



Biomarkers and asthma management: an update

Hannah K. Bayes^{a,b} and Douglas C. Cowan^b

Purpose of review

Asthma is heterogeneous with different endotypes/phenotypes. Response to corticosteroids is variable and novel biological therapies are proving useful. Biomarkers allow individualization of treatment. This review provides an update on available data regarding asthma biomarkers with focus on their utility for prediction of response to steroidal and new biological therapies.

Recent findings

Blood eosinophils are a biomarker with acceptable accuracy as a surrogate for sputum eosinophilia, are associated with relevant outcomes, and are more readily measurable. New evidence supports fraction of exhaled nitric oxide (FENO)-based treatment algorithms for cost-effective maintenance of asthma control/quality of life. Serum and sputum-derived periostin are biomarkers of lung function decline and associated with eosinophilic airway inflammation. Transcriptomics show promise for endotyping; their role in management remains to be determined. Biomarker panels may improve predictive value as shown for the combination of FENO/urinary bromotyrosine in prediction of steroid responsiveness. Novel biological therapies are proving effective in biomarker-selected populations.

Summary

Biomarkers including blood eosinophils and FENO are proving to have utility for the effective administration of steroidal and novel biological therapies in asthma, allowing individualized treatment. Transcriptomics can discriminate subtypes of asthma and may have a role in delivery of individualized therapy.

Keywords

asthma, biomarker, eosinophil, fraction of exhaled nitric oxide, individualized treatment

INTRODUCTION

Asthma is a chronic heterogeneous disease characterized by airway inflammation, typical symptoms, and variable expiratory airflow limitation [1]. It affects 300 million people worldwide, causes 250 000 deaths annually and significantly impacts on healthcare services and society as a whole [2]. Different endotypes (defined by distinct pathophysiological mechanisms) and phenotypes (defined by characteristics of an individual because of interaction of genotype and environment) can be identified [1,3,4] and these have variable responses to currently available treatments [2,5]. Corticosteroids are the mainstay of treatment but a significant minority have 'difficult asthma', defined in the British Asthma Guidelines as persistent symptoms and/or frequent asthma attacks despite regular inhaled or oral corticosteroid-based treatment [6]. Indeed 4–10% of the asthmatic population have severe refractory asthma [7]. Novel therapies predominantly targeting eosinophilic airway inflammation in asthma are increasingly being translated to clinical practice. Identification of

specific biomarker-based asthmatic subtypes will allow delivery of effective targeted standard or novel therapies that are tailored to the individual.

Biomarkers (physical signs or laboratory measurements serving as indicator of underlying pathophysiology [8]) may be used for diagnosis, monitoring, prediction of future risk, and selection of effective treatments. The optimal biomarker will be sensitive and specific, reliable and reproducible, practical, simple to perform, and low in cost.

Biomarkers suggested to have utility in the management of asthma include sputum eosinophils, fraction of exhaled nitric oxide (FENO) and, most

^aInstitute of Infection, Immunity and Inflammation, University of Glasgow and ^bDepartment of Respiratory Medicine, Glasgow Royal Infirmary, Glasgow, UK

Correspondence to Douglas C. Cowan, MBChB, MRCP, PhD, Department of Respiratory Medicine, Glasgow Royal Infirmary, 16 Alexander Parade, Glasgow G31 2ER, UK. Tel: +44 141 211 5451; fax: +44 141 211 1120; e-mail: Douglas.Cowan@ggc.scot.nhs.uk

Curr Opin Allergy Clin Immunol 2016, 16:210–217

DOI:10.1097/ACI.0000000000000263

KEY POINTS

- A significant proportion of patients with asthma have difficulty to control disease.
- Biomarkers will allow individualization of asthma treatment strategies, including directing use of steroids and biological therapies to those who are likely to gain benefit and prevent nonbeneficial use.
- Evidence supports the use of sputum eosinophils, blood eosinophils, FENO and, most recently, periostin as biomarkers of Th2 cell high eosinophilic airway inflammation and in directing steroidal and biological therapies.
- Transcriptomics of asthmatics, both in the airways and blood, is an evolving area of research that may prove useful in phenotyping patients, predicting treatment response, and in elucidating novel mechanisms of disease.
- Combining biomarkers may improve prediction of treatment responsiveness, for example, recent data suggested a role for combination of FENO and urinary bromotyrosine in prediction of steroid responsiveness.

recently, periostin; all biomarkers of Th2 cell high eosinophilic airway inflammation that is considered more steroid-responsive [9,10]. This review will provide an update on research published in 2014/2015 in the field of asthma biomarkers with focus on biomarker utility for prediction of response to steroidal and new nonsteroidal therapies.

ASTHMA BIOMARKERS: RECENT DEVELOPMENTS

Surrogate markers of sputum eosinophilia

Sputum eosinophil count is a useful biomarker in asthma for prediction of steroid responsiveness [5,11] and loss of control after discontinuation of inhaled corticosteroid (ICS) [12], as well as adjusting steroid dose to reduce exacerbations [13,14]. However, sputum induction is demanding on patients, obtained specimens are variable quality, and subsequent processing and analysis require technical expertise that is not widely available. For these reasons, surrogate biomarkers are required.

Peripheral eosinophil count is cheaper and more readily available in the clinical setting. In one study [15], a blood eosinophil count at least 150 cells/ μ l predicted sputum eosinophils at least 2% in nine out of 10 cases with sensitivity 85% and specificity 75%. In another study [16], a blood eosinophil cut-point of at least 0.45×10^9 cells/l had high specificity (97.0%) and positive predictive value (89.2%) for

a sputum eosinophil count at least 2%. The ELEN index, based on eosinophil/lymphocyte and eosinophil/neutrophil ratios, is also proposed for sputum eosinophilia prediction and has recently been demonstrated to have higher predictive accuracy (79.2%) than blood eosinophils alone for sputum eosinophils at least 2% (sensitivity 65.6% and specificity 88.2%) [17].

Blood eosinophils are also associated with asthma outcomes. In a study [18] of 2392 asthmatic adults, blood eosinophils at least 400/mm³ was associated with increased risk of both exacerbations and high reliever use over the following year. In a large primary care cohort study [19] of 130 248 asthmatic individuals, those with peripheral eosinophil count above 400 cells/ μ l had more severe exacerbations and acute respiratory events within a year of follow-up than those with count 400 cells/ μ l or less, and were less likely to achieve asthma control.

Blood eosinophils, FENO, and serum periostin (all Th2-high asthma biomarkers) have been compared for prediction of sputum eosinophilia ($\geq 3\%$) [20**]. Sputum eosinophils were correlated with blood eosinophils and FENO, but not with serum periostin. Furthermore, blood eosinophils and FENO, but not serum periostin, were able to discriminate eosinophilic and noneosinophilic inflammation. Blood eosinophil cut-point of at least 0.27×10^9 /l produced sensitivity 78% and specificity 91%, whereas FENO cut-point of at least 42 ppb gave sensitivity 63% and specificity 92%. Combining biomarkers did not improve diagnostic accuracy. The lack of concordance of periostin with other Th2-high biomarkers is unexpected and suggests that periostin is less tightly associated with eosinophilic airway inflammation than previously thought; this requires clarification in further studies.

Other biomarkers with diagnostic value for sputum eosinophilia prediction include the type 2 innate lymphoid cell count (better than FENO, and comparable with blood eosinophil count in one study [21]) and nasal and pharyngeal eosinophil peroxidase [22]. Interestingly, noninvasive methods for detection of airway eosinophilia are also being developed. In a murine model, noninvasive near-infrared fluorescence imaging, which detected a lectin on eosinophils (Siglec-F) identified eosinophilic airway inflammation [23]. Similarly, in humans the injection of autologous technetium-99m-labelled eosinophils with single photon emission computed tomography has been used to localize eosinophilic lung inflammation [24].

Thus, despite several proposed alternatives, peripheral eosinophil count perhaps shows most

Table 1. Recent results for biomarkers and outcome measures in asthma

Biomarker	First author	Study population	Study design	Duration	Outcome
Blood eosinophils $\geq 400/\text{mm}^3$	Zeiger [18]	2392 adults with asthma	Cohort	1 year	Adjusted rate ratios (95% CI): exacerbations: 1.31 (1.07–1.60), $P=0.009$, ≥ 7 SABA dispensed: 1.17 (1.03–1.33), $P=0.015$
Blood eosinophils $>400/\mu\text{l}$	Price [19]	130 248 primary care patients aged ≥ 12 years with asthma	Cohort	1 year	Adjusted rate ratios (95% CI): severe exacerbations: 1.42 (1.36–1.47); acute respiratory events: 1.28 (1.24–1.33); odds ratio for achieving asthma control: 0.74 (95% CI 0.72–0.77)
FENO <20 ppb	Peirsman [25 [■]]	99 children with allergic asthma	Multicentre, single-blind, RCT, symptom versus FENO-based treatment	1 year	No difference in symptom free days. Significant reductions in asthma exacerbations and unscheduled asthma-related contacts, but increased LRA and ICS use in the FENO-guided group
FENO <10 – 20 ppb depending on atopy status	Petsky [26 [■]]	55 children with asthma from secondary care	Dual-centre, single-blind, RCT, symptoms versus FENO-based treatment	1 year	Significant reduction in those with ≥ 1 exacerbations (6/27 in FENO-based versus 15/28 in the standard care group; $P=0.021$); increased ICS use seen in FENO-guided group
FENO <25 ppb	Honkoop [27 [■]]	611 adults with asthma in primary care	Unblinded, three-arm RCT, symptoms controlled [partly (PCa) or fully (Ca)] versus symptoms fully controlled + FENO (FCa)	1 year	Reduced medication costs for PCa and FCa; FCa had highest probability of cost-effectiveness; no differences in severe exacerbation rate
FENO	Wilson [28]	191 adults with asthma from primary care	Cohort	3 months	No difference in baseline FENO or change in FENO between those successfully reducing ICS dose and those with loss of control/exacerbation
FENO <25 ppb	Bergen [29]	175 children with atopic asthma	Randomized, parallel, treatment adjustment based on FENO + ACT versus standard care + ACT	1 year	No difference in symptoms free days

ACT, asthma control test; Ca, controlled; CI, confidence interval; FCa, fully controlled; FENO, fraction of exhaled nitric oxide; ICS, inhaled corticosteroid; LRA, leukotriene receptor antagonist; PCa, partly controlled; RCT, randomized controlled trial; SABA, short acting beta2 agonist

promise to date as a surrogate of sputum eosinophilia with proven utility in the prediction of outcomes (Table 1). Furthermore, this biomarker has the additional advantages of being cheap, widely available, and easy to obtain.

Fraction of exhaled nitric oxide and outcomes in asthma

Previous guidelines for the use of FENO in asthma management have been conflicting. The 2011 ATS Clinical Practice Guideline recommended FENO for diagnosis of eosinophilic airway inflammation, predicting steroid responsiveness and monitoring airway inflammation in asthma [30]. However, the

2014 European Respiratory Society (ERS)/ATS severe asthma guideline recommended that clinicians do not use FENO to guide therapy in severe disease. Similarly, the 2014/2015 Global Initiative for Asthma (GINA) guidelines stated that FENO cannot be recommended at present for deciding whether to treat patients with possible asthma with ICS and that FENO-guided treatment is not recommended for the general asthma population [1]. However, emerging data support a role for FENO in asthma management (Table 1).

Two recent studies showed significant reductions in exacerbations using a FENO-based treatment algorithm. In one study [25[■]], children with allergic asthma were randomized to FENO-

based or symptoms-based treatment. Over the following year, 23.9% in the FENO group and 47.8% in the symptom group experienced at least one exacerbation ($P=0.017$). However, this was at the cost of significant increases in ICS dose and leukotriene receptor antagonist (LRA) use in the FENO group. Atopy increases FENO [31], and in another study [26[¶]], children with asthma were randomized to treatment based on FENO, adjusted for atopy, versus symptoms-based treatment. Significantly, fewer children in the FENO group had at least one exacerbation over the following year and the number needed to treat to prevent one child from having an exacerbation was four (95% confidence interval 3, 24).

An unblinded randomized trial has compared two symptom-based strategies aiming for partially (Asthma Control Questionnaire, $ACQ < 1.5$) and fully ($ACQ < 0.75$) controlled asthma, and a third symptom and FENO-based strategy aiming for fully controlled (FCa) ($ACQ < 0.75$ and $FENO < 25$ ppb) asthma, in 611 asthmatic adults taking ICS over a year [27^{¶¶}]. The symptom/FENO-based strategy led to asthma control that was significantly better than that for the symptom-based strategy aiming for partial control, and not significantly different from that achieved with the symptom-based strategy aiming for fully controlled asthma. Medication costs were significantly lower if aiming for symptom-based partially controlled or symptom/FENO-based fully controlled asthma than when aiming for symptom-based fully controlled asthma. There were no significant differences between strategies in outcomes such as asthma-related quality of life and severe exacerbation rate.

In contrast to the above studies supporting FENO use, a recent study [28] of adults with well controlled asthma within primary care showed no difference in baseline FENO or in change in FENO between participants successfully reducing ICS dose and those with loss of control or exacerbation over a 3-month period after a 50% reduction in ICS; therefore, FENO was not predictive of loss of control or exacerbation on ICS reduction. In another study [29] of children with atopic asthma followed over a year, the proportion of symptoms free days was similar whether treatment adjustments were based on FENO monitoring and asthma control test (ACT) or just standard care (ACT alone).

Although not fully concordant, these studies do suggest that FENO-based algorithms may have utility for the adjustment of asthma treatment over time with the potential benefits of reducing exacerbations at the expense of increased treatment burden, or reducing medication costs while sustaining asthma control. The recent National Institute for Health and Care Excellence (NICE) guideline states

that the use of FENO in the management of adults with asthma is likely to be cost-effective [32].

Periostin update

Periostin, an extracellular matrix protein induced by IL-4 and IL-13 in airway epithelial cells and lung fibroblasts, has a role in subepithelial fibrosis and accelerating eosinophil tissue infiltration [33]. Serum periostin is proposed as a biomarker of Th2-high asthma. Elevated levels correlate with airway eosinophilia [34] and decline in forced expiratory volume in 1 s [35], and predict response to ICS [36], Th2-targeted monoclonal antibodies, anti-IL-13 (lebrikizumab) [37], and anti-IgE (omalizumab) [38]. Periostin is easily measurable in the systemic circulation, but lacks specificity for asthma, being elevated in several non-Th2-related disease states and produced by other tissue types in addition to airway epithelium [33,39].

Twenty to thirty percent of adult asthmatics are cigarette smokers and as a consequence have poorer disease control, corticosteroid insensitivity, and noneosinophilic/Th2-low airways inflammation compared with never smokers with asthma [40]. A recent study [41] compared serum periostin levels in smokers and never smokers with asthma and in healthy controls. A notable finding was that, although smokers with asthma had significantly lower serum periostin levels than asthmatic never smokers, approximately 40% of smokers with asthma had serum periostin levels above the group median suggesting that subtyping of periostin high smokers with asthma may guide targeted anti-Th2 interventions in this difficult patient group.

Elevated serum periostin has previously been shown to correlate with accelerated lung function decline in asthma [35]. This is further supported by a recent bronchial biopsy study [42] in asthmatics that demonstrated both periostin and osteopontin (another extracellular matrix protein) to be associated with long-standing decline in pulmonary function. In addition, in steroid-naïve asthmatics, ICS led to decreased serum periostin, improved airflow limitation, decreased sputum eosinophils, and reduced airway wall thickness [36]. Thus, collectively, these results strengthen the case for serum periostin as a biomarker for targeting not only eosinophilic inflammation but also the development of airway remodelling in asthma.

Sputum periostin measurement may represent a more organ-targeted asthma-specific biomarker than serum periostin, and one study [43] has confirmed an association between sputum periostin levels and eosinophilic inflammation and airflow obstruction. Finally, combining serum periostin

with additional biomarkers may help in targeting treatment. Patients with elevated FENO and high periostin were found to have accelerated decline in lung function and increased risk of asthma exacerbation despite use of high-dose ICS, compared with FENO-high, periostin-low patients [44].

Transcriptomic endotypes of asthma

Asthma biomarker research has to date focused predominantly on end products of inflammatory pathophysiological pathways; however, transcriptomics provides the opportunity to discriminate asthma subtypes at the level of gene expression. A series of recent publications have begun to dissect the gene transcription profile in the airways and blood of asthmatics linking these to clinical and inflammatory phenotypes [45[•],46,47[•],48[•]].

Measurement of expression of three genes (IL-4, IL-5, and IL-13) within sputum was utilized to classify asthmatics into Th2-low and Th2-high subgroups; the Th2-high group had more severe disease and increased blood and sputum eosinophilia [46]. Analysis of sputum from adult patients has also identified a pattern of expression of six genes that accurately identifies eosinophilic asthma and predicts response to ICS, expression being attenuated with treatment [47[•]].

In another study [45[•]], analysis of the sputum transcriptome identified distinct clusters ('transcriptomic endotypes of asthma') that correlated with clinical and pathophysiological markers of severe and milder asthma phenotypes. These differential patterns of gene expression could also be identified in the peripheral blood of both adults and children, suggesting that there are common and stable systemic changes in gene expression in patients with different asthma subtypes. Within the transcriptomic endotypes of asthma with severe disease and previous near-fatal asthma, differential transcription of genes involved in neuronal function, histamine synthesis, and epithelial cell differentiation was evident [45[•]]. Thus, transcriptomics may facilitate identification of novel molecular mechanisms amenable to therapeutic targeting.

As previously discussed, sputum-based biomarkers may be less practical in the clinical setting. A study [48[•]] utilizing nasal airway brushings in the paediatric asthmatic population has demonstrated that nasal airway gene expression closely mirrors that of bronchial and small airways, including the ability to identify Th2-high and Th2-low subgroups. Future considerations for use of transcriptomics in asthma will include the longitudinal stability of gene expression profiles, influence of intercurrent illness/exacerbations, and relationship

to pathological mechanisms. Transcriptomics may further our understanding of Th2-low disease, and may have utility in predicting response to biological therapies.

Biomarkers for prediction of steroid responsiveness

Corticosteroids remain the mainstay of asthma treatment but response is variable and a proportion of asthmatics are steroid-resistant. Furthermore, in patients taking steroids, not all symptoms are steroid-responsive; dose escalation may increase side-effects while doing little to ameliorate symptoms. Biomarkers of steroid responsiveness would allow appropriate initiation or escalation of steroid treatment, while avoiding or minimizing unhelpful use. As discussed earlier, sputum eosinophil count has proven utility in predicting steroid response and tailoring long-term steroid treatment but is impractical in the clinical setting and other biomarkers are needed.

One study [49[•]] evaluated sputum eosinophils, FENO, and urinary bromotyrosine (a marker of eosinophil activation) for prediction of steroid responsiveness. Receiver-operating characteristic curve analyses area under the curves for prediction of improvement of at least two of the three possible clinical outcomes for sputum eosinophils, FENO, and urinary bromotyrosine were 0.848, 0.754, and 0.691, respectively. The combination of high FENO (≥ 35 ppb) and urinary bromotyrosine (≥ 0.45 ng/mg of creatinine) was associated with a 13-fold increase in the likelihood of a positive response in two clinical outcomes to steroid, suggesting that these two biomarkers have utility in prediction of steroid response while avoiding the need for sputum analysis.

The Medical Research Council UK Refractory Asthma Stratification Programme includes a randomized, multicentre, parallel group study [50] that is underway to evaluate the utility of a composite biomarker scoring system based on FENO, blood eosinophils, and serum periostin for the optimization of corticosteroid treatment in severe asthmatics with low baseline FENO. The results of this study are eagerly awaited.

Biomarkers and biological therapies

Using biomarkers to define specific asthma subtypes that are likely to gain benefit from treatment is key to the success of novel biological agents [37,38,51].

Recent studies [52,53^{••},54^{••},55] have examined the potential of IL-5 blockade, via anti-IL-5/anti-IL-5-receptor monoclonal antibodies, in asthma. IL-5

promotes eosinophil recruitment, persistence, and activation [56], thus studies targeted eosinophilic asthma using blood eosinophil level for selection. Two phase III randomized controlled trials (RCTs) evaluated mepolizumab (anti-IL-5 monoclonal antibody) in patients with severe asthma, frequent exacerbations, and elevated blood eosinophils [53²²,54²²]. Mepolizumab resulted in significant reduction in oral glucocorticosteroid use, reduced exacerbations, and improved symptom control. Two additional RCTs, reported together, examined the role of reslizumab (anti-IL-5 monoclonal antibody) in inadequately controlled asthmatics using medium-to-high doses of ICS-based therapy with elevated blood eosinophils [55]. Both trials demonstrated significant reductions in exacerbation frequency in the active treatment group. Thus, peripheral blood eosinophil count allows effective selection of an anti-IL-5 treatment-responsive patient group.

IL-13, a T2 cytokine, has been linked to airway hyperresponsiveness, mucus hypersecretion, airway remodelling, and corticosteroid resistance [57]. Previously, blockade of IL-13 in moderate-to-severe asthma demonstrated benefit, particularly in patients with evidence of IL-13 pathway activation (either elevated sputum IL-13 [58] or serum periostin levels [37]). Two additional studies have further evaluated treatment effect in patients with uncontrolled severe asthma receiving high-dose ICS [59,60]. The smaller trial of an anti-IL-13 compound, GSK679586, demonstrated no clinical benefit over 12 weeks overall or in the subgroup with elevated serum IgE or blood eosinophil levels [59]. However, neither serum periostin [37] nor sputum IL-13 [58] levels were measured or used to select study patients. Tralokinumab, an anti-IL-13 monoclonal antibody, also failed to significantly reduce exacerbations in a 52-week trial [60]. A post hoc subgroup analysis suggested a possible, but not statistically significant, treatment effect in subgroups with elevated serum dipeptidyl peptidase-4, a factor induced by IL-13 in airway epithelial cells, or serum periostin levels. These recent studies may suggest that IL-13 blockade is less effective in severe disease or that alternative biomarker(s) are required to identify potentially anti-IL-13 responsive patients in the more severe asthma population.

CONCLUSION

It is clear that future management strategies for asthma will be targeted toward specific patient subgroups using specific biomarker(s). The year 2014/2015 has seen exciting developments in this field for Th2-high asthma, including a greater

Table 2. Future directions in asthma biomarker research

Proposed questions for future research

Is a blood eosinophil-based strategy for steroid dose adjustment superior to a standard symptom-based algorithm in long-term management of asthma, and if so, is it comparable with, or better than, a FENO-based algorithm?

What is the role of composite biomarker-based algorithms for adjustment of steroid therapy?

What are the biomarkers of T2-low asthma and can they be used to identify targets for novel treatments in this subtype?

Do transcriptomics have utility in the delivery of individualized asthma therapy?

FENO, fraction of exhaled nitric oxide.

understanding of the roles of blood eosinophils, periostin and FENO as biomarkers, targeting of biological therapies, and the evolving role of transcriptomics in asthma phenotyping. Developing biomarker-directed treatments for Th2-low disease remains a challenge for future research (Table 2).

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Global Initiative for Asthma (GINA) Report. Global Strategy for Asthma Management and Prevention 2015; <http://www.ginasthma.org/documents/4>. [Accessed January 2016]
2. World Health Organisation (WHO) Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. http://www.who.int/gard/publications/GARD_Manual/en/. [Accessed January 2016]
3. Lötvall J, Akdis CA, Bacharier LB, *et al.* Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011; 127:355–360.
4. Haldar P, Pavord ID, Shaw DE, *et al.* Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178:218–224.
5. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Noneosinophilic corticosteroid unresponsive asthma. *Lancet* 1999; 353:2213–2214.
6. British Thoracic Society Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* 2014; 69 (Suppl 1): 1–192.
7. Chung KF, Wenzel SE, Brozek JL, *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43:343–373.
8. Lesko LJ, Atkinson AJ r. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. *Annu Rev Pharmacol Toxicol* 2001; 41:347–366.
9. Smith AD, Cowan JO, Brassett KP, *et al.* Exhaled nitric oxide: a predictor of steroid response. *Am J Respir Crit Care Med* 2005; 172:453–459.
10. Pavord ID, Korn S, Howarth P, *et al.* Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012; 380:651–659.

11. Brown HM. Treatment of chronic asthma with prednisolone; significance of eosinophils in the sputum. *Lancet* 1958; 13:1245–1247.
 12. Deykin A, Lazarus SC, Fahy JV, *et al.* Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. *J Allergy Clin Immunol* 2005; 115:720–727.
 13. Green RH, Brightling CE, McKenna S, *et al.* Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360:1715–1721.
 14. Jayaram L, Pizzichini MM, Cook RJ, *et al.* Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006; 27:483–494.
 15. Ortega H, Katz L, Gunsoy N, *et al.* Blood eosinophil counts predict treatment response in patients with severe eosinophilic asthma. *J Allergy Clin Immunol* 2015; 136:825–826.
 16. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. *J Allergy Clin Immunol* 2015; 135:822–832.
 17. Khatri DB, Gossage DL, Geba GP, *et al.* Discriminating sputum-eosinophilic asthma: accuracy of cutoffs in blood eosinophil measurements versus a composite index, ELEN. *J Allergy Clin Immunol* 2015; 136:812–822.
 18. Zeiger RS, Schatz M, Li Q, *et al.* High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. *J Allergy Clin Immunol Pract* 2014; 2:741–744.
 19. Price DB, Rigazio A, Campbell JD, *et al.* Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Med* 2015; 3:849–858.
 20. Wagener AH, de Nijs SB, Lutter R, *et al.* External validation of blood eosinophils, FENO and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015; 70:115–120.
- A cross-sectional study evaluating the diagnostic accuracy of blood eosinophils, FENO and serum periostin for the prediction of sputum eosinophilia in mild–moderate and moderate–severe asthma cohorts. Blood eosinophils and FENO, but not serum periostin, had diagnostic utility for prediction of sputum eosinophilia. Blood eosinophil count was the biomarker with best overall performance characteristics.
21. Liu T, Wu J, Zhao J, *et al.* Type 2 innate lymphoid cells: a novel biomarker of eosinophilic airway inflammation in patients with mild to moderate asthma. *Respir Med* 2015; 109:1391–1396.
 22. Rank MA, Ochkur SI, Lewis JC, *et al.* Nasal and pharyngeal eosinophil peroxidase levels in adults with poorly controlled asthma correlate with sputum eosinophilia. *Allergy* 2015; doi: 10.1111/all.12817. [Epub ahead of print]
 23. Markus MA, Dullin C, Mitkovski M, *et al.* Non-invasive optical imaging of eosinophilia during the course of an experimental allergic airways disease model and in response to therapy. *PLoS One* 2014; 9:e90017.
 24. Loutsios C, Farahi N, Simmonds R, *et al.* Clinical application of autologous technetium-99m-labelled eosinophils to detect focal eosinophilic inflammation in the lung. *Thorax* 2015; 70:1085–1086.
 25. Peirsman EJ, Carvelli TJ, Hage PY, *et al.* Exhaled nitric oxide in childhood allergic asthma management: a randomised controlled trial. *Pediatr Pulmonol* 2013; 49:624–631.
- A multicentre, single-blind, RCT of symptom-based versus FENO-based asthma management over 12 months in 99 allergic asthmatic children. FENO-based treatment was associated with a significant reduction in exacerbations but significant increase in ICS dose and leukotriene receptor antagonist use.
26. Petsky HL, Li AM, Au CT, *et al.* Management based on exhaled nitric oxide levels adjusted for atopy reduces asthma exacerbations in children: a dual centre randomized controlled trial. *Pediatr Pulmonol* 2014; 50:535–543.
- A dual centre, single-blind, RCT of symptoms-based versus FENO-based (adjusted for atopy) asthma management over 12 months in 63 children with asthma. FENO-based treatment led to a significant reduction in children experiencing at least one exacerbation but a significantly higher ICS dose. The number needed to treat to prevent one child from having an exacerbation in 12 months was four.
27. Honkoop PJ, Loijmans RJB, Termeer EH, *et al.* Symptom and fraction of exhaled nitric oxide-driven strategies for asthma control: a cluster-randomized trial in primary care. *J Allergy Clin Immunol* 2015; 135:682–688.
- A nonblind, pragmatic randomized trial of three treatment strategies over 12 months in 611 asthmatic adults in primary care aiming at: partial control (ACQ < 1.5), complete control (ACQ < 0.75), and FENO-driven control (ACQ < 0.75, FENO < 25 ppb). The FENO-driven strategy led to significantly reduced medication use compared with the symptom-driven complete control strategy and, of the three strategies, had the highest probability of cost-effectiveness while sustaining asthma control and quality of life.
28. Wilson E, McKeever T, Hargadon B, *et al.* Exhaled nitric oxide and inhaled corticosteroid dose reduction in asthma: a cohort study. *Eur Respir J* 2014; 44:1705–1707.
 29. Bergen SV-V, Vaessen-Verberne AA, Landstra AM, *et al.* Fractional exhaled nitric oxide monitoring does not improve asthma management in children with concordant and discordant asthma phenotypes. *Am J Respir Crit Care Med* 2015; 192:1016–1018.
 30. Dweik RA, Boggs PB, Erzurum SC, *et al.* An Official ATS Clinical Practice Guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. *Am J Respir Crit Care Med* 2011; 184:602–615.
 31. Nadif R, Rava M, Decoster B, *et al.* Exhaled nitric oxide, nitrite/nitrate levels, allergy, rhinitis and asthma in the EGEA study. *Eur Respir J* 2014; 44:351–360.
 32. The National Institute for Health and Care Excellence (NICE). Measuring fractional exhaled nitric oxide concentration in asthma: NIOX MINO, NIOX VERO and NObreath 2014. <https://www.nice.org.uk/guidance/dg12>. [Accessed January 2016]
 33. Parulekar AD, Atik MA, Hanania NA. Periostin, a novel biomarker of TH2-driven asthma. *Curr Opin Pulm Med* 2014; 20:60–65.
 34. Jia G, Erickson RW, Choy DF, *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012; 130:647–654.
 35. Kanemitsu Y, Matsumoto H, Izuahara K, *et al.* Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013; 132:305–312.
 36. Hoshino M, Ohtawa J, Akitsu K. Effect of treatment with inhaled corticosteroid on serum periostin levels in asthma. *Respirology* 2016; 21:297–303.
 37. Corren J, Lemanske RF Jr, Hanania NA, *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011; 365:1088–1098.
 38. Hanania NA, Wenzel S, Rosén K. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013; 187:804–811.
 39. Chiappori A, De Ferrari L, Folli C, *et al.* Biomarkers and severe asthma: a critical appraisal. *Clin Mol Allergy* 2015; 13:20.
 40. Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. *Eur Respir J* 2013; 41:716–726.
 41. Thomson NC, Chaudhuri R, Spears M, *et al.* Serum periostin in smokers and never smokers with asthma. *Respir Med* 2015; 109:708–715.
 42. Kanemitsu Y, Ito I, Niimi A, Izuahara K. Osteopontin and periostin are associated with a 20-year decline of pulmonary function in patients with asthma. *Am J Respir Crit Care Med* 2014; 190:472–474.
 43. Bobolea I, Barranco P, del Pozo V, *et al.* Sputum periostin in patients with different severe asthma phenotypes. *Allergy* 2015; 70:540–546.
 44. Nagasaki T, Matsumoto H, Kanemitsu Y. Using exhaled nitric oxide and serum periostin as a composite marker to identify severe/steroid-insensitive asthma. *Am J Respir Crit Care Med* 2014; 190:1449–1452.
 45. Yan X, Chu J-H, Gomez J, *et al.* Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. *Am J Respir Crit Care Med* 2015; 191:1116–1125.
- Comprehensive study of the transcriptome in asthma demonstrating common patterns of gene expression in the sputum and blood of children and adults that are associated with the clinical phenotypes of near-fatal, severe, and milder asthma.
46. Peters MC, Mekonnen ZK, Yuan S, *et al.* Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014; 133:388–394.
 47. Baines KJ, Simpson JL, Wood LG, *et al.* Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. *J Allergy Clin Immunol* 2014; 133:997–1007.
- The authors identified a six-gene expression signature within sputum that could identify patients with eosinophilic asthma compared with other inflammatory phenotypes, as well as predicting response to and being attenuated by ICS therapy.
48. Poole A, Urbanek C, Eng C, *et al.* Dissecting childhood asthma with nasal transcriptomics distinguishes subphenotypes of disease. *J Allergy Clin Immunol* 2014; 133:670–678.
- The authors demonstrate that the nasal airway transcriptome mirrors that of the bronchial and small airway transcriptome in children with asthma, and thus is suggested as a potential alternative, more accessible means of gene expression profiling. Nasal transcriptome profile distinguished Th2-low and Th2-high subgroups, and the expression of nasal 'IL-13' was linked to asthma exacerbations.
49. Cowan DC, Taylor DR, Peterson LE, *et al.* Biomarker-based asthma phenotypes of corticosteroid response. *J Allergy Clin Immunol* 2015; 135:877–883.
- A 4-week trial of ICS in steroid-naïve adult asthmatics with measurement of a panel of biomarkers (sputum eosinophils, FENO, and urinary bromotyrosine) before and after treatment, and also in healthy controls. Each of the biomarkers individually had utility for the prediction of steroid responsiveness, but the combination of high FENO and high urinary bromotyrosine had the greatest power to predict a favourable response.
50. Heaney LG, Djukanovic R, Woodcock A, *et al.* Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). *Thorax* 2016; 71:187–189.
 51. Hilvering B, Pavord ID. What goes up must come down: biomarkers and novel biologicals in severe asthma. *Clin Exp Allergy* 2015; 45:1162–1169.
 52. Castro M, Wenzel SE, Bleeker ER, *et al.* Benralizumab, an anti-interleukin 5 receptor α monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2014; 2:879–890.
 53. Ortega HG, Liu MC, Pavord ID, *et al.* Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014; 371:1198–1207.
- Large RCT of anti-IL-5 monoclonal antibody demonstrating patients with severe eosinophilic asthma, selected on the basis of elevated peripheral blood eosinophil counts, have a significant reduction in oral glucocorticosteroid dose, reduction in annual exacerbation frequency, small improvement in forced expiratory volume in 1 s and improved symptom control in response to treatment. Further demonstrated the efficacy of subcutaneously administered therapy.

54. Bel EH, Wenzel SE, Thompson PJ. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014; 371:1189–1197. RCT of anti-IL-5 monoclonal antibody demonstrating patients with severe eosinophilic asthma, selected on the basis of elevated peripheral blood eosinophil counts, have a significant reduction in oral glucocorticosteroid dose, reduction in annual exacerbation frequency and improved symptom control in response to treatment.
55. Castro M, Zangrilli J, Wechsler ME, *et al.* Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015; 3:355–366.
56. Hilvering B, Xue L, Pavord ID. Evidence for the efficacy and safety of antiinterleukin-5 treatment in the management of refractory eosinophilic asthma. *Ther Adv Respir Dis* 2015; 9:135–145.
57. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004; 202:175–190.
58. Piper E, Brightling C, Niven R, *et al.* A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. *Eur Respir J* 2013; 41:330–338.
59. De Boever EH, Ashman C, Cahn AP, *et al.* Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: a randomized trial. *J Allergy Clin Immunol* 2014; 133:989–996.
60. Brightling CE, Chanez P, Leigh R, *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; 3:692–701.