

REVIEW

Biomarkers of therapy responsiveness in asthma: pitfalls and promises

S. J. H. Vijverberg^{1,2}, L. Koenderman², E. S. Koster¹, C. K. van der Ent³, J. A. M. Raaijmakers¹ and A.-H. Maitland-van der Zee¹

¹Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, The Netherlands, ²Department of Respiratory Medicine, University Medical Centre Utrecht, Utrecht, The Netherlands and ³Department of Paediatric Respiratory Medicine, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands

Clinical & Experimental Allergy

Summary

Asthma is one of the most common chronic diseases worldwide. There is a large inter-individual variability in response to asthma treatment. Most patients respond well to standard therapy; however, a small proportion of the patients remain symptomatic despite treatment with high dosages of corticosteroids. Uncontrolled asthma leads to a decreased quality of life. Therefore, it is important to identify individuals who will respond poorly to standard asthma medication, especially to standard maintenance therapy with inhaled corticosteroids, at an early stage. Response to anti-inflammatory therapy is generally monitored by the assessment of clinical symptoms, which only partially correlates with underlying airway inflammation. The identification of specific inflammatory biomarkers might help to guide treatment or predict a corticosteroid response more accurately. Some inflammatory biomarkers are already finding their way into clinical practice (e.g. fraction of nitric oxide in exhaled breath), whereas others are predominantly used as a research tool (e.g. profiles of volatile organic compounds). Currently, there is no inflammatory biomarker used in routine clinical practice to predict a corticosteroid response. More knowledge on the underlying biological mechanism(s) of heterogeneous therapeutic responses could help to identify novel biomarkers. This review will focus on inflammatory patterns and genetic variations that may underlie differences in treatment response in patients with asthma, and will provide an overview of inflammatory biomarkers that could potentially serve as response predictors.

Correspondence: Dr Anke-Hilse Maitland-van der Zee, Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands.

E-mail: a.h.maitland@uu.nl

Cite this as: S. J. H. Vijverberg, L. Koenderman, E. S. Koster, C. K. van der Ent, J. A. M. Raaijmakers and A.-H. Maitland-van der Zee, *Clinical & Experimental Allergy*, 2011 (41) 615–629.

Introduction

Asthma is one of the most common chronic illnesses affecting approximately 300 million individuals worldwide and resulting in an estimated 250 000 asthma-related deaths annually [1, 2]. A dominant feature of asthma is airway inflammation [3]. Bronchoscopy with biopsies and bronchoalveolar lavage (BAL) are considered to be gold standards to assess airway inflammation. Nevertheless, these methods are invasive, and therefore, not commonly applied in routine clinical practice [4]. In recent years, several potential diagnostic tools to assess airway inflammation in a less invasive manner have been investigated (e.g. inflammatory markers in peripheral blood, sputum and exhaled breath). However, much debate remains on the validity and value of various methods and markers.

Standard treatment for persistent asthma is generally based on the regular use of inhaled corticosteroids (ICS) to inhibit chronic inflammatory response, combined with short- or, in more severe cases, long-acting β_2 -agonists

to reduce bronchoconstriction [5]. An alternative class of anti-inflammatory medications are the leukotriene receptor antagonists (LTRA), though they are generally less effective as maintenance therapy than ICS [2]. Most patients diagnosed with asthma will have a beneficial response to asthma therapy. However, there is a large inter-individual variability [6], and approximately 5–10% of the patients with asthma remain symptomatic despite high dosages of ICS and even oral corticosteroids (OC). Uncontrolled asthma leads to a reduced quality of life (QOL), may induce lung damage and can be fatal [2, 7]. Therefore, it is important to identify individuals who will respond poorly to standard asthma medication, especially to ICS, at an early stage. A better understanding of biological processes underlying the treatment outcome could help to identify clinical applicable biomarkers and improve asthma diagnosis and management. In addition, the identification of new predictive biomarkers could contribute to a more efficient utilization of health care resources, and provide leads for innovative therapeutic approaches.

The aim of this paper is to review the current knowledge of clinically applicable biomarkers for therapy responsiveness in asthma. First, we will describe the current understanding of how variations in inflammatory patterns or genetic variations may underlie a poor therapeutic response. In the second part, we will focus on inflammatory biomarkers and their potential to predict a response to corticosteroids.

Biological factors involved in response to asthma therapy

It is well known that poor therapy adherence, misdiagnosis or continued exposure to allergens may limit the response to anti-asthma therapy [8]. In addition, biological factors such as distinct inflammatory patterns and genetic polymorphisms may influence treatment response.

Inflammatory patterns in asthma

The majority of asthma patients have atopic asthma with specific IgE antibodies, which are responsible for the initiation of an inflammatory cascade upon allergen re-exposure.

A prolonged presence of activated inflammatory cells in the airways may lead to chronic inflammation and might induce alterations in the composition, content and organization of the airways (airway remodelling). Airway remodelling involves subepithelial basement membrane thickening, epithelial cell disruption, neoangiogenesis, goblet cell metaplasia, enlarged submucosal glands and an increase in airway smooth muscle mass [9], and has been observed in chronic asthmatics [10]. However, it is thought that airway remodelling may also occur in parallel with, or even precede, airway inflammation, as structural changes in bronchial tissue may already be present in paediatric asthma patients [9, 11].

Inflammation in asthma is often described as eosinophilic, based upon the presence of primed eosinophils in airways. In addition, various studies have shown that inflammation may also occur in the absence of increased levels of eosinophils [12–15]. As a consequence, a concept has evolved that more than one inflammatory pattern in asthma pathogenesis exists, and that the heterogeneity of the clinical expression of asthma may be associated with distinct underlying inflammatory patterns [12]. A study by Wenzel *et al.* [13] identified at least two specific inflammatory patterns in the airways of difficult-to-treat asthmatic adults based on BAL samples: an (classical) eosinophilic phenotype with high levels of eosinophils and a non-eosinophilic phenotype with few or no eosinophils. The eosinophilic and non-eosinophilic phenotype had distinct physiological and clinical features; the non-eosinophilic group had less basement membrane thickening, a lower forced expiratory volume in 1 s (FEV₁), a

decreased FVC/slow VC ratio, as well as more episodes of respiratory failure requiring intubation.

Green *et al.* [15] analysed the sputum samples of 259 asthmatic adults and identified a subgroup of 60 patients who had elevated sputum neutrophil counts ($\geq 65.3\%$) and normal eosinophil counts ($< 1.9\%$). Compared with the total group of patients these patients were older, tended to have developed asthma at a later age and were more often female and non-atopic. However, physiological features, such as FEV₁ and FVC/slow VC ratio, did not significantly differ between the patients with and without sputum neutrophilia.

In addition, Simpson *et al.* [14] identified four inflammatory phenotypes within an adult asthma population treated with ICS:

- (1) Neutrophilic asthma: marked by an increased neutrophil proportion ($> 61\%$);
- (2) Eosinophilic asthma: marked by an increased eosinophil proportion ($> 1.0\%$);
- (3) Mixed granulocytic asthma: marked by increased neutrophils ($> 61\%$) and increased eosinophils levels ($> 1.0\%$) and;
- (4) Paucigranulocytic asthma: marked by normal levels of neutrophils and eosinophils.

Furthermore, they showed that the majority of the individuals, who were classified as having a non-eosinophilic phenotype at their first study visit, were classified again as having a non-eosinophilic phenotype at their second visit (after 4 weeks or 5 years). The authors did not find differences in clinical features between the groups.

In contrast, a recent study by Porsbjerg *et al.* [16] did observe differences in airway hyperresponsiveness and nitric oxide fraction levels in exhaled breath (FeNO), between the four subtypes in 62 adults with asthma not on anti-inflammatory therapy. The differences in clinical features suggest that distinct inflammatory phenotypes may involve specific pathogenetic mechanisms, a hypothesis that is reinforced by a recent study by Baines *et al.* [17]. They showed that peripheral blood neutrophils, isolated from eight adults with eosinophilic asthma and nine adults with non-eosinophilic asthma who were not using ICS had distinct gene expression profiles. In the neutrophils of non-eosinophilic asthma patients, genes involved in neutrophil chemotaxis, neutrophil survival and activation of the nuclear factor kappa B (NF- κ B) cascade were up-regulated. Furthermore, peripheral blood neutrophils of non-eosinophilic asthma patients released significantly higher levels of IL-8. These findings suggest that neutrophils may be activated differently in the distinct inflammatory phenotypes.

Few studies assessed the heterogeneity of airway inflammation in children; nonetheless, distinct eosinophilic and non-eosinophilic inflammatory patterns have also

been identified in the paediatric population. Lovett et al. [18] assessed the sputum of 113 Australian children (age: 6–17 years) with a doctor's diagnosis of asthma. Of these children, 46% were classified as having eosinophilic asthma based upon an increased sputum eosinophil concentration ($>2.5\%$ of sputum leucocytes), while the remaining 54% of the children were classified as non-eosinophilic asthmatics. Drews et al. [19] investigated the presence of eosinophils and neutrophils in the sputum of 54 Brazilian schoolchildren who were not using ICS or oral steroids. Children with non-atopic asthma showed a significantly higher number of sputum neutrophils compared with the children with atopic asthma and the controls. Furthermore, only 24% of the non-atopic asthmatics showed sputum eosinophilia, in contrast to 81% of the atopic asthmatics. These results suggest that inflammation in children with non-atopic asthma might be predominantly neutrophilic.

Heterogeneity in inflammatory response has also been observed in children with difficult asthma. De Blic et al. [20] investigated bronchial inflammatory profiles in 28 children with difficult asthma (age: 9–15 years). Bronchial biopsies showed that eosinophil and neutrophils counts in the epithelium were significantly higher in the children with persistent symptoms compared with children with few symptoms. In addition, BAL showed a trend for higher percentages of neutrophils in children with persistent symptoms.

Nevertheless, one should be careful extrapolating results from paediatric studies to an adult population and vice versa. Longitudinal studies on the progression of paediatric inflammatory phenotypes to adult inflammatory phenotypes are lacking [21]. Furthermore, preliminary data from Fleming et al. [22] have shown that inflammatory phenotypes in children with severe asthma can change over time.

Moreover, corticosteroid treatment can influence the inflammatory phenotype. Studies by Cowan et al. [23] and Luijk et al. [24] showed that corticosteroid treatment could lead to an increase of sputum neutrophils. This increase was observed after an allergen challenge [24], as well as during regular treatment [23]. Cowan and colleagues analysed the sputum of 88 asthmatics after steroid withdrawal and subsequently after a period of ICS use. Upon steroid withdrawal, none of the asthmatics were classified as neutrophilic. However, after a (28+ day) trial of fluticasone two of the 63 asthmatics originally classified as eosinophilic, and three of the 29 asthmatics originally classified as paucigranulocytic, were classified as neutrophilic. Nevertheless, in total 32 asthmatics presented with an altered inflammatory phenotype after steroid treatment.

The effect of corticosteroids on inflammatory patterns might be explained by the differential effect of these drugs on the survival of distinct types of inflammatory cells. *In*

vitro studies have shown that corticosteroids induce apoptosis in eosinophils while they inhibit apoptosis in neutrophils [25–27]. Thus, the neutrophilic phenotype observed after corticosteroid treatment might be a consequence of the selective mechanism of action of the steroids. Nevertheless, the neutrophilic phenotype may also occur in the absence of steroid treatment, as sputum neutrophilia has also been observed in steroid-naïve patients [17, 19].

Inflammatory patterns and therapy response

Considering that eosinophils and neutrophils are not equally sensitive to corticosteroids, it is likely that patients with distinct inflammatory phenotypes may respond differently to anti-inflammatory therapy. Therefore, poor response to steroid therapy could be mediated by an inflammatory pattern that is intrinsically insensitive to steroids. However, the relationship between sputum eosinophilia and corticosteroid response remains inconclusive (see Table 1).

Other studies have assessed whether monitoring airway inflammation through the assessment of sputum eosinophils might be a better marker to guide therapy than the assessment of clinical symptoms. Petsky et al. [28] evaluated three randomized-controlled trials that compared adjusting asthma therapies based on sputum eosinophils to adjustment based upon clinical symptoms. All three studies [29–31] included asthmatic adults and used asthma exacerbations, defined as the requirement for rescue OC, as the primary study outcome. The studies differed slightly in the eosinophil cut-off level used to guide therapy, yet all studies showed a significant reduction in the frequency and the severity of asthma exacerbations in the group for whom sputum eosinophils counts were used to guide therapy. Remarkably, clinical symptoms (visual analogue scale score, QOL scores and β -agonist use) did not significantly differ between groups.

This is in line with work by Haldar et al. [32] and Nair et al. [33] that showed that eosinophilic inflammation plays a role in asthma exacerbations, but is not necessarily involved in asthma symptoms or lung function. Both studies were randomized clinical trials that assessed the effect of Mepolizumab on adults with refractory asthma and sputum eosinophilia. Mepolizumab is a monoclonal antibody directed against IL-5, an important regulator of eosinophil production, activation and survival. By neutralizing IL-5, Mepolizumab inhibits eosinophilic inflammation. The studies by Haldar and colleagues and Nair and colleagues showed that compared with a placebo, treatment with Mepolizumab led to a reduction in the number of severe exacerbations in asthmatics with sputum eosinophilia despite high doses of corticosteroids. This may suggest that patients with high sputum eosinophils respond preferentially to Mepolizumab with fewer

Table 1. Overview of studies assessing markers in induced sputum for steroid responsiveness in individuals with asthma

Study	Marker	Cut-off value	Study population	Design	Definition of response	Study outcome	Association/ predictor?
Meijer et al. [58]	ECP	120 µg/L	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$ / $\Delta PC_{20}Mch \geq 1$ / $\Delta QOL \geq 0.5$	Baseline values of the clinical parameters used as outcome were the major predictors of clinical response. Sputum ECP provided no additional information compared with other predictors	-
Pavord et al. [64]	Eosinophilia	3%	23 asthmatic adults	2-month trial of ICS	Improvement in $PC_{20}Mch$	Sputum eosinophilia was correlated with improvement in $PC_{20}Mch$ ($r: 0.41$; $P < 0.05$)	+
Little et al. [69]	Eosinophilia	4%	37 asthmatic adults	2-week trial of OC	ΔFEV_1 of $\geq 15\%$	There was no correlation between sputum eosinophil count and ΔFEV_1 ($p: 0.19$, $P: 0.36$). Sputum eosinophilia had a NPV of 68% and a PPV of 64% for $\Delta FEV_1 \geq 15\%$	+/-*
Godon et al. [68]	Eosinophilia	1%	46 adults with mild uncontrolled asthma	1-month trial of ICS	Improvement in symptom score, QOL, FEV_1 , $PC_{20}Mch$	There was no correlation between sputum eosinophilia and improvement in symptom score, $PC_{20}Mch$ or QOL. Sputum eosinophilia did show a correlation with improvement of FEV_1 ($p: 0.52$, $P: 0.002$)	+/-
Meijer et al. [58]	Eosinophilia	3%	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$ / $\Delta PC_{20}Mch \geq 1$ / $\Delta QOL \geq 0.5$	Baseline values of the clinical parameters used as an outcome were the major predictors of clinical response. Sputum eosinophilia provided additional information for ΔFEV_1 ($\beta: 0.32$), $\Delta PC_{20}Mch$ ($\beta: 0.20$) and for ΔQOL ($\beta: 0.22$). Prediction of response of individual patients remained poor (correct classification: 57–65%).	+
Szeffler et al. [65]	Eosinophilia	NA	26 asthmatic adults	24-week trial with ICS	$\Delta FEV_1 \geq 15\%$ (good), $\Delta FEV_1 < 5\%$ (poor)/ $\Delta PC_{20}Mch > 3$ (excellent), $\Delta PC_{20}Mch < 1$ (poor)	Patients with an excellent improvement in $PC_{20}Mch$ had higher sputum eosinophil levels compared with patients with a poor improvement in $PC_{20}Mch$ ($P: 0.013$)	+
Bacci et al. [70]	Eosinophilia	3%	67 asthmatic adults	4-week trial of ICS	$\Delta FEV_1 \geq 12\%$, $\Delta PC_{20}Mch \geq 1$	Eosinophilic patients showed significant improvement in ΔFEV_1 and $\Delta PC_{20}Mch$, while non-eosinophilic patients did not. Sputum eosinophils had a high NPV (100%) for $\Delta FEV_1 \geq 12\%$, but a low PPV (34%)	+
Berry et al. [66]	Eosinophilia	1.9%	16 asthmatic adults	Cross-over design of 8 weeks of ICS and 8 weeks of placebo	Improvement in $PC_{20}Mch$, QOL	Eosinophilic patients showed a greater improvement in $PC_{20}Mch$ ($P: 0.018$) and QOL ($P: 0.008$) upon treatment with ICS (compared with placebo) than non-eosinophilic patients	+
Lex et al. [71]	Eosinophilia	2.5%	17 children with difficult asthma	OC for 14 days or one single intramuscular dose of steroids	$\Delta FEV_1 \geq 9\%$ and/or overall subjective improvement	There was a similar clinical improvement in eosinophilic and non-eosinophilic patients.	-

Martin et al. [67]	Eosinophilia	NA	72 adult asthmatics	6-week trial of ICS	$\Delta FEV_1 > 5\%$	Levels of sputum eosinophils did not differ between responders ($\Delta FEV_1 > 5\%$) and non-responders ($\Delta FEV_1 \leq 5\%$) ($P: 0.09$)	–
Cowan et al. [23]	Eosinophilia	2%	88 asthmatics	28-day trial of ICS	$FEV_1 \geq 12\%$ and/or $\Delta ACQ \geq 0.5$ point decrease and/or $\Delta PC_{20}AMP \geq 2$ and/or $\Delta FeNO \geq 40\%$ decrease	Patients with sputum eosinophilia showed more improvement in ACQ ($P: 0.001$), FEV_1 ($P < 0.001$), $PC_{20}AMP$ ($P: 0.008$) and $FeNO$ ($P < 0.001$), although a proportion of the non-eosinophilic patients also showed a response	+ / –
Green et al. [15]	Neutrophilia	65.3%	49 adult asthmatics	2-month trial with ICS	Improvement in symptom score, improvement in FEV_1 , improvement in $PC_{20}Mch$	Patients with sputum neutrophilia showed less improvement in symptom score ($P: 0.04$), FEV_1 ($P: 0.026$) and $PC_{20}Mch$ ($P: 0.029$)	+

*Little and colleagues showed that the combination of FeNO and sputum eosinophilia gave the highest predictive value; however, the predictive values for the combined measurements are not shown here.

ACQ, asthma control questionnaire; ECP, eosinophil cationic protein; FeNO, fraction of nitric oxide in exhaled breath; FEV_1 , forced expiratory volume in 1 s; ICS, inhaled corticosteroids; NPV, negative predictive value; OC, oral corticosteroids; $PC_{20}AMP$, doubling provocative dose of adenosine monophosphate; $PC_{20}Mch$, doubling provocative dose of methacholine; PPV, positive predictive value; QOL, quality of life; r , Pearson's correlation coefficient; β , standardized regression coefficient; ρ , Spearman's rank-correlation coefficient.

exacerbations; nevertheless, neither of the studies addressed the effect of Mepolizumab on asthmatic patients with low sputum eosinophils. Moreover, Mepolizumab had no effect on asthma symptoms, FeNO or lung function. This implies that eosinophilic inflammation might not always underlie classical asthma symptoms (e.g. bronchial hyperresponsiveness).

Genetic variations and therapy response

Almost a decade ago, Drazen et al. [6] suggested that up to 80% of the inter-individual variance in treatment response of Caucasian asthmatics might be due to genetic variations. These variations could lead to an altered expression or function of therapeutic targets and pathways. Since then, several genetic variants have been described in genes that are involved in the action or metabolism of anti-asthma drugs [34–36]. Various pharmacogenetic studies have focused on the influence of genetic variations on the response of asthmatics to β_2 -agonists [37–43], though fewer have focused on corticosteroid response. This is probably due to the complex mechanism of action and the wide spectrum of activity of these drugs [35]. Corticosteroids are thought to exert their anti-inflammatory effects by binding to the corticosteroid receptor in the cytoplasm [44]. Upon binding, the protein complex migrates to the nucleus where it binds to specific regions of the DNA and subsequently induces or represses the gene transcription of a wide variety of genes. In addition to direct gene regulation by binding to the cytoplasmic corticosteroid receptor, corticosteroids are also able to suppress inflammation through alternative mechanisms [44, 45].

Most pharmacogenetic studies have focused on polymorphisms that may alter the glucocorticoid receptor (*NR3C1*) gene or the corticotrophin-releasing hormone receptor type 1 (*CRHR1*) gene [34]. Various polymorphisms in *NR3C1* have been associated with an altered response to steroid therapy, yet results have been inconsistent and studies have not specifically focused on patients with asthma rather on patients with primary cortisol resistance, familial glucocorticoid resistance, psoriasis, allergy or inflammatory bowel disease [34]. In contrast, studies investigating variations in *CRHR1* did address asthmatic patients. Tantisira et al. [46] and Weiss et al. [47] showed that variations in *CRHR1* were associated with lung function improvement after 8 weeks of treatment with ICS in asthmatic adults and children in three large clinical trial populations. On the other hand, an observational study by Dijkstra et al. [48] could not replicate this finding in a group of 164 patients with asthma. Other genes for which genetic variants have been associated with a corticosteroid response in asthmatics include *TBX21*, encodes for T-bet a transcription factor for T lymphocyte production [49, 50]; *FCER2*, encodes for

low-affinity IgE receptor [51]; *DUSP1*, encodes for an enzyme involved in the regulation of proinflammatory cytokines [52]; *STIP1*, encodes for a component of the glucocorticoid receptor complex [53]; and *NK2R*, encodes for neurokinin receptor 2 [50]. Nevertheless, these associations need to be replicated in other asthma cohorts.

Because of the complex mechanism of action of corticosteroids, it is likely that multiple genetic variations, rather than a single or just a few variations, will influence a therapeutic response. Hakonarson *et al.* [54] examined gene expression profiles in peripheral blood mononuclear cells from glucocorticoid-sensitive and glucocorticoid-resistant asthma patients. They showed that an expression pattern of 11 genes could predict the corticosteroid response in asthmatic patients with an accuracy of 84%. Notably, the expression of one single gene at baseline, *NFKB1*, encoding for the DNA-binding subunit of the transcription factor NF- κ B, could already predict corticosteroid response with an accuracy of 81%. A study by Donn *et al.* [55] examined gene expression profiles in T lymphoblasts from glucocorticoid-sensitive and glucocorticoid-resistant healthy individuals but identified a different discriminatory gene set than Hakonarson and colleagues had done.

Pharmacogenetic and pharmacogenomic approaches have shown significant associations between genetic and genomic variations and corticosteroid response; nevertheless, to date, genotyping or assessing gene expression profiles of asthma patients before starting asthma medication has no clinical relevance. An important obstacle is the apparent lack of alternative anti-inflammatory treatment strategies. Furthermore, identified associations need to be further validated by replication in other asthma cohorts and more knowledge is required about the functional role of the identified genetic variants and the interaction of these variants with other factors. Unbiased approaches such as Genome-Wide Association Studies and whole-exome sequencing could play an important role in uncovering additional relevant variants. As advances in genomic technologies and bioinformatics are rapidly expanding and the costs of DNA screening are decreasing, genotyping might become part of routine clinical practice in the future [56].

Inflammatory markers as markers for therapeutic response

In order to improve the diagnosis and treatment of asthma, there is significant interest in the development of inflammatory biomarkers. A number of putative biomarkers have been studied, including inflammatory markers in peripheral blood, induced sputum, urine and exhaled breath (condensate). Most studies have focused on the relationship between these markers and the presence of asthma symptoms or disease severity; nevertheless, sev-

eral studies have also evaluated the association with corticosteroid response (Tables 1–3).

Peripheral blood

Few studies have investigated inflammatory biomarkers in relation to therapy response in peripheral blood, plasma or serum samples and the results are inconclusive (Table 2). Szeftler *et al.* [57] showed that asthmatic children with increased levels of serum eosinophil cationic protein, serum total IgE levels and blood eosinophil counts were more likely to respond to ICS. In addition, Meijer *et al.* [58] reported that the eosinophil percentage in the blood was a predictor of a therapeutic response in adults with asthma, yet the baseline values of clinical parameters used as outcome parameters (i.e. FEV₁, PC₂₀Mch and QOL) were the major predictors of corticosteroid response and prediction of an individual response based on blood eosinophilia remained poor.

The identification of inflammatory mediators (e.g. chemokines and cytokines) in peripheral blood, plasma or serum that relate to specific asthma phenotypes or therapy response has been difficult. These mediators can be studied in multiplex immunoassays allowing complex statistics to identify unique profiles associated with particular clinical phenotypes of the disease. However, an inherent weakness of these studies [59, 60] lies in the fact that the choice of biomarkers determines the (lack of) success of this approach. An important bias is the focus on pro-inflammatory mediators. Relatively little attention is given to the fact that (1) many anti-inflammatory proteins (e.g. receptors antagonists such as IL-1RA) are not measured, (2) pro-inflammatory mediators can antagonize each others function [61] and (3) several inflammatory mediators may still be unknown.

An alternative approach to study inflammatory mediators is to examine shifts in activation profiles of inflammatory cells. The function of inflammatory cells is (in part) regulated by the functionality of their receptors, which can be modulated upon priming with inflammatory mediators. Studies in asthmatics have shown that various clinical phenotypes are characterized by the presence of primed inflammatory cells in peripheral blood (for a review see [12]). These differences in cellular phenotypes are, however, quite subtle showing that it is not so important what the expression of certain receptors is, but rather the activation status of these receptors. Two recent proof-of-principle studies have shown that the expression of active integrins [62] and FcRs [63], rather than the overall expression of these receptors on neutrophils, can identify specific clinical phenotypes in allergic asthma. More knowledge on how inflammatory mediators influence the activation phenotypes of inflammatory cells, might lead to the identification of novel inflammatory

Table 2. Overview of studies assessing markers in peripheral blood for steroid responsiveness in individuals with asthma

Study	Marker	Cut-off value marker	Study population	Design	Definition of response	Study outcome	Association/predictor?
Meijer et al. [58]	ECP	15 µg/L	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$ / $\Delta PC_{20}Mch \geq 1$ / $\Delta QOL \geq 0.5$	Baseline values of the clinical parameters used as an outcome were the major predictors of clinical response. Serum ECP provided no additional information compared with other predictors	–
Szeffler et al. [57]	ECP	15 µg/L	126 asthmatic children	Cross-over design of 8 weeks of ICS and 8 weeks of LTRA	$\Delta FEV_1 \geq 7.5\%$	Improvement of FEV ₁ upon ICS was associated with higher serum ECP (OR: 2.8; $P < 0.01$)	+
Meijer et al. [58]	Eosinophilia	3%	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$ / $\Delta PC_{20}Mch \geq 1$ / $\Delta QOL \geq 0.5$	Baseline values of the clinical parameters used as an outcome were the major predictors of clinical response. Blood eosinophils provided some additional information for ΔFEV_1 (β : 0.35) and ΔQOL (β : 0.29), but not for $\Delta PC_{20}Mch$. Prediction of individual patients responses remained poor (correct classification: 44–63%)	+ / –
Szeffler et al. [57]	Eosinophilia	350 cells/mm ³	126 asthmatic children	Cross-over design of 8 weeks of ICS and 8 weeks of LTRA	$\Delta FEV_1 \geq 7.5\%$	Improvement of FEV ₁ $\geq 7.5\%$ upon ICS was associated with higher eosinophil blood counts (OR: 2.3, $P < 0.05$)	+
Bacci et al. [70]	Eosinophilia	5%	67 asthmatic adults	4-week trial of ICS	$\Delta FEV_1 \geq 12\%$, $\Delta PC_{20}Mch \geq 1$	Both patients with high and low blood eosinophils showed a significant improvement in FEV ₁ and symptom scores. Improvement in $\Delta PC_{20}Mch$ was only significant in the high blood eosinophil group.	–
Meijer et al. [58]	IgE	–	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$ / $\Delta PC_{20}Mch \geq 1$ / $\Delta QOL \geq 0.5$	Predictive values were poor (NPV: 35%, PPV: 54%) Baseline values of the clinical parameters used as an outcome were the major predictors of clinical response. Serum IgE provided some additional information for ΔQOL when combined with other predictors (β : 0.29; 0.22)	+ / –
Szeffler et al. [57]	IgE	200 kU/L	126 asthmatic children	Cross-over design of 8 weeks of ICS and 8 weeks of LTRA	$\Delta FEV_1 \geq 7.5\%$	Improvement of FEV ₁ $\geq 7.5\%$ upon ICS was associated with higher IgE levels (OR: 2.9, $P < 0.01$)	+

ECP, eosinophilic cationic protein; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonist; NPV, negative predictive value; OR, odds ratio; PC₂₀Mch, doubling provocative dose of methacholine causing 20% fall in FEV₁; PPV, positive predictive value; QOL, quality of life; β , standardized regression coefficient.

Table 3. Overview of studies assessing markers in exhaled breath for steroid responsiveness in individuals with asthma

Study	Marker	Cut-off value	Study population	Design	Definition of response	Study outcome	Association/predictor?
Little et al. [69]	FeNO	10 p.p.b.	37 adults with stable asthma	2-week trial of OC	$\Delta FEV_1 \geq 15\%$	There was a correlation between FeNO and improvement in FEV_1 ($p: 0.47$, $P: 0.003$). FeNO had a NPV of 72% and a PPV of 83% for $\Delta FEV_1 \geq 15\%$	+
Meijer et al. [58]	FeNO	N/A	118 adults with unstable asthma	2-week trial of ICS	$\Delta FEV_1 \geq 9\%$, $\Delta PC_{20}Mch \geq 1$, $\Delta QOL \geq 0.5$	Baseline values of the clinical parameters used as an outcome were the major predictors of clinical response. FeNO provided no additional information	–
Szeffler et al. [65]	FeNO	N/A	26 asthmatic adults	24-week trial with ICS	$\Delta FEV_1 \geq 15\%$ (good), $\Delta FEV_1 < 5\%$ (poor)/ $\Delta PC_{20}Mch > 3$ (excellent), $\Delta PC_{20}Mch < 1$ (poor)	$\Delta FEV_1 \geq 15\%$ was associated with higher median FeNO ($P: 0.002$)	+
Smith et al. [117]	FeNO	47 p.p.b.	52 patients (children and adults) with undiagnosed respiratory symptoms	4-week trial of ICS	$\Delta FEV_1 \geq 12\%$ /mean morning peak flow $\geq 15\%$ / $\Delta PC_{20}AMP \geq 2$ /composite symptom score reduction of ≥ 1	High FeNO levels were associated with a better steroid response on all of the assessed endpoints. AUC: 0.76 (PPV: 47%, NPV: 89%) for $\Delta FEV_1 \geq 12\%$; AUC: 0.81 (PPV: 53%, NPV: 94%) for increase in morning peak flow, AUC: 0.64 (PPV: 35%, NPV: 77%) for decrease in symptom score; AUC: 0.91 (PPV: 82%, NPV: 91%) for $PC_{20}AMP$	+
Szeffler et al. [57]	FeNO	25 p.p.b.	126 asthmatic children	Cross-over design of 8 weeks of ICS and 8 weeks of LTRA	$\Delta FEV_1 \geq 7.5\%$	Favourable response to fluticasone alone was associated with higher FeNO (OR: 2.8, $P < 0.05$)	+
Martin et al. [67]	FeNO	N/A	72 asthmatic adults	6-week trial of ICS	$\Delta FEV_1 > 5\%$	FeNO levels did not differ between responders ($\Delta FEV_1 > 5\%$) and non-responders ($\Delta FEV_1 \leq 5\%$) ($P: 0.68$)	–
Cowan et al. [23]	FeNO	33 p.p.b.	88 asthmatics	28-day trial of ICS	$FEV_1 \geq 12$ and/or $\Delta ACQ \geq 0.5$ point decrease and/or $\Delta PC_{20}AMP \geq 2$ and/or $\Delta FeNO \geq 40\%$ decrease	In non-eosinophilic patients FeNO was a predictor of improvement of $\Delta PC_{20}AMP$ (AUC: 0.810), but not for improvement of ACQ or FEV_1 . In eosinophilic patients FeNO was a predictor of improvement of $\Delta PC_{20}AMP$ (AUC: 0.778) and of improvement in ACQ (AUC: 0.727)	+
Pérez-de-Llano et al. [118]	FeNO	30 p.p.b.	102 adults with difficult-to-treat asthma	Stepwise increase in ICS for 1 month. Patients who remained uncontrolled received OC for 1 month	$ACT \geq 20$	FeNO was a good predictor of improvement in ACT (AUC: 0.925, PPV: 88%, NPV: 91%)	+

*Little and colleagues showed that the combination of FeNO and sputum eosinophilia gave the highest predictive value; however, the predictive values for the combined measurements are not shown here.

ACQ, asthma control questionnaire; ACT, asthma control test; AUC, area under the curve; FeNO, fraction of nitric oxide in exhaled breath; FEV_1 , forced expiratory volume in 1 s; ICS, inhaled corticosteroids; NPV, negative predictive value; OC, oral corticosteroids; OR, odds ratio; PPV, positive predictive value; $PC_{20}AMP$, doubling provocative dose of adenosine monophosphate; $PC_{20}MCh$, doubling provocative dose of metacholine; p.p.b., parts per billion; p , Spearman's rank-correlation coefficient.

markers in peripheral blood, which could potentially serve as response predictors.

Expectorated sputum

Sputum induction is a semi-invasive method to assess inflammation in the lower airways. Studies investigating markers in sputum for corticosteroid response in asthmatics have mainly focused on sputum eosinophilia (Table 1). Some studies have shown that higher levels of sputum eosinophils are associated with a better response to corticosteroids [64–66]; in contrast, others could not find, or only find a poor correlation [67, 68]. Little et al. [69] showed that baseline sputum eosinophil count did not significantly correlate with an improvement in FEV₁ in 30 adults asthmatics who received OC. However, sputum eosinophilia ($\geq 4\%$) could predict a response in lung function (FEV₁ $\geq 15\%$) upon OC treatment with a positive predictive value (PPV) of 64% and negative predictive value (NPV) of 68%. Nevertheless, the sensitivity of the marker was low (54%). Combining sputum eosinophilia with FeNO led to the highest PPV, NPV and sensitivity/specificity values. Cowan et al. [23] reported that asthmatics with sputum eosinophilia ($\geq 2\%$) were more likely to respond to ICS than asthmatics without sputum eosinophilia. The PPV, NPV and sensitivity of sputum eosinophilia to predict ICS response were high ($\geq 80\%$) in this study; however, the specificity was only 67%. This indicates that the absence of sputum eosinophilia does not necessarily imply a poor therapy response. In contrast, Bacci et al. [70] found that sputum eosinophilia ($> 3\%$) had a low PPV (34%) for lung function response in adult asthmatics on ICS treatment, but had a very high NPV (100%). In two complementary studies, Lex et al. [71] and Meijer et al. [58] found that sputum eosinophilia could not, or only poorly, predict corticosteroid response in asthmatics. Green et al. [15] assessed the relationship between sputum neutrophilia and short-term response to ICS. Patients with sputum neutrophilia ($> 65.3\%$) showed less improvement in asthma symptoms, FEV₁ and methacholine airway responsiveness after 2 months of ICS treatment compared with patients without sputum neutrophilia.

The use of measuring markers in expectorated sputum in routine clinical practice will remain complicated; sputum induction is technically demanding and time consuming and infants and young children are generally not able to expectorate sputum.

Urine

Sampling urine is non-invasive and easy to do. The most promising biomarker in urine for therapeutic response, particularly for a response to LTRA, seems to be the level of cysteinyl-leukotrienes. Higher levels of urine leuko-

triene E₄ (uLTE₄) have been associated with increased likelihood of a response to LTRA in asthmatic children and adults [57, 72]. In addition, a recent study by Rabinovitch et al. [73] showed that asthmatic children from two asthma trials with high uLTE₄/FeNO ratios were more likely to respond to LTRAs than to ICS. However, there was no significant relationship with therapy response when LTE₄ was assessed alone. This suggests that uLTE₄ might be a marker to guide therapy, especially when combined with FeNO.

Exhaled breath condensate

Exhaled breath condensate (EBC) is the liquid phase of exhaled air and is sampled by cooling. EBC mainly consists of condensed water vapour, but it also contains non-volatile substances that are released in aerosol particles and volatile substances in a gas phase [74]. It is thought that changes in EBC content reflect biochemical changes in the airway surface fluid.

Various markers in EBC have been reported to be elevated in patients with asthma compared with healthy individuals, such as adenosine concentration [75] and markers of oxidative stress (i.e. hydrogen peroxide [76], nitric oxide (NO)-related products [77], isoprostanes and leukotrienes [78]). Furthermore, the pH of EBC has been reported to be decreased in acute asthmatics, though reports on the acidification of EBC of stable asthmatics remain inconsistent [79]. There is evidence that some of the markers in EBC also correlate with disease severity. Al Obaidi et al. [80] assessed the EBC of 153 adults with atopic asthma after 4 weeks of ICS. Patients, who remained poorly controlled while on treatment, showed significantly increased levels of hydrogen peroxide and a decreased EBC pH. Moreover, Tseliou et al. [81] compared 29 severe refractory asthmatics with 27 moderate asthmatics and 17 healthy controls. EBC pH in the group of difficult-to-control patients was significantly lower compared with the pH of patients with moderate asthma and healthy subjects.

Other studies have focused on the measurement of cytokines, chemokines, adhesion molecules and high-sensitivity C-reactive protein (hs-CRP) in EBC. Zietkowski and colleagues found that hs-CRP [82] and eotaxin-1 [83] are associated with disease severity in atopic asthmatics. Hs-CRP and eotaxin-1 were elevated in unstable asthmatics compared with stable (ICS naïve as well as ICS treated) asthmatics and healthy controls. However, a study by Leung et al. [84] could not find an association between eotaxin-1 levels and disease severity in asthmatic children. Robroeks et al. [85] compared the levels of various cytokines, chemokines and adhesion molecules in EBC between 31 asthmatic children and 29 healthy controls with the use of multiplex immunoassay technique. Asthmatic children showed elevated levels of cytokines (IL-2,

IL-4, IL-5, IL-6, IFN- γ), chemokines (MIP1- α , MIP, RANTES, IP10, IL-8 and MCP1) and adhesion molecules (sICAM and sVCAM) compared with the controls. In addition, Matsunaga et al. [86] measured 40 cytokines and chemokines in 16 steroid-naïve asthmatics and 10 non-smoking healthy individuals and showed that the expressions of IL-4, IL-8, IL-17, TNF- α , RANTES, IFN- γ -inducible protein 10, TGF- β and macrophage inflammatory protein 1 α and 1 β were up-regulated in asthmatics.

Collecting EBC is completely non-invasive and relatively easy to perform [87]. Nevertheless, the measurement of markers in EBC is still in its experimental phase, and many methodological problems need to be resolved before measuring biomarkers in EBC can enter clinical practice [88, 89]. There is a lack of a standardized methodology for EBC collection, complicating the comparability of measurements among studies. In addition, various factors are likely to influence biomarker measurements in EBC such as type of the condenser equipment, cooling temperature, condenser tube coating, cleaning procedures, breathing patterns and pollution in the ambient air. Furthermore, it remains uncertain from which compartments of the respiratory system specific markers originate and how the dilution of distinct markers should be assessed. Moreover, established reference values for markers in EBC are not yet available [90].

Exhaled breath

Measuring inflammatory biomarkers in the exhaled breath is most appealing, because it is totally non-invasive, simple to obtain and measurements can be easily repeated.

Fraction of nitric oxide in exhaled breath. In the last few years, FeNO has emerged as one of the most promising biomarkers for airway inflammation [91, 92]. Measurement of FeNO is non-invasive, easy and highly reproducible [93]. Several FeNO analysers, approved for clinical use, are currently available. However, the clinical usefulness of FeNO remains an issue because the direct association with airway inflammation is still unclear and other factors can influence FeNO [94].

NO is a highly reactive gaseous molecule that is produced in the airways when the amino acid L-arginine is oxidized to the amino acid L-citrulline. The synthesis of NO is catalysed by nitric oxide synthases (NOS), of which currently three isoforms are known: two forms of constitutive NOS and one form of inducible NOS (iNOS). Inducible nitric oxide synthase is activated by a wide range of inflammatory cytokines and has been found in several tissues, such as macrophages, type II alveolar epithelial cells, fibroblasts, airway and vascular smooth muscle cells, respiratory epithelial cells, mast cells, endothelial cells, neutrophils, hepatocytes and chondrocytes [91, 92].

In 1993, Alving et al. [95] were the first to show that FeNO was increased in mild atopic asthmatics compared with individuals without asthma. It is thought that the increased FeNO in asthmatics is caused by up-regulated iNOS in the inflamed airways. Upon synthesis in the airways, NO diffuses into the airway lumen that is essentially NO free, thereby causing the rise in FeNO. Various studies have confirmed the results of Alving and colleagues and have shown increased levels of FeNO in adult and paediatric asthma patients [96–99]. Furthermore, several studies have shown that anti-inflammatory therapy reduces FeNO in asthmatic patients [100–104]. It has been suggested that FeNO is specifically a marker for eosinophilic inflammation and that increased levels of FeNO might reflect uncontrolled asthma. Nevertheless, studies assessing the relation between FeNO and the presence of eosinophils in sputum and bronchial specimens remain inconclusive [105–108]. In addition, FeNO seems to be associated with blood eosinophilia, but only in individuals with atopic asthma [109, 110]. Silvestri et al. [109] compared 66 atopic asthmatic children with 26 non-atopic asthmatic children (both steroid naïve) and found a positive relation between FeNO and blood eosinophilia, but only in the atopic children. Steerenberg et al. [110] found the same relationship in a sample of 450 schoolchildren. Other studies showed that atopic subjects with asthma have higher levels of FeNO than non-atopic subjects [111, 112]; therefore, the question arises whether FeNO might be more closely associated to atopy than asthma. Scott et al. [113] addressed this question in a community sample of 864 adults. They showed that the presence of atopy alone was already associated with higher levels of FeNO. The coexistence of atopy and asthma was associated with even higher FeNO levels, though FeNO levels were not significantly different between non-atopic individuals with or without asthma. These findings suggest that atopy, rather than asthma itself, is driving FeNO. However, a study by Leung et al. [84] showed no difference in FeNO levels between atopic and non-atopic children without asthma. Furthermore, several other factors have been described to influence FeNO measurements, such as corticosteroid use, diet (food containing L-arginine or nitrate), age, height, race, season, smoking, exercise, respiratory viral infections and to a lesser extent, repeated spirometric measurements [92, 94, 114].

Studies that assessed the value of FeNO in the management of asthma have shown that the use of FeNO to adjust treatment does not lead to a major improvement in asthma control or significant reduction in exacerbations [94, 115, 116]. A large RCT by Szeffler et al. [116] with 546 adolescents and young adults with asthma (age: 12–20 years) showed that the addition of FeNO monitoring to guideline-based management did not lead to better asthma control, yet did result in treatment with higher doses of ICS. In contrast, an RCT by Smith et al. [115] in 97 asthmatics (age:

12–75 years) showed that the addition of FeNO monitoring to guideline-based management resulted in treatment with reduced doses of ICS. There was no significant difference in asthma control between the two treatment arms.

However, FeNO might be a valuable marker to predict relapse upon ICS reduction or predict corticosteroid responsiveness [69, 117–120]. A study by Zacharasiewicz et al. [120] investigated whether FeNO could be used to predict success or failure of ICS reduction in 40 children with stable asthma (age: 6–17 years). They showed that increased levels of FeNO and the percentage of sputum eosinophils were significant predictors for exacerbation upon steroid reduction. Still, the predictive value of FeNO alone was limited. More than 30% of the children with elevated FeNO levels remained stable upon ICS reduction. Szeffler et al. [57] and Knuffman et al. [119] showed that paediatric asthma patients with elevated FeNO levels were more likely to respond to corticosteroids than to montelukast. Smith et al. [117] showed that elevated FeNO levels could predict steroid responsiveness in children and adults with non-specific airway symptoms. FeNO was shown to be a better predictor than spirometry, bronchodilator response or AHR measurements. Several other studies have found a relationship between FeNO levels before treatment with corticosteroids and subsequent treatment response in adults and children with asthma (see Table 3). Generally, higher FeNO levels at baseline seem to be associated with a better response to therapy [23, 57, 65, 69, 117–119]; however, others have reported a lack of association between FeNO levels and therapy outcome [58, 67]. Study designs (including duration of treatment), study populations, cut-off values of FeNO and definitions of therapeutic response vary considerably between studies, which makes it difficult to compare findings.

Volatile organic compounds. A complex mixture of volatile organic compounds (VOCs) is present in exhaled breath. These compounds are partly produced by oxidative stress and lipid peroxidation, and these processes are likely to be influenced by the presence of airway inflammation. Pattern recognition of these compounds may function as disease-specific fingerprints. Currently, there are two main methods to detect and analyse VOC profiles: polymer-based gas sensor array (electronic nose), or gas chromatography-mass spectrometry (GC-MS). The electronic nose assesses the spectrum of volatiles present in exhaled breath (smellprint), without determining the individual molecular components. Dragonieri et al. [121] have shown that an electronic nose could discriminate adults with asthma from non-asthmatic controls based upon their VOC profiles. However, the electronic nose was less successful in discriminating mild from severe asthmatics. Dallinga et al. [122] showed by the use of GC-MS that the measurement of a limited set of VOCs in exhaled air could distinguish asthmatic children from healthy

controls with high sensitivity (95%) and high specificity (89%).

Assessing VOC profiles might be promising for asthma diagnosis and management, especially when knowledge of asthma-related VOCs is integrated with the user convenience of a handheld measurement device such as the electronic nose. Yet, robust validation of the assessment of VOC patterns in a large population of asthmatics is necessary, as well as longitudinal assessment of inter-individual variation of VOC patterns. In addition, the modifying effect of therapy on VOC profiles should also be addressed.

Conclusions and future perspectives

A consensus is present in the asthma field that new and non-invasive biomarkers are necessary to better diagnose and stage the various asthma phenotypes in clinical practice and thus improve asthma treatment. In the clinical setting, asthma control is often used as a surrogate endpoint for treatment response and refers to the absence of clinical manifestations. Nevertheless, asthma may be characterized by recurrent periods of symptoms, and the absence of clinical symptoms does not necessarily correlate with the absence of underlying airway inflammation. Furthermore, not only biological factors (such as inflammatory patterns and variations in drug receptors) may play a role in a therapeutic response, but psychological, sociological and environmental factors may also be involved. More knowledge on the pathobiological mechanism underlying asthma aetiology may be of great importance to identify relevant biomarkers for asthma diagnosis and management. Currently, various surrogate markers for airway inflammation have been identified, though few studies have addressed whether these markers are also associated with response to corticosteroids. Studies that have been performed varied considerably in study design and definition of treatment response and are therefore difficult to compare.

FeNO does not seem to be clinically applicable for diagnosing uncontrolled asthma or adjusting asthma treatment, but it still might be a promising marker to predict corticosteroid responsiveness in routine clinical practice. FeNO is easy to measure (with hand-held analysers already commercially available), highly reproducible and non-invasive. Nevertheless, findings on the relationship of FeNO with therapy response remain inconsistent. These inconsistent data might be due to the heterogeneity of the disease and the lack of individual 'normal' FeNO values. More research is necessary to assess the value of FeNO in distinct asthma phenotypes.

In addition, it is likely that the measurement of a combination of markers might evaluate therapy response more accurately, and studying patterns or profiles of inflammatory markers instead of assessing single

substances is becoming increasingly popular in asthma research. Patterns of VOCs or gene expression profiles might be interesting examples of such a systems approach. In addition, unbiased approaches with a focus on proteomics, metabolomics, epigenetics or pharmacogenomics might lead to further identification of novel biomarkers, especially because technologies in these areas are rapidly advancing.

In conclusion, further validation and evaluation of inflammatory biomarkers for therapy response are necessary. At a later stage, the cost-effectiveness of implementing a specific marker in routine clinical practice should also be assessed.

Acknowledgements

Financial and competing interest disclosure: Susanne J. H. Vijverberg is paid by an unrestricted grant from Glaxo-SmithKline (GSK). Leo Koenderman has no financial

relationship with a commercial entity that has an interest in the subject of this manuscript. Ellen S. Koster is paid by an unrestricted grant from GSK. Cornelis K. van der Ent received unrestricted grants from GSK and Grunenthal. Jan A. M. Raaijmakers is a part-time professor at the Utrecht University and vice president external scientific collaborations for GSK in Europe, and holds stock in GSK. Anke-Hilse Maitland-van der Zee is funded by a Veni grant from the Netherlands Organization for Scientific Research and received an unrestricted grant from GSK. Furthermore, the division of Pharmacoepidemiology and Clinical Pharmacology employing authors Susanne J. H. Vijverberg, Ellen S. Koster, Jan A. M. Raaijmakers and Anke-Hilse Maitland-van der Zee, has received unrestricted funding for pharmacoepidemiologic research from GSK, Novo Nordisk, the private-public funded Top Institute Pharma (<http://www.tipharma.nl>; includes co-funding from universities, government and industry), the Dutch Medicines Evaluation Board and the Dutch Ministry of Health.

References

- Masoli M, Fabian D, Holt S *et al*. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. *Allergy* 2004; **59**:469–78.
- Global Initiative for Asthma (GINA). *Global strategy for asthma management and prevention*. GINA, 2009. Available at <http://www.ginasthma.org> (accessed November 2010).
- Holgate ST. Pathogenesis of Asthma. *Clin Exp Allergy* 2008; **38**:872–97.
- Connett GJ. Bronchoalveolar lavage. *Paediatr Respir Rev* 2000; **1**:52–6.
- Rottier BL, Duiverman EJ. Anti-inflammatory drug therapy in asthma. *Paediatr Respir Rev* 2009; **10**:214–9.
- Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. *Br Med Bull* 2000; **56**:1054–70.
- van Gent R, van Essen LE, Rovers MM *et al*. Quality of life in children with undiagnosed and diagnosed asthma. *Eur J Pediatr* 2007; **166**:843–8.
- Fleming L, Wilson N, Bush A. Difficult to control asthma in children. *Curr Opin Allergy Clin Immunol* 2007; **7**:190–5.
- Mauad T, Bel EH, Sterk PJ. Asthma therapy and airway remodeling. *J Allergy Clin Immunol* 2007; **120**:997–1009.
- Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008; **8**:183–92.
- Baena-Cagnani C, Rossi GA, Canonica GW. Airway remodelling in children: when does it start? *Curr Opin Allergy Clin Immunol* 2007; **7**:196–200.
- Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. *Proc Am Thorac Soc* 2009; **6**:256–9.
- Wenzel SE, Schwartz LB, Langmack EL *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; **160**:1001–8.
- Simpson JL, Scott R, Boyle MJ *et al*. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; **11**:54–61.
- Green RH, Brightling CE, Woltmann G *et al*. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; **57**:875–9.
- Porsbjerg C, Lund TK, Pedersen L *et al*. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. *J Asthma* 2009; **46**:606–12.
- Baines KJ, Simpson JL, Bowden NA *et al*. Differential gene expression and cytokine production from neutrophils in asthma phenotypes. *Eur Respir J* 2009; **35**:522–31.
- Lovett CJ, Whitehead BF, Gibson PG. Eosinophilic airway inflammation and the prognosis of childhood asthma. *Clin Exp Allergy* 2007; **37**:1594–601.
- Drews AC, Pizzichini MMM, Pizzichini E *et al*. Neutrophilic airway inflammation is a main feature of induced sputum in nonatopic asthmatic children. *Allergy* 2009; **64**:1597–601.
- de Blic J, Tillie-Leblond I, Tonnel AB *et al*. Difficult asthma in children: an analysis of airway inflammation. *J Allergy Clin Immunol* 2004; **113**:94–100.
- Bush A, Menzies-Gow A. Phenotypic differences between pediatric and adult asthma. *Proc Am Thorac Soc* 2009; **6**:712–9.
- Fleming L, Wilson N, Regamey N *et al*. Are inflammatory phenotypes in children with severe asthma stable? *Eur Respir J* 2007; **30**:483S.
- Cowan DC, Cowan JO, Palmay R *et al*. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 2010; **65**:384–90.
- Luijk B, Van Den Berge M, Kerstjens HAM *et al*. Effect of an inhaled adenosine A2A agonist on the allergen-induced late asthmatic response. *Allergy* 2008; **63**:75–80.
- Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* 1995; **154**:4719–25.

- 26 Meagher LC, Cousin JM, Seckl JR *et al.* Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophil granulocytes. *J Immunol* 1996; 156:4422–8.
- 27 Belvisi MG. Regulation of inflammatory cell function by corticosteroids. *Proc Am Thorac Soc* 2004; 1:207–14.
- 28 Petsky HL, Kynaston JA, Turner C *et al.* Tailored interventions based on sputum eosinophils versus clinical symptoms for asthma in children and adults. *Cochrane Database Syst Rev* 2007; CD005603.
- 29 Green RH, Brightling CE, McKenna S *et al.* Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360:1715–21.
- 30 Chlumsky J, Striz I, Terl M *et al.* Strategy aimed at reduction of sputum eosinophils decreases exacerbation rate in patients with asthma. *J Int Med Res* 2006; 34:129–3.
- 31 Jayaram L, Pizzichini MM, Cook RJ *et al.* Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006; 27:483–94.
- 32 Haldar P, Brightling CE, Hargadon B *et al.* Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; 360:973–84.
- 33 Nair P, Pizzichini MMM, Kjarsgaard M *et al.* Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009; 360:985–93.
- 34 Koster ES, Maitland-van der Zee AH, Vijverberg SJH *et al.* Pharmacogenetics of anti-inflammatory therapy in asthma: corticosteroids and leukotriene antagonists. *Antiinflamm Antiallergy Agents Med Chem* 2010; 9:267–75.
- 35 Turner SW. Genetic predictors of response to therapy in childhood asthma. *Mol Diagn Ther* 2009; 13:127–35.
- 36 Lima JJ, Blake KV, Tantisira KG *et al.* Pharmacogenetics of asthma. *Curr Opin Pulm Med* 2009; 15:57–62.
- 37 Basu K, Palmer CNA, Tavendale R *et al.* Adrenergic beta2-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. *J Allergy Clin Immunol* 2009; 124:1188–94, e1183.
- 38 Wechsler ME, Kunselman SJ, Chinchilli VM *et al.* Effect of beta2-adrenergic receptor polymorphism on response to longacting [beta]2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, cross-over trial. *Lancet* 2009; 374:1754–6.
- 39 Israel E, Chinchilli VM, Ford JG *et al.* Use of regularly scheduled albuterol treatment in asthma: Genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004; 364:1505–12.
- 40 Bleecker ER, Postma DS, Lawrance RM *et al.* Effect of ADRB2 polymorphisms on response to longacting [beta]2-agonist therapy: a pharmacogenetic analysis of two randomised studies. *Lancet* 2007; 370:2118–25.
- 41 Wechsler ME, Lehman E, Lazarus SC *et al.* Beta2-Adrenergic receptor polymorphisms and response to salmeterol. *Am J Respir Crit Care Med* 2006; 173:519–26.
- 42 Bleecker ER, Yancey SW, Baitinger LA *et al.* Salmeterol response is not affected by beta2-adrenergic receptor genotype in subjects with persistent asthma. *J Allergy Clin Immunol* 2006; 118:809–16.
- 43 Israel E, Drazen JM, Liggett SB *et al.* The effect of polymorphisms of the beta2-adrenergic receptor on the response to regular use of albuterol in asthma. *Am J Respir Crit Care Med* 2000; 162:75–80.
- 44 Lowenberg M, Stahn C, Hommes DW *et al.* Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. *Steroids* 2008; 73:1025–9.
- 45 Ito K, Chung KF, Adcock IM. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 2006; 117:522–43.
- 46 Tantisira KG, Lake S, Silverman ES *et al.* Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. *Hum Mol Genet* 2004; 13:1353–9.
- 47 Weiss ST, Lake SL, Silverman ES *et al.* Asthma Steroid Pharmacogenetics: A Study Strategy to Identify Replicated Treatment Responses. *Proc Am Thorac Soc* 2004; 1:364–7.
- 48 Dijkstra A, Koppelman GH, Vonk JM *et al.* Pharmacogenomics and outcome of asthma: no clinical application for long-term steroid effects by CRHR1 polymorphisms. *J Allergy Clin Immunol* 2008; 121:1510–3.
- 49 Tantisira KG, Hwang ES, Raby BA *et al.* TBX21: a functional variant predicts improvement in asthma with the use of inhaled corticosteroids. *Proc Natl Acad Sci USA* 2004; 101:18099–104.
- 50 Ye YM, Lee HY, Kim SH *et al.* Pharmacogenetic study of the effects of NK2R G231E G>A and TBX21 H33Q C>G polymorphisms on asthma control with inhaled corticosteroid treatment. *J Clin Pharm Ther* 2009; 34:693–701.
- 51 Tantisira KG, Silverman ES, Mariani TJ *et al.* FCER2: a pharmacogenetic basis for severe exacerbations in children with asthma. *J Allergy Clin Immunol* 2007; 120:1285–91.
- 52 Jin Y, Hu D, Peterson EL *et al.* Dual-specificity phosphatase 1 as a pharmacogenetic modifier of inhaled steroid response among asthmatic patients. *J Allergy Clin Immunol* 2010; 126:618–25, e612.
- 53 Hawkins GA, Lazarus R, Smith RS *et al.* The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. *J Allergy Clin Immunol* 2009; 123:1376–83, e1377.
- 54 Hakonarson H, Bjornsdottir US, Halapi E *et al.* Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. *Proc Natl Acad Sci USA* 2005; 102:14789–94.
- 55 Donn R, Berry A, Stevens A *et al.* Use of gene expression profiling to identify a novel glucocorticoid sensitivity determining gene, BMPRII. *FASEB J* 2007; 21:402–14.
- 56 Koster ES, Rodin AS, Raaijmakers JAM *et al.* Systems biology in pharmacogenomic research: the way to personalized prescribing? *Pharmacogenomics* 2009; 10:971–8.
- 57 Szeffler SJ, Phillips BR, Martinez FD *et al.* Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005; 115:233–42.
- 58 Meijer RJ, Postma DS, Kauffman HF *et al.* Accuracy of eosinophils and eosinophil cationic protein to predict steroid improvement in asthma. *Clin Exp Allergy* 2002; 32:1096–103.
- 59 Kato M, Yamada Y, Maruyama K *et al.* Serum eosinophil cationic protein and 27 cytokines/chemokines in acute ex-

- acerbation of childhood asthma. *Int Arch Allergy Immunol* 2010; 152:62–6.
- 60 Fitzpatrick AM, Higgins M, Holguin F *et al*. The molecular phenotype of severe asthma in children. *J Allergy Clin Immunol* 2010; 125:851–7, e818.
 - 61 Oudijk E-JD, Lo Tam Loi AT, Langereis JD *et al*. Functional antagonism by GM-CSF on TNF- α -induced CD83 expression in human neutrophils. *Mol Immunol* 2008; 46:91–6.
 - 62 Johansson MW, Barthel SR, Swenson CA *et al*. Eosinophil $\beta 1$ integrin activation state correlates with asthma activity in a blind study of inhaled corticosteroid withdrawal. *J Allergy Clin Immunol* 2006; 117:1502–4.
 - 63 Kanter D, ten Hove W, Luijk B *et al*. Expression of activated Fc γ RII discriminates between multiple granulocyte-priming phenotypes in peripheral blood of allergic asthmatic subjects. *J Allergy Clin Immunol* 2007; 120:1073–81.
 - 64 Pavord ID, Brightling CE, Woltmann G *et al*. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet* 1999; 353:2213–4.
 - 65 Szefer SJ, Martin RJ, King TS *et al*. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002; 109:410–8.
 - 66 Berry M, Morgan A, Shaw DE *et al*. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007; 62:1043–9.
 - 67 Martin RJ, Szefer SJ, King TS *et al*. The Predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial. *J Allergy Clin Immunol* 2007; 119:73–80.
 - 68 Godon P, Boulet LP, Malo JL *et al*. Assessment and evaluation of symptomatic steroid-naïve asthmatics without sputum eosinophilia and their response to inhaled corticosteroids. *Eur Respir J* 2002; 20:1364–9.
 - 69 Little SA, Chalmers GW, MacLeod KJ *et al*. Non-invasive markers of airway inflammation as predictors of oral steroid responsiveness in asthma. *Thorax* 2000; 55:232–4.
 - 70 Bacci E, Cianchetti S, Bartoli M *et al*. Low Sputum Eosinophils Predict the Lack of Response to Beclomethasone in Symptomatic Asthmatic Patients. *Chest* 2006; 129:565–72.
 - 71 Lex C, Jenkins G, Wilson NM *et al*. Does sputum eosinophilia predict the response to systemic corticosteroids in children with difficult asthma? *Pediatr Pulmonol* 2007; 42:298–303.
 - 72 Cai C, Yang J, Hu S *et al*. Relationship between urinary cysteinyl leukotriene E4 levels and clinical response to anti-leukotriene treatment in patients with asthma. *Lung* 2007; 185:105–12.
 - 73 Rabinovitch N, Graber NJ, Chinchilli VM *et al*. Urinary leukotriene E4/exhaled nitric oxide ratio and montelukast response in childhood asthma. *J Allergy Clin Immunol* 2010; 126:545–51, e544.
 - 74 Hoffmeyer F, Raulf-Heimsoth M, Bruning T. Exhaled breath condensate and airway inflammation. *Curr Opin Allergy Clin Immunol* 2009; 9:16–22.
 - 75 Lazar Z, Vass G, Huszar A *et al*. Exhaled breath condensate: adenosine, ATP and other purines. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 183–95.
 - 76 Loukides S, Bakakos P, Kostikas K. Exhaled breath condensate: hydrogen peroxide. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 162–7.
 - 77 Donnelly LE. Exhaled breath condensate: nitric oxide-related compounds. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 207–16.
 - 78 Montuschi P. Exhaled breath condensate: 8-isoprostane and eicosanoids. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 196–206.
 - 79 Bikov A, Antus B, Losonczy G *et al*. Exhaled breath condensate pH. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 173–82.
 - 80 Al Obaidi AH, Samarai AMA. Biochemical Markers as a Response Guide for Steroid Therapy in Asthma. *J Asthma* 2008; 45:425–8.
 - 81 Tseliou E, Bessa V, Hillas G *et al*. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. *Chest* 2010; 138:107–13.
 - 82 Zietkowski Z, Tomasiak-Lozowska MM, Skiepkowski R *et al*. High-sensitivity C-reactive protein in the exhaled breath condensate and serum in stable and unstable asthma. *Respir Med* 2009; 103:379–85.
 - 83 Zietkowski Z, Tomasiak-Lozowska M, Skiepkowski R *et al*. Eotaxin-1 in exhaled breath condensate of stable and unstable asthma patients. *Respir Res* 2010; 11:110.
 - 84 Leung TF, Wong GWK, Ko FWS. Increased macrophage-derived chemokine in exhaled breath condensate and plasma from children with asthma. *Clin Exp Allergy* 2004; 34:786–91.
 - 85 Robroeks CMHHT, Rijkers GT, Jöbsis Q *et al*. Increased cytokines, chemokines and soluble adhesion molecules in exhaled breath condensate of asthmatic children. *Clin Exp Allergy* 2010; 40:77–84.
 - 86 Matsunaga K, Yanagisawa S, Ichikawa T *et al*. Airway cytokine expression measured by means of protein array in exhaled breath condensate: correlation with physiologic properties in asthmatic patients. *J Allergy Clin Immunol* 2006; 118:84–90.
 - 87 Rosias P, Dompeling E, Hendriks HJE *et al*. Exhaled breath condensate in children: pearls and pitfalls. *Pediatr Allergy Immunol* 2004; 15:4–19.
 - 88 Horvath I, Hunt J, Barnes PJ. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005; 26:523–48.
 - 89 Dompeling E, Rosias PPR, Jobsis Q. Exhaled breath condensate sample collection: standards and open issues. In: Horvath I, de Jongste JC, eds. *European respiratory monograph: exhaled biomarkers*, Volume 49. Sheffield, UK: European Respiratory Society Journals Ltd, 2010; 152–61.
 - 90 Kostikas K, Koutsokera A, Papiris S *et al*. Exhaled breath condensate in patients with asthma: implications for application in clinical practice. *Clin Exp Allergy* 2008; 38:557–65.
 - 91 Pijnenburg MWH, Jongste JCD. Exhaled nitric oxide in childhood asthma: a review. *Clin Exp Allergy* 2008; 38:246–59.
 - 92 Rodway GW, Choi J, Hoffman LA *et al*. Exhaled nitric oxide in the diagnosis and management of asthma: clinical

- implications. *Chron Respir Dis* 2009; 6:19–29.
- 93 Kharitonov SA, Gonio F, Kelly C *et al.* Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003; 21:433–8.
 - 94 Kerckmar C. Exhaled nitric oxide in the diagnosis and management of childhood asthma. *Ther Adv Respir Dis* 2010; 4:71–82.
 - 95 Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993; 6:1368–70.
 - 96 Lehtimäki L, Turjanmaa V, Kankaanranta H *et al.* Increased bronchial nitric oxide production in patients with asthma measured with a novel method of different exhalation flow rates. *Ann Med* 2000; 32:417–23.
 - 97 Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. *Chest* 2003; 123:751–6.
 - 98 Buchvald F, Eiberg H, Bisgaard H. Heterogeneity of FeNO response to inhaled steroid in asthmatic children. *Clin Exp Allergy* 2003; 33:1735–40.
 - 99 Tamasi L, Bohacs A, Bikov AS *et al.* Exhaled nitric oxide in pregnant healthy and asthmatic women. *J Asthma* 2009; 46:786–91.
 - 100 Bratton DL, Lanz MJ, Miyazawa N *et al.* Exhaled nitric oxide before and after montelukast sodium therapy in school-age children with chronic asthma: a preliminary study. *Pediatr Pulmonol* 1999; 28:402–7.
 - 101 Piacentini LG, Peroni GD, Miraglia Del Giudice M *et al.* Effect of montelukast on exhaled NO in asthmatic children exposed to relevant allergens. *Pediatr Allergy Immunol* 2002; 13:137–9.
 - 102 Lönnkvist K, Anderson M, Hedlin G *et al.* Exhaled NO and eosinophil markers in blood, nasal lavage and sputum in children with asthma after withdrawal of budesonide. *Pediatr Allergy Immunol* 2004; 15:351–8.
 - 103 Cohen J, Douma WR, ten Hacken NHT *et al.* Ciclesonide improves measures of small airway involvement in asthma. *Eur Respir J* 2008; 31:1213–20.
 - 104 Erin EM, Zacharasiewicz AS, Nicholson GC *et al.* Rapid effect of inhaled ciclesonide in asthma a randomized, placebo-controlled study. *Chest* 2008; 134:740–5.
 - 105 Lim S, Jatakanon A, Meah S *et al.* Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000; 55:184–8.
 - 106 Payne DNR, Adcock IM, Wilson NM *et al.* Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001; 164:1376–81.
 - 107 Lemièrre C, Ernst P, Olivenstein R *et al.* Airway inflammation assessed by invasive and noninvasive means in severe asthma: Eosinophilic and noneosinophilic phenotypes. *J Allergy Clin Immunol* 2006; 118:1033–9.
 - 108 Pontin J, Blaylock MG, Walsh GM *et al.* Sputum eosinophil apoptotic rate is positively correlated to exhaled nitric oxide in children. *Pediatr Pulmonol* 2008; 43:1130–4.
 - 109 Silvestri M, Sabatini F, Sale R *et al.* Correlations between exhaled nitric oxide levels, blood eosinophilia, and airway obstruction reversibility in childhood asthma are detectable only in atopic individuals. *Pediatr Pulmonol* 2003; 35:358–63.
 - 110 Steerenberg PA, Janssen NAH, de Meer G *et al.* Relationship between exhaled NO, respiratory symptoms, lung function, bronchial hyperresponsiveness, and blood eosinophilia in school children. *Thorax* 2003; 58:242–5.
 - 111 Ludviksdottir D, Janson C, Hogman M *et al.* Exhaled nitric oxide and its relationship to airway responsiveness and atopy in asthma. *Respir Med* 1999; 93:552–6.
 - 112 Nordvall SL, Janson C, Kalm-Stephens P *et al.* Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. *Allergy* 2005; 60:469–75.
 - 113 Scott M, Raza A, Karmaus W *et al.* Influence of atopy and asthma on exhaled nitric oxide in an unselected birth cohort study. *Thorax* 2010; 65:258–62.
 - 114 Gabriele C, Pijnenburg MWH, Monti F *et al.* The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children. *Pediatr Allergy Immunol* 2005; 16:243–7.
 - 115 Smith AD, Cowan JO, Brassett KP *et al.* Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005; 352:2163–7.
 - 116 Szeffler SJ, Mitchell H, Sorkness CA *et al.* Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet* 2008; 372:1065–72.
 - 117 Smith AD, Cowan JO, Brassett KP *et al.* Exhaled nitric oxide: a predictor of steroid response. *Am J Respir Crit Care Med* 2005; 172:453–9.
 - 118 Perez-de-Llano LA, Carballada F, Castro Anon O *et al.* Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. *Eur J Immunol* 2010; 35:1221–7.
 - 119 Knuffman JE, Sorkness CA, Lemanske Jr RF *et al.* Phenotypic predictors of long-term response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma. *J Allergy Clin Immunol* 2009; 123:411–6.
 - 120 Zacharasiewicz A, Wilson N, Lex C *et al.* Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. *Am J Respir Crit Care Med* 2005; 171:1077–82.
 - 121 Dragonieri S, Schot R, Mertens BJA *et al.* An electronic nose in the discrimination of patients with asthma and controls. *J Allergy Clin Immunol* 2007; 120:856–62.
 - 122 Dallinga JW, Robroeks CMH, Berkel JJ *et al.* Volatile organic compounds in exhaled breath as a diagnostic tool for asthma in children. *Clin Exp Allergy* 2010; 40:68–76.