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Abstract

Recognition of asthma as a heterogeneous disease revealed different potential molecular targets and urged the development of targeted, customized treatment modalities. Evidence was provided for different inflammatory subsets of asthma and more recently, further refined to T helper (Th)2-high and Th2-low subphenotypes with different responsiveness to standard and targeted pharmacotherapy. Given these differences in immunology and pathophysiology, proof of concept studies of novel treatment modalities for asthma should be performed in adequate, well-defined phenotypes. In this review, we describe both existing and novel biomarkers of Th2-inflammation in asthma that can be applied to classify asthma subphenotypes in clinical studies and for treatment monitoring.

Introduction

Current guidelines acknowledge the heterogeneity of asthma and the potential value of biomarkers to aid diagnosis and adequate treatment [1]. The concept of different asthma phenotypes has been recognized by clinicians since a long time, exemplified by the subdivision into intrinsic (non-allergic) and extrinsic (allergic) asthma [2,3]. In the late 1990s, Rosi and colleagues showed by a factor analysis that clinical parameters, physiological tests and inflammatory biomarkers provide complimentary information and that combining these data could help to define an individual's asthma phenotype and subsequent customized treatment [4]. Ever since a number of cluster analyses have been performed [5,6]. Furthermore, several attempts have been made to define asthma (sub)phenotypes based on clinical or physiological presentations or provoking stimuli, yielding overlapping characteristics. Although none of the proposed phenotyping systems has been validated so far, inflammatory phenotyping (*i.e.*, eosinophilic, neutrophilic, mixed, paucigranulocytic) offers the potential of linking a specific inflammatory phenotype to (targeted) treatment options and disease monitoring [5, 7]. More recently, asthma phenotypes have been defined based on their T-helper 2 (Th2)-gene expression, yielding at least two major phenotypes: *i.e.*, ''Th2- high'' and ''Th2-low'' subsets [8,9]. Although not fully confirmed, some of these insights have already been successfully implemented into clinical practice, treatment monitoring and clinical trials [10]. In this context, periostin is an emerging biomarker of eosinophilic and Th2-driven inflammation in asthmatic patients [11].

In the current asthma exacerbation model of Th2-driven airway inflammation used to study targeted therapies [Figure 1], inhaled allergen initiates activation of mast cells with subsequent release of pro-inflammatory mediators, including leukotrienes (LTs)

and prostaglandins (PGs) during the early asthmatic airway response (EAR). During the subsequent late asthmatic response (LAR), activated Th2-cells release interleukin (IL)-4, IL-5, IL-13, eotaxin and TARC [12], with subsequent IgE isotype switching in B cells, eosinophil activation including pro-inflammatory mediator release and airway smooth muscle (ASM) cell proliferation [13]. In addition, airway epithelial cells secrete IL-25 and IL-33, thus activating dendritic cells and promoting the release of IL-5 and IL-13 from innate lymphoid cells [14,15,16,17,18,19,**20,21**]. IL-13 induces the migration and survival of eosinophils [20], activation of macrophages [**22**], mucus hypersecretion [**23**], production of inducible nitric oxide (NO) synthase (iNOS) by airway epithelial cells [**24**], and transformation of airway fibroblasts into myofibroblasts promoting collagen deposition [23]. Apart from its role in the pathophysiology of airway remodelling, IL-13 also has been shown to induce non-specific airways hyperresponsiveness (AHR) [25].

In this review we discuss the most important Th2-derived biomarkers currently used in clinical practice and clinical research of asthma and potential future applications.

Systemic Th2-biomarkers
Blood eosinophils

For decades, peripheral blood eosinophil counts have been used for indirect assessment of airway inflammation and to aid the diagnosis of asthma. Evidence has been provided that total peripheral blood eosinophil counts can guide corticosteroid treatment, to predict asthma exacerbations [26] and to be sensitive markers of fatal asthma [27].

In healthy adults, blood eosinophil counts generally range from 0.015 to 0.65 × 10^9/L [28]. There is a substantial diurnal variation (over 40%), with the lowest counts in the morning and the highest at night [29]. Blood eosinophil counts and serum IL-5 and IL-13 levels were reduced after 16 weeks of treatment with omalizumab (anti-IgE) [30,31].

Similarly, 50 weeks of treatment with mepolizumab (anti–IL-5) significantly decreased blood eosinophil counts, sputum eosinophils and the number of severe exacerbations in patients with severe eosinophilic asthma refractory to high dose of inhaled corticosteroids (ICS) or systemic corticosteroids [32, 33, 34]. Thus, peripheral blood eosinophil counts are believed to reflect Th2-driven inflammation within the airways.

Soluble Th2-markers from peripheral blood

Immunoglobulin E (IgE)

IgE is a key mediator of allergic conditions including asthma and allergy [35]. Hence, total serum IgE level is a specific biomarker of atopy and Th2-driven inflammation. In a multicenter trial, 182 patients with mild-to-severe asthma (approx 30% receiving ICS) were followed for 2.5 years. While correcting for FEV_1, sex, and age, total serum IgE level was the only independent variable tested predicting corticosteroid-induced improvement in AHR [36]. In another study of 562 patients with mild-to-severe allergic asthma, total serum IgE levels were related to asthma severity and inversely correlated with lung function (FEV_1 % predicted) [37]. Additionally, despite a substantial inter-subject variability, there was only minor overlap in serum IgE levels between the asthmatic (554 ± 447 IU/mL) and the control population (69 ± 33 IU/mL). In fact, a cut-off of 200 IU/mL would indicate sensitivity of 93% and specificity of 91% among these subjects.

Eosinophil cationic protein (ECP)

Eosinophil cationic protein (ECP) and eosinophilic peroxidase (EPO) are released during degranulation of eosinophils and, hence, increased serum levels of these products are

indicative of systemic eosinophilic inflammation. Serum ECP levels are often increased in asthmatic subjects, with a sensitivity of 70% and a specificity of 74% (efficiency 73%) and have been indicative for the presence of asthma [38]. Seasonal increases in serum ECP levels have been observed in sensitized, corticosteroid-naive asthmatics [39]. In this study, the increase in serum ECP levels significantly correlated with changes in parameters of asthma activity: *i.e.,* increases in symptom scores and blood eosinophils, and decreases in PEF values and PD20 methacholine [39]. In another observational study, patients with seasonal allergic rhinitis (AR) who developed asthma-like symptoms over 6 years, initially presented with significantly higher serum ECP levels (16.7 microg/L *vs* 8.2 microg/L) and higher serum EPO levels (17.9 microg/L *vs* 8.8 microg/L) compared with rhinitics who did not develop lower airway symptoms over time [40]. Hence, in these subjects with seasonal AR, serum ECP and EPO levels measured outside of season showed a high predictive ability for the development of asthma [40]. In addition, both treatment with ICS [41] and targeted therapies, including omalizumab [42] and mepolizumab [34], decreased serum ECP levels in asthmatic patients.

Eotaxin

The chemokine eotaxin (CCL11) is a potent and selective chemoattractant for human eosinophils [43]. Serum eotaxin levels were higher in asthmatic patients (175.8±49.3 pg/mL) compared to controls (109.6±56.1 pg/mL), and correlated to the ECP levels in the asthmatics [44]. In a study investigating serum samples from 944 individuals of 218 asthma-affected families, eotaxin levels were higher in asthmatic parents than in asthmatic children, but no difference was found between healthy and asthmatic individuals [45]. In addition, serum eotaxin has been useful in predicting the severity of

symptoms that patients develop during steroid-tapering and could therefore be evaluated in guiding asthma treatment [46].

TARC

Thymus and activation-regulated chemokine (TARC; CCL17) is another biomarker suggested to be involved in the Th2-inflammatory response. Mean serum concentrations of TARC has been shown to be primarily increased in atopic dermatitis (325 pg/mL), but in some studies also in bronchial asthma (271 pg/mL) and allergic rhinitis (147 pg/mL; *versus* healthy volunteers (31.9 pg/mL) [47,48].

In asthmatic subjects, serum TARC concentrations were found to correlate with serum eotaxin concentrations [49]. In addition, some authors also report correlations with total IgE [50], while this was not found by others [48,49]. TARC in plasma was also increased in asthmatic children who were sensitized to cat allergen but not in those sensitized to other aeroallergens [50], and increased after allergen challenge [51]. Plasma TARC concentration may be responsive to corticosteroid treatment as increased levels (mean 131.0 pg/mL) have been found in asthmatic children not on inhaled corticosteroids compared with those treated with ICS (97.5 pg/mL) and healthy controls (76.0 pg/mL) [50].

Interleukins (IL) 4,5 and 13

Th2 cytokines such as IL-4, IL-5 and IL-13 play important roles in allergic diseases. However, due to their overall low levels in peripheral blood especially in stable clinical state, it is often difficult to detect them using traditional assays [48]. In some studies, increased serum IL-4 and IL-5 levels were found in asthmatic subjects compared to

healthy controls, but no relationship was found between these cytokine levels and clinical parameters of asthma [52]. In the aforementioned study investigating asthma-affected families [45], serum levels of IL-4 and IL-5 were lower in asthmatic adults compared to asthmatic children, with no difference between healthy and asthmatic individuals. However, IL-5 was the best predictor for extrinsic asthma and allergic rhinitis in children, and frequent asthma exacerbations in children were associated with increased serum IL-5 levels. In a study comparing fluticasone propionate (FP) and salmeterol (Salm) *versus* FP only, inhaled allergen challenge increased mean serum IL-5 from 0.7 to 5.9 pg/mL in sensitized asthmatics. Compared to FP only, pretreatment with the combination (FP/Salm) significantly reduced serum IL-5 and blood eosinophils 1 to 6 h post-allergen challenge [53]. Similarly, treatment with omalizumab reduced serum levels of IL-5 and IL-13 in atopic asthmatics [31].

In a recent study, a combined anti-IL4/13 antibody (dupilumab®) was used to treat patients with moderate-severe asthma with elevated blood eosinophil counts ($\geq$300 cells/µL) or increased sputum eosinophils ($\geq$3%). In addition to a significant clinical effectiveness: *i.e.,* reducing numbers of exacerbations and improving ACQ-5 scores, Th2-associated biomarkers as TARC, 3-eotaxin, IgE, and FeNO were all significantly reduced following dupilumab treatment [54].

Periostin

Periostin is a matrix protein associated with fibrosis; its expression in airway structural cells is upregulated by recombinant IL-4 and IL-13 [9]. Within the airways of asthmatics, periostin expression has been found to correlate with several aspects of airway remodeling [11]. In patients with severe uncontrolled asthma unresponsive to maximal

ICS treatment, increased serum periostin levels were indicative of persistent eosinophilic airway inflammation sampled by sputum and bronchial biopsies. In this study population, serum periostin was the single best predictor of airway eosinophilia compared to other Th2-markers tested: *i.e.,* IgE levels, blood eosinophil counts and fractionated exhaled NO (FENO) levels [11]. In a recent interventional study with lebrikizumab (anti-IL-13), baseline serum periostin levels were used to subphenotype patients [55]. After 12 weeks of treatment, improvements in FEV_1 and asthma exacerbations were more evident in the lebrikizumab-treated group compared with the placebo group, while the increase in FEV_1 after lebrikizumab-treatment was even more pronounced in the high-periostin subgroup compared to the low-periostin group (serum periostin levels above and below the median for the 212 study subjects). Similarly, lebrikizumab treatment was associated with a significant reduction in FENO levels compared with those seen in the placebo group, especially in the high-periostin subgroup [55]. These data suggest that serum periostin levels might be a useful tool for identifying responders to anti–IL-13 therapies. Following a similar subphenotyping of patients with uncontrolled severe persistent allergic asthma, a decrease in exacerbation frequency was seen following treatment with omalizumab in the high *versus* the low group referring to three Th2-biomarkers: FeNO, blood eosinophils and serum periostin (< or >50 ng/mL) [56].

Th2-biomarkers in urine

In asthmatic children, eosinophil protein X (EPX) measurements in urine were applied for the monitoring of eosinophilic inflammation [57]. Similar to other eosinophil-derived markers, excretion of EPX/EDN shows circadian rhythms with the highest levels occurring at night [58]. Increased urine concentrations of EPX/EDN were detected in

asthmatics and reductions were shown following anti-inflammatory (ICS) treatment [59].

Increased urine levels of EPX/EDN ($\geq$100 microg/mMol creatinine) in wheezing children during the first year may predict the development of allergic sensitization or asthma in later life [60].

Cysteinyl leukotrienes (CysLTs: LTC_4, LTD_4 and LTE_4) are eicosanoids produced through the 5-lipoxygenase pathway by a variety of cells associated with allergic inflammation, including eosinophils, mast cells, and basophils [61]. CysLTs are excreted in urine as LTE_4 (normal levels about 50 pg/μg creatinine), which is an indirect marker of CysLTs activity within the airways. Urinary LTE_4 levels increase with both spontaneous asthma exacerbations, aspirin and allergen challenges, and in nocturnal asthma during the night [62,63,64, 65,66]. In contrast to corticosteroids [63], drugs inhibiting CysLTs synthesis, such as leukotriene synthesis inhibitors, significantly reduce urinary LTE_4 levels [67;66]. As anticipated, urinary LTE_4 can help predict the clinical response to leukotriene modifiers [68].

Th2-biomarkers in airway samplings

Eosinophils in bronchial tissue

Approximately 50% of the asthmatics across different severities consistently show airway eosinophilia on airway sampling (bronchial biopsies, sputum) [7]. However, within the eosinophil phenotype, various subphenotypes exist with different responsiveness to gold standard therapy as was shown by a cluster analysis [5]. Eosinophils within the airway mucosa are also known to be increased in Th2-driven, allergic asthma, with further increases following relevant allergen challenge [69] and in patients with aspirin-sensitive asthma [70]. In pollen sensitized allergic rhinitics,

segmental allergen challenge with grass pollen extract increased eosinophils both in the challenged and unchallenged bronchial mucosa as well as in peripheral blood and nasal biopsies [71]. Of interest, the presence of eosinophils in bronchial biopsies may help to predict responsiveness to gold standard therapy with ICS [72]. Alternatively, in allergic asthmatics, anti-leukotriene treatment (montelukast 10 mg QID) for 8 weeks was shown to effectively decrease eosinophils in bronchial biopsies to a similar degree as low-dose fluticasone (2x100 mcg), while fluticasone was significantly superior in reducing serum ECP [73]. Other targeted therapies, such as omalizumab effectively reduced eosinophils both in sputum and in bronchial biopsies of allergic asthmatics following allergen challenge [74].

Soluble markers

Bronchoalveolar lavage & bronchial washings

Several Th2-biomarkers can be quantified in bronchoalveolar lavage (BAL) fluid. The levels of ECP (and EPX) are increased in BAL fluid of asthmatics (increases dependent on asthma severity/activity/treatment) *versus* healthy controls [75,76]. Repeated low doses of cat allergen increased ECP levels in BAL (from mean 0.8 to 3.1 microg/L) and nonspecific AHR in sensitized mild asthmatics, without inducing clinical symptoms [77]. In addition, increased ECP and specific IgE levels in BAL may be related to the pathogenesis of the ragweed-induced late airway response (LAR) in ragweed-sensitized asthmatics [78].

 In an observational study of patients with eosinophilic bronchitis, asthmatics and healthy controls, only a minority of subjects yielded baseline eotaxin values above the detection limit (0.21 pg/mL in BAL fluid and 6.25 pg/mL in BW, respectively), and

hence, no difference could be found between the 3 study populations [79]. In a longitudinal study, a sustained increase in BAL eotaxin levels was found in asthmatics *versus* healthy controls regardless of ICS treatment, while BAL IL-5 levels seemed more ICS-responsive [80].

IL-4, IL-5 and IL-13 are typically involved in atopic asthma, and they have all been demonstrated to increase after allergen provocation [81, 82, 83, 84, 85, 86, 87,88], where the effect on IL-4 persisted for 2 weeks while IL-13 levels were back to baseline after 1 week [89]. A similar effect was seen in allergic rhinitics [81].

Similar to other Th2-cytokines, TARC is increased in BAL following allergen challenge [51,84], and is correlated to both IL-5 and IL-13. Moreover, TARC levels are higher in patients with an allergen-induced LAR compared to those without [90]. More recently, a BAL study revealed a Th2-high phenotype, with severe poorly controlled asthma despite corticosteroids, with upregulation of the PGD2 pathway [91].

Leukotrienes

A limited number of studies have been published on leukotrienes measurements in BAL. Involvement of LTs (*i.e.,* LTB_4 and CysLTs) in the inflammation and the physiology of nocturnal asthma has been demonstrated as increased BAL LTB_4 and CysLT levels were found at 4:00 AM in nocturnal asthmatics compared to healthy controls. In parallel, urinary LTE_4 were also significantly higher in nocturnal asthmatics *versus* controls. In contrast, no differences in BAL LT-levels could be demonstrated in both groups at 4 PM [92]. Following allergen challenge, no difference was found in BAL CysLT levels between patients with a LAR compared to those without a LAR (3,2-8,7 pg/mL) [90], possibly due to the fact that CysLTs can be released during both the early and the late response to allergen, by mast cells and eosinophils, respectively. However, a more pronounced

difference was seen between patients with aspirin-sensitive *versus* aspirin-tolerant asthma, possibly due to the marked airway eosinophilia in the aspirin-sensitive group [93, 94]. In one study, CysLTs have been shown to respond to ICS, as BAL LTC$_4$ levels of asthmatic subjects were lower after 2.5 years ICS therapy [95], while leukotriene modifiers, as exemplified by the 5-lipoxygenase inhibitor, zileuton, in the aforementioned study by Wenzel et al, effectively blocked all LTs while improving nocturnal manifestations of asthma [92].

Sputum

Given the invasive nature of bronchial biopsies, sputum analysis has offered a valid alternative allowing non-invasive asthma phenotyping and customized treatment [7,96]. Induced sputum is feasible in the majority of patients, yields repeatable cell counts (mainly eosinophils and neutrophils) across all asthma severities [96] and allows mRNA analysis [97]. Across the literature, eosinophilic airway inflammation is defined by at least ≥2% sputum eosinophils. Following allergen-induced LAR, repeatable increases in sputum eosinophils have been demonstrated in allergic asthmatics with superior outcomes if expressed as %non-squamous cells *versus* cells/mL [98]. So far, sputum eosinophils have been successfully applied as outcome variables in many proof of concept and (treatment) monitoring studies. The pre-requisite for successful implementation of this technique implies harmonization of standard operating procedures (both during induction and processing) across research centers and adequate analysis in a qualified, central laboratory [99].

Soluble markers

Similarly to BAL, several studies showed increased sputum ECP levels in asthmatics *versus* healthy controls [100 , 101 , 102 , 103] correlating with asthma activity/severity/treatment status [104, 105]. Asthmatics in remission and asthma patients with a slow FEV_1 decline had similar sputum ECP levels [106], while patients with a fast FEV_1 decline and exacerbating asthmatics had higher ECP levels [103]. Patients with seasonal allergic asthma and rhinitis with nonspecific AHR showed increased sputum ECP levels during season with persistent increases off season [120] and inhaled allergen increased sputum ECP levels in sensitized asthmatics [107,108]. Several treatments have been shown to decrease sputum ECP levels: *e.g.* prednisolone [100], beclometasone [109], montelukast [110] and roflumilast [111].

Sputum Th2-cytokines including IL-4, IL-5, IL-13, eotaxin and TARC are increased in asthmatic subjects as compared with healthy controls, with further increases during both spontaneous and modeled (*e.g.* allergen challenge) exacerbations [12, 101 , 103 ,112, 113, 121]. Sputum TARC levels were increased in asthmatics (1117 pg/mL) as compared with healthy controls (31,8 pg/mL), and were positively correlated with sputum eotaxin. However, in contrast to sputum eotaxin, which showed a strong positive correlation with the sputum ECP, sputum TARC did not correlate with the ECP levels [49]. Ample evidence has been provided for a major role of IL-13 in the pathophysiology of airway remodeling [114], which clinically translates into e.g. rapid FEV1 decline and nonspecific AHR [113]. Smoking and/or its immunological effects evidently affect IL-13 as sputum IL-13 levels were higher in smokers than non-smokers regardless of concomitant asthma [101]. IL-13 production appears to be reciprocally regulated by corticosteroid therapy, since sputum IL-13 levels were overall lower in corticosteroid-treated asthmatics

compared with untreated controls [115]. Similarly, following inhaled allergen challenge, increases in IL-4, IL-5, IL-13, eotaxin and TARC were found in ultracentrifuged sputum of dual responders at 7 and 24 h post-challenge, while these levels were significantly decreased by pretreatment with a short course of high dosed fluticasone, corresponding with complete blockade of the allergen-induced LAR [12]. In this study, ultracentrifugation of sputum (at 35,000 rpm) was applied since Th2-cytokines are usually undetectable if measured under baseline conditions [12].

Sputum CysLTs were increased in asthmatics (median, 9.5 ng/mL) *versus* healthy controls (6.4 ng/mL), and higher in subjects with persistent asthma requiring ICS (median, 11.4 ng/mL) or within 48 h of an acute, severe exacerbation (13 ng/mL) *versus* subjects with episodic asthma (7.2 ng/mL) [116,117, 118, 119,120]. In addition, sputum CysLTs and urinary LTE_4 concentrations were higher in asthmatics with eosinophilic airway inflammation [116]. Seasonal (allergic) asthmatics could be differentiated from those with rhinitis with or without AHR by higher sputum CysLT levels [geometric mean: 3.3 (lower 95%-upper 95% confidence interval (CI) of geometric mean: 1.9-5.1) *vs.* 1.4 (0.9-2.2) and 0.7 (0.3-1.6) pg/microg total protein, respectively] during pollen season, while levels declined off-season [121]. In addition, susceptible asthmatics to exercise-induced bronchoconstriction [122] and aspirin-sensitive asthmatics have increased sputum CysLTs [123]. Fluticasone combined with montelukast produced greater reductions in sputum CysLTs than fluticasone with salmeterol [124], while zafirlukast did not affect sputum CysLTs levels [125].

Exhaled markers

Exhaled nitric oxide

In untreated asthma, FeNO has been shown to correlate with airway eosinophilic inflammation sampled by induced sputum and bronchial biopsies [126]. Based on FeNO levels, Dweik and colleagues identified two asthma phenotypes: high (≥35 ppb) and low (<35 ppb) subphenotype; the FeNO-high subphenotype characterized by longer disease duration, nonspecific AHR, atopy and airway eosinophila [127].

The lack of correlation between sputum eosinophils and FeNO in patients treated with anti-IL-5 (mepolizumab®) indicates that NO production is related to IL-13 and Th2-response rather than to the eosinophilic inflammation *per se* [128]. Indeed, a link exists between serum periostin levels and FeNO, with high FeNO levels being indicative of Th2-inflammation within the airways. In line with these observations, treatment with anti–IL-13 therapy (lebrikizumab®) has shown to reduce FeNO levels in high-periostin and high-FeNO asthmatics [55]. In addition, 12 weeks of lebrikizumab treatment was associated with improvements in lung function and exacerbations in patients with Th2-high phenotype (*i.e.*, high serum periostin and high FeNO at baseline) compared to those with a low Th2-profile [55].

FeNO measured according to ERS/ATS recommendations (50mL/s) mainly reflects NO-production within central airways and is closely associated with the expression of iNOS within bronchial epithelial cells [129]. Alternatively, the origin of NO from the peripheral airways (alveolar NO; CANO) is not completely known. In an *in vitro* study on small airway epithelial cells, the Th1-cytokines (*i.e.,* IL-1beta, TNF-alpha, and IFN-gamma) provided a pronounced iNOS expression compared to IL-13 inducing a modest increase [130]. This is somewhat in contrast to bronchial epithelial cell known to respond very well to the Th2-cytokine IL-13 [131]. In asthmatics *in vivo*, CANO seems to correlate with

distal lung eosinophilic inflammation [72]. Increased CANO levels were found (7.1 ppb) in patients refractory to high doses of ICS, but decreased following treatment with prednisolone (30 mg QID). There was a close correlation between BAL eosinophils and CANO while single FeNO measurements correlated with eosinophil counts in BW [72].

Exhaled breath condensate (EBC)

In theory, EBC allows sampling from the entire airways, including small airways. However, in practice, the method is associated with a number of methodological issues hampering interpretation. Due to their size, proteins, including cytokines, are invariably present in low levels close to or under detection limit. Increased levels of reactive oxidative markers and eicosanoid mediators (8-isoprostane and leukotrienes) can be more readily and reproducibly measured [132,133].

Saliva

Saliva can be easily obtained and biomarkers have been proposed to reflect systemic inflammation making saliva samplings a promising tool for larger population screenings and epidemiological surveys [134]. Even though clearly detectable levels of pro-inflammatory mediators and cytokines can be measured in saliva [135], surprisingly few studies have been published on the topic.

Conclusions

Apart from more traditional biomarkers, including blood and sputum eosinophils, serum IgE and FeNO levels, novel emerging Th2-related markers, such as periostin, TARC and others, have been identified. Combinations of different biomarkers may further help to

refine the Th2-subphenotypes and hence, may serve as useful tools to aid diagnosis and to define clinical phenotypes suitable for Th2-targeted therapies. Already today, there are very promising clinical examples proofing that it is possible to use Th2-related biomarkers to identify responders, with a high degree of accuracy. However, the optimal biomarker or combination of biomarkers awaits validation. In addition, there is an additional need to define the value of adequate biomarkers, not only to identify potential responders to therapy, but also as true primary outcome measures being closely related to disease activity and thereby competing with traditional variables as lung function and disease control.

Compliance with Ethics Guidelines

Conflict of Interest

Zuzana Diamant has served on an advisory board (Aerocrine) and served as a consultant for various companies (Hall Allergy Hexal, Mundipharma, Urogenix, Profess, QPS Netherlands).

Leif Bjermer has served on advisory boards and received payment for giving lectures.

Ellen Tufvesson decalres that she has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Figure1.

Immunological interactions within Th-2 driven airway inflammation in asthma

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