarding underlying immunopathology, associated biomarkers and targeted therapies (18). Airway eosinophilic inflammation constitutes the main characteristic of this type of asthma, so the latter is often classified merely as eosinophilic or non-eosinophilic (19). Eosinophilic inflammation is driven predominantly by T2 immune responses regulated by T2-helper (Th2) cells and group 2 innate lymphoid cells (ILC-2)

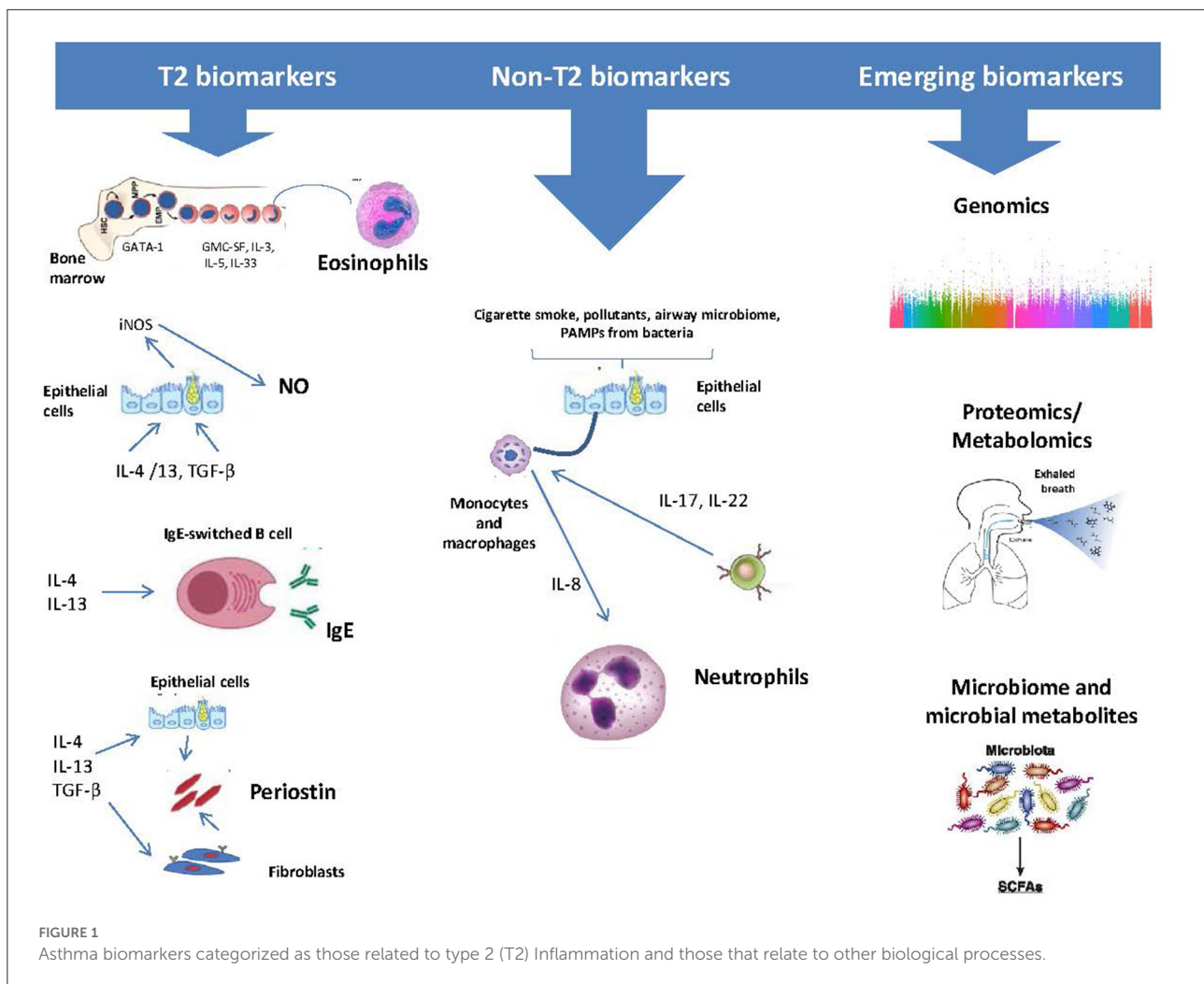
and their relative Th2 cytokines, and it is reflected, to some extent, by sputum or blood eosinophilia and elevated FeNO concentration (20).

Eosinophils

Among the asthmatic patients, the prevalence of eosinophilic inflammation is referred as almost as 50% in various studies (21). Upon their activation, eosinophils release a number of inflammatory mediators from their intracellular granules, that are responsible not only for the appearance of asthmatic symptoms, but also, in more advanced stages of the disease, for the observed fixed airflow obstruction and airway remodeling (22). IL-5, IL-4, and IL-13 are key cytokines in the eosinophilic inflammatory process, as they regulate the eosinophilic migration, activation and survival into the asthmatic airway (23).

The association between asthma and eosinophilia is well-established. Airway eosinophilia is significantly increased in asthmatic patients compared with healthy subjects, and has been found to correlate with the degree of airway hyperreactivity (AHR), exacerbation severity and poor clinical outcomes (24, 25). Sputum eosinophilia as an index of airway eosinophilia, has been found to predict response to treatment with corticosteroids (26) and may be used as a therapeutic target for guiding the intensity of treatment with inhaled corticosteroids (ICS) (27). Additionally, treatment adjustments according to sputum eosinophil levels have been associated with reductions in the rate of severe exacerbations and with decreased need for hospital admissions, compared to the standard clinical-guided management, in patients with moderate-to-severe asthma (28).

Pizzichini et al., in a small cross-sectional study suggested that sputum eosinophilia (cut-off >3%) is a more reflective biomarker of asthmatic airway inflammation than the absolute peripheral blood eosinophil count (BEC) (29). However, sputum eosinophil levels have been found to vary over time and after treatment (30), and induced sputum technique may also be laborious and time-consuming. Moreover, a recent *post-hoc* analysis of data from patients who had provided both baseline sputum and blood samples participating in DREAM study (31) revealed that, among the placebo group, increasing baseline sputum eosinophil count predicted a decrease in exacerbation rate in the following 52 weeks, while, on the contrary, predicted exacerbations increased with increasing baseline BEC (32). The authors of the study suggest that this finding may be due to the small number of patients included in the analysis; however, this may also represent an interesting new finding suggesting that sputum eosinophils may not represent a more useful predictive biomarker than BEC in patients with severe eosinophilic asthma. Furthermore, BEC is considered superior to FeNO in identifying sputum eosinophilia (33) and, most importantly, is easily measured and widely used in the context



of whole blood count testing; therefore a recent (or historical) BEC is usually available for most the patients. For these reasons, the use of BEC as the basic biomarkers of T2 inflammation has now prevailed in daily clinical practice.

From a clinical standpoint, BEC are useful in the early detection of exacerbation risk, loss of asthma control and lung function deterioration in patients with severe asthma (34, 35). Baseline BEC have been shown to predict the degree of reduction in severe exacerbation rate in patients with severe eosinophilic asthma treated with ICS (36). BEC values between 150 and 400 cells/ μ L have also been used to predict response to treatment with monoclonal antibodies (mAbs) targeting IL-5 and its receptor IL-5R (16, 37–39). In the pooled analysis of the DREAM and MENSA studies (37), a better response to treatment with the anti-IL5 mAb mepolizumab was observed in patients with severe asthma and BEC of ≥ 150 cells/ μ L in baseline. Similarly, data from the pooled analysis of the CALIMA and SIROCCO studies showed that BEC ≥ 300 cells/ μ L, along with the rate

of exacerbations during the previous year, were the stronger predictors of treatment response to benralizumab, an anti-IL5R biologic (16). In the early efficacy studies of the anti-IL5 mAb reslizumab, significant reductions in asthma exacerbation rate (38) and improvements in lung function (39) were observed in patients with persistent asthma and BEC ≥ 400 cells/ μ L. However, BEC should be considered a continuous variable, with higher levels predicting a greater response to treatment (40).

The inconsistency observed between sputum and blood eosinophil counts may be attributed to the heterogeneity of the studied populations in several studies (41). Moreover, a single BEC measurement may be insufficient for asthma diagnosis and therapeutic decisions, with greater variability observed for BEC values between 150 and 299 cells/ μ L (42). Finally, the utility of BEC also seems to be restricted by their considerably low specificity, as elevated levels can be observed in several autoimmune and atopic diseases and in many parasitic infections (43).

FeNO

In asthma, the bronchial airway is usually rich in Nitric oxide (NO), produced from inducible nitric oxide synthases (iNOS) under the mediation of the T2 inflammatory cytokines IL-4 and IL-13 (44). NO has been used as an indirect marker of asthmatic inflammation, as its fraction in exhaled breath (FeNO) can be measured non-invasively with the use of a hand-held analyzer, making it a valuable clinical tool in everyday clinical practice (45).

However, FeNO can only modestly predict airway eosinophilia, with many studies suggesting moderate sensitivity and specificity for detecting sputum eosinophilia $\geq 3\%$ (46). Several cut-off values have been proposed, though it has become evident that values < 25 ppb generally rule out airway eosinophilic inflammation, whereas values > 50 ppb are strongly suggestive (47).

Elevated FeNO levels may reflect ongoing airway inflammation and may predict an increased risk for asthma exacerbations and decline in lung function (48, 49). In steroid-naive asthma patients, FeNO levels are higher in patients with allergic asthma and are associated with greater AHR and reversibility of airway obstruction (50). A FeNO level > 50 ppb has been found predictive of response to ICS (47) and, in the appropriate clinical setting, may support the decision for the initiation of ICS. FeNO levels are usually responsive to ICS therapy and their changes have been used to monitor adherence to treatment in patients with uncontrolled asthma (51). In an 8-week, randomized, placebo-controlled trial, FeNO levels significantly decreased and then remained stable after only 2 weeks of treatment with ICS compared with placebo, while they again significantly increased shortly after the 2-week washout period (52). In an Australian RCT including 220 pregnant, non-smoking asthmatic women, 53% of whom had non-eosinophilic, treatment adjustments made according to FeNO levels resulted in more significant reductions in exacerbation rates compared to clinical assessment only guided-therapy (53). A 36-week double-blind, parallel RCT comparing FeNO-guided vs. symptom-based management in 80 asthmatic adults with a history of atopy in two-thirds (54), demonstrated a significant decrease in AHR at the end of the study in the FeNO group compared with the control group. In a more recent double-blind, multicenter RCT, baseline FeNO levels were useful in predicting response to treatment with ICS in patients with no previous asthma diagnosis, non-specific respiratory symptoms (cough, wheeze, or dyspnea), and $< 20\%$ bronchodilator reversibility, mean BEC < 260 cells/ μ L, after exclusion of COPD diagnosis (55). The findings of these studies indicate that the use of FeNO in guiding asthma management may be more appropriate in atopic patients.

However, although airway eosinophilia and T2-inflammation may overlap, they are not synonymous. That has been clearly illustrated in the early mepolizumab

(anti-IL5 mAb) and Dupilumab (anti-IL4R α mAb) studies, in which blood eosinophil and FeNO levels, respectively, were affected by therapy to a different extent (15, 17). In addition, the inconsistency between FeNO levels and asthma control may be, at least partially, explained by the fact that numerous factors, such as age, gender, body weight, atopy, smoking, food and upper respiratory allergic diseases, can affect FeNO and should be taken into account in clinical decisions (56). Therefore, the 2021 National Asthma Education and Prevention Coordinating Committee (NAEPPCC) made a conditional recommendation for FeNO use in clinical practice, suggesting its use in combination with compatible clinical parameters including history, symptoms and clinical and spirometric findings (46).

IgE

The role of IgE in atopic asthma has been well-described for many decades (57). However, although robust evidence of the observed immunological changes produced by anti-IgE therapies have been produced by the times of the first clinical studies of omalizumab (14, 58), the utility of IgE as a biomarker of allergic inflammation in asthma has not yet been fully clarified. However, it has been suggested that total IgE levels can serve as a biomarker for monitoring IgE production and treatment guidance in an individual level (59).

In allergic asthmatics, several studies have shown an association between allergic sensitization in early life and lung function impairment in adulthood". (60–63). Total serum IgE levels have been shown to correlate with asthma severity, and with increased risk for loss of asthma control and severe exacerbations both in children and adults (64–66). However, an old study by the TENOR study group, did not demonstrate a relationship between total IgE geometric mean and severity of asthma, although IgE total levels were higher in children with severe asthma compared to those with mild-moderate disease (67). Moreover, therapeutic efficacy of omalizumab has been shown to be comparable in patients with baseline IgE levels between 30 and 700 UI/mL (68, 69), therefore IgE cannot reliably be used as a biomarker for prediction of treatment response in omalizumab.

On the contrary, measurement of specific IgE (sIgE) to aeroallergens has shown relatively high sensitivity concerning asthma diagnosis (70). Furthermore, Matsui et al. (71), demonstrated that sIgE levels were associated with more severe asthma in a large cohort of young inner-city asthmatics. Similarly, in a more recent study, the number of specific allergen sensitizations correlated with asthma severity and exacerbation risk in children (72). As total serum IgE is not allergen-specific and may be influenced by several extrinsic factors and pathologic conditions, sIgE may be considered a more reliable biomarker both for asthma diagnosis and severity assessment.

Periostin

Periostin is a secreted extracellular matrix protein produced by bronchial epithelial cells and fibroblasts under the stimulus of IL-4 and IL-13, therefore it is also considered to be a T2-related biomarker (73). However, periostin differentiates from other T2 biomarkers in that it is implicated in airway remodeling, therefore it is considered to be associated with chronic rather than acute T2 inflammation (74).

Serum periostin has been associated with fixed airflow limitation and subepithelial fibrosis in a number of clinical studies (75, 76). An RCT evaluating the effect of ICS therapy on serum periostin levels revealed that, ICS significantly lowered both serum periostin and sputum eosinophil counts, and that decrease was associated with improvements in lung function and airway remodeling (77).

However, several studies underline the existing discordance between serum periostin and sputum eosinophilia. Findings from the BOBCAT study showed that periostin was a good predictor of airway eosinophilic inflammation, with an AUROC of 0.84 (78), however, in another study, periostin was unable to differentiate eosinophilic from non-eosinophilic asthma (33). Moreover, wide periostin use is confined by the lack of standardized measurement techniques and validated predicted values (79).

Anti-IL-13 therapies have not so far been authorized for the treatment of severe eosinophilic asthma. However, in a phase-2 RCT including 219 patients with uncontrolled asthma, lebrikizumab, an anti-IL13 mAb, significantly increased FEV1 compared with placebo, only in patients with high serum periostin levels at baseline (80). In a 52-week RCT of tralokinumab, another anti-IL13 mAb, significant improvements in asthma exacerbation rate, lung function, and asthma symptoms were also found in patients with increased pre-treatment periostin serum levels (81). Therefore, periostin is also an emerging biomarker of T2 inflammation.

A summary of the main characteristics of the T2-biomarkers presented above is illustrated in Table 1.

Biomarkers in non-T2 asthma

Non-eosinophilic asthma has traditionally been defined as asthma without evidence of T2 inflammation, and in general is characterized by the predominance of neutrophils or the absence (or normal levels) of other granulocytes in induced sputum (82).

Neutrophilic phenotype constitutes a proportion of non-T2 asthma (83), although until recently it was thought to be a misdiagnosis of chronic obstructive pulmonary disease (COPD) or a consequence of corticosteroid treatment (84). The normal range of neutrophils in induced sputum in healthy individuals ranges between 30 and 50%, and subsequently airway neutrophilia is defined as between 51 and 70% (85).

However, age-specific reference values are warranted, as airway neutrophilia varies with age (86). The neutrophilic phenotype is widely associated with smoking, obesity, acute airway infections, underlying anti-inflammatory therapies and various forms of air pollution (87, 88). Airway neutrophilia has been found to be facilitated by local, IL-17 mediated (89) and systemic inflammatory pathways (90).

Paucigranulocytic asthma comprises another component of non-T2 asthma, and is by default defined as asthma without T2 biomarkers (91). Therefore, targeted treatment options, as well as clinically applicable biomarkers, are still largely under exploration (92). In this context, the clinically relevant issue of whether “true” non-T2 asthma really exists has been raised, as high-dose ICS and oral corticosteroids (OCS) may potentially minimize blood eosinophils and FeNO levels, therefore masking preexisting T2 inflammation (93).

However, “prototype” non-T2 asthma is associated with poor response to corticosteroid treatment (94), therefore some authors suggest that high doses of ICS may be reduced in the majority of patients with non-eosinophilic asthma (95), and in the absence of targeted therapies, other treatable traits should be investigated e.g., exposure to smoking, obesity, chronic respiratory infections, and airway smooth muscle dysfunction (96). Although smoking cessation is favorable for all asthmatic patients, independently of their inflammatory phenotype, this intervention might be even more important for asthmatic patients with neutrophilic inflammation (97). Similarly, although obesity has been linked with neutrophilic inflammation, there is evidence that weight loss interventions may reduce symptom burden in obese asthmatics through inflammation-independent pathways (98). Long-term, low-dose macrolide therapy may also be a therapeutic option for this subset of patients. In the AMAZES study (99), long-term, low dose oral azithromycin therapy significantly decreased exacerbations and improved quality of life in patients with uncontrolled asthma, irrespectively of the underlying inflammatory phenotype. A range of biologics targeting non-T2 cytokines, such as IL-17, IL-6, IL-1, and TNF α , have been tested in several clinical trials, but none of them has shown particular benefits for non-T2 asthma (100).

Emerging biomarkers

The omics approach

As our understanding of asthma heterogeneity increases, it has become evident that best clinical practice demands new approaches in the era of personalized medicine. The omics technologies currently emphasize on the identification of clinically applicable proteomic and genomic-based biomarkers to facilitate health-care development (101).

TABLE 1 Summary of characteristics of major T2-biomarkers.

Biomarker	Utility	Advantages	Limits
Sputum eosinophils counts	Define the airway eosinophilic phenotype (29). Predict AHR, severity of exacerbations and disease outcomes (24, 25). Predict response to treatment with CS (26).	Non-invasive	Time consuming, requires specialized equipment, not all patients can provide adequate samples. Time and treatment-dependent variations (30)
Blood eosinophils counts	Define the eosinophilic phenotype (33). Predictive of exacerbations, loss of asthma control and lung function decline (32, 34, 35). Predict response to biologic therapies (16, 36–40)	Minimally invasive Easily measured in the clinical setting. Good correlations with sputum eosinophils compared to FeNO (33)	Varying cut-off points, variable stability over time (42), relatively low specificity (43)
FeNO	Identifies airway eosinophilic inflammation (45). Prognostic of exacerbations, lung function decline, and degree of AHR (48–50). Predicts response to treatment with CS and biologics (17, 47, 55) and detects adherence to treatment (51) and treatment success (5–54).	Non-invasive. Easily collected in clinical setting	Moderate sensitivity and specificity for sputum eosinophilia $\geq 3\%$ (46). Affected by multiple confounders (56)
Total IgE	Predictive of asthma severity and risk for exacerbations and loss of asthma control (64–66).	Minimally invasive. Identifies patients eligible for anti-IgE therapy (14, 58)	Not-predictive of response to anti-IgE therapy (68, 69). Not asthma-specific; outperformed by sIgE in diagnosing asthma and predicting asthma severity and exacerbation risk (70–72)
Periostin	Identifies T2-inflammation (73), airway remodeling and fixed airway obstruction (74–76). Predicts response to treatment with anti-IL-13 biologics (80, 81).	Not currently available in clinical practice. Lack of standardized measurement techniques and reference values (79)	Poorly associated with sputum eosinophilia (33, 78)

FeNO, fraction of nitric oxide in exhaled air; IgE, immunoglobulin E; sIgE, specific immunoglobulin E; CS, corticosteroids; AHR, airway hyperreactivity.

Genomics

The chromosome 17q21 is in the epicenter of current genomic epidemiological studies in asthma, as it encloses many genes and single nucleotide polymorphisms (SNPs) of interest (102). Several genes (CLC, EMR4P, IL-5RA, FRRS1, HRH4, SLC29A1, SIGLEC8, and IL1RL1) have been identified to be overexpressed in allergic diseases such as asthma, dermatitis and rhinitis, with IL-5/JAK/STAT and IL-33/ST2/IRAK/TRAF pathways being central in disease multimorbidity, both in children and adolescents (103). Similarly, genetic variations of the interleukin-1 receptor-like 1 (IL1RL1) gene have also been related with asthma exacerbations in children (104).

However, although transcriptomics studies have been valuable in the characterization of genome variations between the different asthma subtypes, asthma susceptibility cannot be linked to just a number of genetic signatures, due to the complex underlying disease-pathogenetic mechanisms (105). Therefore, genome-wide, large-scale approaches are essential, such as those performed in the U-BIOPRED consortium (106), which evaluated over 1,500 differentially expressed genes from

large asthma cohorts and revealed three distinct clusters of disease: (i) an eosinophilic cluster, characterized by the IL-33R, CCR3, and thymic stromal lymphopoietin (TSLP) receptor (TSLPR) transcript expression, that was associated with the highest sputum eosinophilia, more elevated FeNO levels, and more severe asthma; (ii) a neutrophilic cluster, characterized by overexpression of genes related to interferon (IFN) and tumor necrosis factor (TNF), sputum neutrophilia, high levels of systemic inflammation and prevalence of eczema; and (iii) a paucigranulocytic-eosinophilic cluster, characterized by genes regulating various metabolic pathways and mitochondrial functions, that shows the lowest prevalence in severe asthma.

Proteomic/metabolomics

In recent years, both proteomics and metabolomics technologies have been increasingly used for the recognition of novel biomarkers in asthma. Exhaled breath, mainly comprised of water vapor and inert gases, also contains thousands of volatile organic compounds (VOCs) (107), with

pH, H₂O₂, and several indirect by-products of oxidation, like 8-isoprostane and ethane in exhaled breath, being the most often analyzed by breathomics technology in asthma (108). Exhaled VOC analysis technologies include two main methodological headings approaches: (a) pattern recognition sensors termed “electronic noses” (e-noses), which “signal” the differential of multiple overlapping VOCs, and (b) chemical analytical techniques, typically with the use of mass spectrometry, that measure ions created by VOCs according to their mass/charge ratio (109). Emerging evidence supports the hypothesis that measurement of VOCs concentrations alterations in exhaled air of asthmatic patients may reflect different disease states, suggesting potential usefulness in non-invasive, early diagnosis and effective management (110–112).

Abdel-Aziz et al. (113) used an electronic nose tool to successfully distinguish healthy from asthmatic subjects, while Nieto-Fontarigo et al. (114), combining liquid chromatography with mass spectrometry, identified 18 serum proteins as potential biomarkers of asthma phenotypes (e.g., FCN2 and MASP1 for non-allergic asthma, or HSPG2 and IGFALS for allergic asthma) and disease severity. Moreover, exhaled breath pH has been found significantly lower in severe refractory asthma compared to moderate disease and healthy subjects (115) and in asthmatic smokers compared with non-smokers (116). Lower EBC pH values have been associated with high BMI, high BAL neutrophil counts, impaired lung function, and increased allergic and gastroesophageal reflux symptoms (117). Brinkman et al. have also illustrated differences in exhaled volatiles such as methanol, acetonitrile, and bicyclooctan-1-ol, 4-methyl in patients with loss of asthma control compared with the recovery period (111). On the contrary, the results of the ALLIANCE study failed to show differences in exhaled VOCs and inflammatory markers in asthmatic patients (118). Although further work is required to validate these preliminary findings, several systematic reviews suggest the large potential for the breathomics biomarker approach (112).

Microbiota and respiratory infections biomarkers

Modern lifestyle has fundamentally disrupted the human microbiome ecology and this may explain, at least in part, the increased incidence of immune-mediated diseases such as allergy and asthma (119). These highly sophisticated host–microbe interactions are currently being intensely studied by many researchers around the world.

It is evident that early-life alterations in gut microbiome composition may be involved in asthma pathogenesis (120). In adult asthmatics, a positive correlation between the increased abundance of histamine-secreting bacteria in the gut and asthma

severity has been found (121). Similarly, in murine models of asthma, bacterial-derived histamine released within the gut has been demonstrated to mediate significant inflammatory responses in the lungs (122). In addition to increased abundance, more interestingly, decreased microbiome diversity in the gut has been associated with dysbiosis and enhanced prevalence of allergies and asthma (123, 124). Therefore, due to the close interaction of intestinal microbiota on the mucosal immune system, the gut can be considered as an essential site of immune cross-talk, with an opposing impact on allergy development and treatment, the so-called gut-lung axis (125).

In addition to the gut microbiota, several studies have also showed significant alterations in the microbial communities in the asthmatic airways. Zhou et al., have shown that the relative abundance of *Veillonella* and *Prevotella spp* in the airways of newborns was associated with increased risk of asthma incidence by the age of 6 years (126). In another study, alterations in the microbiome composition in the upper airways in infants were associated with a higher risk of severe asthma exacerbation in asthmatic children (127). In adults, predominance of the phylum *Proteobacteria* in the lungs may be associated with more severe asthma or with loss of asthma control (128), with more profound microbial changes observed in obese asthmatics (129). Microbial metabolites may also potentially serve as valuable biomarkers in asthma, as dysfunctional relationships have been found between respiratory microbes and their circulating metabolites (130).

Limitations and future perspectives

Unfortunately, at the moment, all the available asthma biomarkers used in severe asthma lack of specificity for the identification of the precise disease endotype that will respond to the existing targeted treatments i.e., blood eosinophilia predicts therapeutic response to all currently available biological therapies (10). Similarly, biomarkers cannot differentiate eosinophilic asthma from eosinophilic COPD, although the relationship between airway and systemic eosinophilia and T2 inflammation appears stronger in severe asthma than in COPD (131). In line with the above, the use of blood eosinophils as the sole biomarker for the eosinophilic phenotype may be misleading, as the amount of eosinophils in the blood is both time and treatment-dependent (132, 133), therefore a single low BEC may not necessarily rule out airway eosinophilia (134).

In recognition of the inherent heterogeneity of asthma phenotypes, Pavord et al. (135), proposed a gradient rather than a dichotomous approach for the classification of severe asthma phenotypes. Similarly, Heaney et al. (136), using data of 1,716 patients from the International Severe Asthma Registry (ISAR), proposed an eosinophilic-probability algorithm to assess severe asthma phenotypes, classifying eosinophilic status from Grade 3 (most likely eosinophilic) to Grade 0 (non-eosinophilic).

With the use of this approach, the authors showed that the eosinophilic phenotype prevails in the majority of severe asthmatics, with 83.8% of subjects belonging into the “most likely” eosinophilic phenotype and only 1.6% characterized as “non-eosinophilic”. Supportive evidence on this issue comes from the real-world Wessex AsThma CoHort of difficult asthma (WATCH) study (137), that searched historical electronic blood count records of patients with difficult asthma over a period of 10 years, and reported a strikingly increased prevalence of “eosinophilia ever” when viewed longitudinally.

The combination of different biomarkers may add additional discriminatory value in predicting exacerbations and response to treatment. Price et al., in an observational study with participation of primary care patients, demonstrated that the combination of both high FeNO (>50 ppb) and high BEC (>300 cells/ μ L) resulted in an almost 4-fold greater exacerbation risk the preceding year, compared to patients with the low biomarkers reference group (138).

More interestingly, the combination of biomarkers with relevant clinical characteristics may be more accurate in the characterization of asthma phenotypes. Recently, a severe asthma expert consensus proposed a set of diagnostic criteria for severe eosinophilic asthma, however, this approach has not been validated in a clinical setting (139). To this point, the ISAR study group (140), developed a multicomponent algorithm for use in real life, incorporating many variables associated with severe eosinophilic asthma (BEC FeNO, atopic status, age of asthma onset, asthma related comorbidities, and OCS use), while both the Severe Asthma Research Program (SARP) and the Leicester cohorts have also used this approach (141). Based on the combination of feasible biomarkers with clinical meaningful disease characteristics, current asthma guidelines have now adapted algorithms for the initial choice of targeted biologic treatments and for the monitoring of subsequent treatment response (142–144).

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Conclusions

Biomarkers are significant elements of precision medicine, as they may provide valuable information, in terms of characterization of disease phenotypes and endotypes, selection of the appropriate targeted therapy, and monitoring of treatment efficacy. The advent of new techniques, combined with biomarker combination strategies, will likely assist the identification of novel functional traits for patients non-responsive to currently available targeted treatment options, including patients with non-T2 asthma.

Author contributions

EF contributed to conception of the manuscript and wrote the first draft of the manuscript. EF, FG, and MA wrote sections of the manuscript. KD and KP have also contributed to conception of the manuscript, apart from revision, read and approve. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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