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**Title: SPUTUM INTERLEUKIN 13 AS A BIOMARKER FOR THE EVALUATION OF ASTHMA CONTROL**

**Short title/running head: Sputum interleukin (IL)-13 and asthma control**

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## ABSTRACT

**Background:** Asthma control refers to the extent to which the manifestations of asthma have been reduced or eradicated by treatment. Interleukin-13 (IL-13) has a central role in Th2 response and serves as a possible therapeutic target in uncontrolled asthma. Fraction of exhaled nitric oxide (FeNO) and sputum eosinophils have modest performance in the evaluation of asthma control

**Objective:** To assess the diagnostic performance of sputum IL-13 for the evaluation of asthma control. Furthermore, to investigate the performance of sputum eosinophils and FeNO.

**Methods:** One hundred seventy patients with asthma were studied. All subjects underwent assessment of asthma control by asthma control test (ACT), lung function tests, FeNO measurement and sputum induction for cell count identification and IL-13 measurement in supernatants.

**Results:** IL-13 (pg/ml) levels in sputum supernatant differed significantly among patients with well controlled asthma and those with not well controlled asthma (median IRQ 78 [66-102] vs 213[180-265],  $p<0.001$ ). ROC analysis showed that, for the whole study population, the diagnostic performance of IL-13 was superior to both sputum eosinophils and FeNO levels (AUC 0.92, 95%CI 0.87 to 0.95 vs AUC 0.65, 95%CI 0.58 to 0.72 vs AUC 0.65, 95%CI 0.55 to 0.72 respectively).

**Conclusion:** The diagnostic performance of sputum IL-13 was superior to both sputum eosinophils and FeNO levels for the identification of well-controlled asthma. Sputum IL-13 levels could serve as a useful biomarker for asthma control assessment.

## Introduction

Current asthma guidelines have focused on asthma control, both for the classification and for the proper management of individual patients [1]. Asthma control test (ACT) is a simple questionnaire that consists of five simple questions with five possible response options (rated from 1–5) for each question. It includes questions about limitations of everyday activity due to asthma, the presence of daytime or nocturnal symptoms, the frequency of use of rescue medications [2]. ACT has been extensively used in clinical practice. Despite the central role of inflammation in the pathogenesis and natural history of asthma, no biomarker of airways inflammation has been recommended in the assessment of asthma control in current guidelines [1]. Symptoms are subjective estimations of the patients and often reflect their expectations, whereas biomarkers have the advantage of being more objective measurements of airway inflammation. Despite this, no biomarker has reached the routine use in everyday clinical practice.

In recent years intense research has focused on the use of biomarkers as a guide to treat asthma and achieve disease control. Sputum eosinophils have been used to guide treatment in addition to guideline-recommended standard care [3, 4]. Such management resulted in the achievement of similar levels of asthma control but fewer exacerbations [5]. Fraction exhaled nitric oxide ( $F_{eNO}$ ) failed to show an overall benefit in clinical control or exacerbation rates compared to guided treatment strategy [5, 6] but some recently published data supported that alterations in the levels of  $F_{eNO}$  are related to alterations in the level of asthma control and that it may predict exacerbations [7, 8]. Despite the above data both biomarkers showed a modest

performance in predicting asthma control in a study including asthmatics with a broad spectrum of severity [9].

Interleukin 13 (IL-13) is a T-helper type 2 (Th2) cytokine with a central role in asthma pathogenesis and shares similar role, structure and receptor with IL-4 [10]. IL-13 is expressed in the asthmatic airways, has been related to underlying severity and has been shown to predict asthma control as assessed by ACQ [11]. Targeting IL-13 with monoclonal antibodies showed a beneficial effect in lung function that appeared to be greater in patients with higher levels of serum periostin [12]. Patient-reported outcomes, as measured by symptom scores and control questionnaires, were not significantly altered, although patients who had detectable IL-13 levels in their sputum showed trends toward greater improvements in ACQ [13]. Considering the above evidence it is likely that IL-13 is implicated in uncontrolled asthma.

We hypothesized that the diagnostic performance of sputum IL-13 would be superior to both sputum eosinophils and FeNO and could serve as a useful biomarker for the assessment of asthma control.

The aim of this study was to assess the diagnostic performance of sputum IL-13 for the identification of well-controlled asthma and compare it with eosinophils in induced sputum and FeNO. Furthermore the effect of underlying asthma severity was also assessed.

## **Materials and Methods**

### ***Subjects***

Patients with asthma were recruited from an on-going cohort of asthmatic patients who are followed up in two University asthma clinics: 1<sup>st</sup> and 2<sup>nd</sup> Respiratory

Medicine Departments of the University of Athens. The recruitment period was between June 2012 and September 2014. Diagnosis of asthma was established according to GINA guidelines [1] whereas diagnosis of Severe Refractory Asthma (SRA) was established according to ATS guidelines [14].

All patients included were treated with treatment provided by an asthma specialist in order to achieve the best level of asthma control and following an at least 6-month follow up and were adherent to therapy. We excluded patients under 18 or over 70 years old, pregnant women, patients with any other respiratory disease, any concomitant malignant, heart, renal, liver or collagen disease. Patients with a respiratory tract infection or asthma exacerbation in the past 8 weeks prior to the study entry were also excluded. The study was approved by the ethics committees of both hospitals and all subjects provided an informed consent.

### ***Evaluation of Asthma Control***

Asthma control was evaluated with a validated questionnaire, the Asthma Control Test (ACT). According to the sum of scores (5–25), asthma control was categorized as follows: well controlled (20–25 points) and not well controlled (<20 points) [2].

### ***Sputum induction***

Sputum was induced as previously described using all the modifications for safe measurements according to the underlying asthma severity [15, 16]. Patients inhaled 3% saline at room temperature nebulized by an ultrasonic nebulizer (DeVilbiss Co., Heston, UK) at the maximal saline output (4 ml/min). The total period of sputum induction was 15 min. Subjects were encouraged to cough deeply at 3-min intervals until the 15-min induction time had been completed. Sputum was processed using selected plugs as previously described [17]. Dithiothreitol (DTT) was added in a

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volume equal to four times the weight of the sputum specimen and it was further diluted with phosphate buffered saline (PBS) in a volume equal to the sputum plus DTT. Total cell counts were performed on a hemacytometer using Trypan blue stain. Slides were prepared by using cytopsin (Shandon, Runcorn, UK) and were stained with May-Grunwald and Giemsa for differential cell counts. Cell counting was performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each sample. A sample was considered adequate when the patient was able to expectorate at least 2 ml of sputum and the slides contained <10% squamous cells on differential cell counting. Total cell count expressed as the number of cells  $\times 10^6$  and the percentage (%) of sputum inflammatory cells were used for analysis. Sputum supernatants were kept at  $-70^{\circ}\text{C}$  for further measurement of IL-13.

### ***Lung Function***

Forced expiratory volume in 1<sup>st</sup> s (FEV1) and forced vital capacity (FVC), were measured using Master Screen Body (Viasys Healthcare, Jaeger, Hoechberg, Germany) according to the American Thoracic Society guidelines [18].

### ***FeNO measurement***

The fraction of exhaled nitric oxide (FeNO) was measured using a portable NO analyzer (NIOX MINO, Aerocrine; Sweden) as previously described [19].

### ***Atopic Status***

A positive skin prick test (mean wheal diameter of 3mm or greater) to any of twenty common aeroallergens (including mites, grasses, trees, fungus, domestic animals) was used to confirm atopy.

### ***Mediator Assays***

IL-13 was measured using an enzyme-linked immunosorbent assay kit (ELISA, R&D systems, Minneapolis, Minnesota, USA with a detection limit of 32 pg/ml.

### ***Study design***

All subjects were assessed by two experienced physicians (SL & PB). On day one they provided a medical history and underwent a physical examination. At the same day spirometry was performed, FeNO was measured and ACT was assessed. Skin prick tests were performed if they had not been assessed at least one year before entering the study. Sputum induction was performed on the following day.

### ***Statistical analysis***

Normally distributed data are presented as mean  $\pm$  SD, skewed data as median (interquartile ranges), and categorical data as n (%). Normality of distribution was checked with Kolmogorov-Smirnov test. Differences in numerical variables between two groups were evaluated with unpaired t-tests or Mann-Whitney U-tests for normally and skewed data respectively, whereas comparisons of proportions were performed using chi-square tests. Correlations of IL-13 levels were performed with Spearman's rank correlation coefficient ( $r_s$ ). For the assessment of IL-13, FeNO and sputum eosinophils performance as predictors of asthma control, receiver operating characteristic (ROC) curves were created. Areas under the ROC curves (AUC) with 95% confidence intervals (CI) and their differences from 0.5 were calculated. Sensitivities, specificities, positive (PPV) and negative (NPV) predictive values were calculated for the optimal cut-points. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA, USA) and MedCalc 9 (MedCalcSoftware, Mariakerke, Belgium).

## Results

One hundred seventy patients with asthma (123 with mild-moderate and 47 with SRA) were enrolled in the study. According to ACT score, 139 asthma patients were characterized as having well controlled asthma (ACT score  $\geq 20$ ), whereas 31 patients were characterized as suffering from not well controlled asthma (ACT score  $< 20$ ).

Demographic characteristics of study participants are summarized in Table 1. Characteristics for the subgroups of mild to moderate asthma and SRA are also provided in Table 1.

### Inflammatory variables according to asthma control & severity

IL-13 (pg/ml) levels in sputum supernatant were significantly higher among patients with not well controlled asthma compared to those with well controlled asthma (median IRQ 213[180-265] vs 78 [66-102],  $p < 0.001$  Table 2, Figure 1A). The levels of IL-13 presented a significant, yet weak correlation ( $r_s^2 = 0.33$ ,  $p < 0.001$ ). Both sputum eosinophils and FeNO levels also differed between well-controlled and not well-controlled asthma being higher in the latter group (Table 2).

The majority of patients with mild-moderate asthma were well controlled (81.7% with ACT score  $\geq 20$ ). On the other hand, approximately half of the patients with SRA were well controlled (53.2% with ACT score  $\geq 20$ ). IL-13 (pg/ml) in sputum supernatant was significantly higher in patients with SRA compared to those with mild to moderate asthma (median IRQ 156 [80-245] vs 78[66-103],  $p < 0.001$ , Table 2, Figure 1B). There were no differences in IL-13 levels in patients with high ( $\geq 3\%$ ) and low ( $< 3\%$ ) sputum eosinophils within the subgroups of patients with mild to moderate asthma ( $p = 0.618$ ) or SRA ( $p = 0.734$ ) (Figure 1B). Both sputum eosinophils and FeNO levels were higher in SRA compared to mild to moderate asthma (Table 2).

A negative correlation between sputum IL-13 and FEV1 was found ( $r=-0.228$ ,  $p=0.003$ ).

### **ROC analysis.**

#### **Diagnostic performance of IL-13 in sputum supernatant, sputum eosinophils and FeNO for the identification of well-controlled asthma.**

##### ***Whole study population***

The diagnostic performance of IL-13, FeNO and sputum eosinophils for the identification of patients with well-controlled asthma is presented in Table 3 and the corresponding ROC curves are shown in Figure 2. For the whole study population, the diagnostic performance of IL-13 was superior to both sputum eosinophils and FeNO levels (AUC 0.92, 95%CI 0.87 to 0.95 vs AUC 0.65, 95%CI 0.58 to 0.72 vs AUC 0.65, 95%CI 0.55 to 0.72 respectively, Table 3, figure 2). Pair wise comparison of ROC curves showed a difference between areas 0.204 and 0.267 for the comparisons between IL-13 and FeNO and IL-13 and sputum eosinophils respectively,  $p<0.001$  for both. No significant difference area was observed between FeNO and sputum eosinophils (0.06 and  $p=0.320$ ).

##### ***Subgroups according to underlying severity***

The diagnostic performance of IL-13, FeNO and sputum eosinophils for the identification of mild to moderate asthma patients with well-controlled asthma is presented in Table 3 and the corresponding ROC curves are shown in Figure 3A. The diagnostic performance of IL-13 was superior to both sputum eosinophils and FeNO levels (AUC 0.80, 95%CI 0.72 to 0.86 vs AUC 0.58, 95%CI 0.5 to 0.67 vs AUC 0.71, 95%CI 0.64 to 0.78 respectively, Table 2, figure 3A). Pairwise comparison of ROC

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curves showed a difference between areas 0.151 and 0.204 for the comparisons between IL-13 and FeNO and IL-13 and sputum eosinophils respectively,  $p=0.032$  and  $<0.001$  respectively. No significant difference area was observed between FeNO and sputum eosinophils (0.06 and  $p=0.660$ ).

The diagnostic performance of IL-13, FeNO and sputum eosinophils for the identification of SRA patients with well-controlled asthma is presented in Table 3 and the corresponding ROC curves are shown in Figure 3B. The diagnostic performance of IL-13 was superior to both sputum eosinophils and FeNO levels (AUC 0.98, 95%CI 0.899 86 vs AUC 0.64, 95%CI 0.49 to 0.78 vs AUC 0.69, 95%CI 0.53 to 0.81 respectively, Table 2, figure 3B). Pairwise comparison of ROC curves showed a difference between areas 0.293 and 0.336 for the comparisons between IL-13 and FeNO and IL-13 and sputum eosinophils respectively,  $p<0.001$  for both. No significant difference area was observed between FeNO and sputum eosinophils (0.04 and  $p=0.607$ ).

## Discussion

The main finding of our study is that IL-13 levels in sputum supernatant were significantly higher in patients with not well controlled asthma compared to those with well controlled asthma. Moreover, IL-13 levels were also higher in patients with SRA compared to those with mild to moderate asthma. The diagnostic performance of IL-13 was superior to both sputum eosinophils and FeNO levels for the identification of well-controlled asthma. This applied irrespective of asthma severity as the superior diagnostic performance of IL-13 was observed both in mild to moderate and SRA.

The level of asthma control is defined as the extent to which the manifestations of asthma can be observed in a patient or have been reduced or removed by treatment. The long-term goals of asthma management are to achieve good symptom control and to minimize future risk of exacerbations, fixed airflow limitation and side effects of treatment. The current concept of asthma management includes pharmacological and non-pharmacological treatment adjusted in a continuous cycle that involves assessment of control, adjustment of treatment to achieve control and review of the response[1].

There is compelling evidence that IL-13 is a central mediator in the pathogenesis of asthma arising from studies both in animal models and humans [20]. Increased IL-13 mRNA expression has been found in bronchial biopsies from subjects with moderate asthma [21, 22]. Similarly, increased IL-13 mRNA expression has been found in sputum cells from both corticosteroid-naïve and inhaled corticosteroid-treated asthmatic subjects [23]. Although the production of IL-13 is inhibited by ICS, some patients with uncontrolled asthma continue to present elevated levels of IL-13 in sputum, despite the use of systemic and inhaled corticosteroids [24]. A monoclonal antibody to IL-13, lebrikizumab, was associated with improvement in lung function, when added to standard therapy in patients with asthma that was inadequately controlled despite inhaled glucocorticoid therapy indicating an indirect association of IL-13 with lack of asthma control [25]. Interestingly in two studies where asthma control was assessed after an anti-IL-13 based intervention no significant differences were observed in asthma control [12, 13]. However when the patients were stratified according to the expression/detection of IL-13 in their sputum supernatant significant alterations in asthma control were detected.

Our finding of higher IL-13 levels in SRA compared to mild to moderate asthma is of particular interest. Saha et al, firstly reported IL-13 overexpression in patients with SRA [11]. Moreover, in this study, the number of IL-13+ cells in the bronchial submucosa was increased in all asthma severity groups in comparison to healthy control subjects without any differences across disease severity. Sputum IL-13 levels were higher in the severe asthma group when compared to the control group but there was no significant difference compared to milder forms of the disease. SRA is characterized by increased airway inflammation and remodeling compared to milder forms of the disease and based on our data this might be partly reflected in the over-expression of sputum IL-13.

Our results refer to Th2-related asthma, as IL-13 is central to the Th2 response. However, our findings may be relevant to both allergic and non-allergic severe asthma, due to the recently described Th2-associated inflammation that is present in some forms of non-allergic asthma, such as the late-onset eosinophilic asthma developed in adulthood. The mechanism behind this type of inflammatory response involves type 2 innate lymphoid cells that, after stimulation with the epithelial cytokines thymic stromal lymphopoietin (TSLP) and IL-33, produce TH2-associated cytokines, including high amounts of IL-5 and IL-13. This potential central role in late-onset non-allergic asthma may additionally explain the excellent diagnostic performance of IL-13 for the identification of well-controlled asthma [26].

Both sputum eosinophils and FeNO levels, have been previously associated with asthma control in numerous studies. Sputum eosinophilia is observed in a significant proportion of asthmatic patients irrespective of underlying severity [27]. A treatment

strategy directed at normalization of the induced sputum eosinophil count has been reported to reduce asthma exacerbations and admissions without the need for additional anti-inflammatory treatment [3]. FeNO which has been primarily related to TH2 inflammation has also been prospectively used to guide asthma treatment, with conflicting results [5, 28]. In a study by Volbeda et al, it was shown that asthmatic subjects with well-controlled asthma had lower FeNO levels than the subjects with uncontrolled asthma [29]. In a study including 381 asthmatic subjects, the addition of FeNO to ACQ-7 increased by 14.8% the detectability of not well controlled asthma upon adjustment of maintenance therapy [30]. In another study evaluating the longitudinal assessment of FeNO and sputum eosinophils it was shown that both FeNO and sputum eosinophils are useful for titrating treatment in asthmatic patients in order to achieve better long-term control of the disease. However, FeNO decrease seems to reflect adequately the reduction of sputum eosinophils only after 6 months [31]. In a systematic review and meta-analysis evaluating the effect of tailoring asthma treatment to eosinophil markers it was concluded that tailoring of treatment based on sputum eosinophils is effective in decreasing asthma exacerbations. However, tailoring of asthma treatment based on FeNO levels has not been shown to be effective in improving asthma outcomes in children and adults [32]. In a recent study a symptom- plus FeNO-driven strategy was found to reduce asthma medication use while sustaining asthma control and quality of life and was suggested to be the preferred strategy for adult asthmatic patients in primary care [33]. It is important to note that any tool used in addition to the validated asthma control questionnaires provides benefits at least for particular outcomes e.g exacerbations. However, we focused on asthma control because the achievement and maintenance of control is the main target of current asthma medication. In our study, the diagnostic performance of

IL-13 was superior to both sputum eosinophils and FeNO levels for the identification of well-controlled asthma in the whole study population as well as in the subgroups of mild to moderate and SRA. The relationship between IL-13 and asthma control is presumably based on an indirect effect of airway remodeling to both airflow limitation and symptom driven airway obstruction [10]. Periostin is among the most highly differentially expressed genes in asthma after IL-13 exposure and serum levels of periostin may also be an attractive biomarker for assessing asthma control. The different and sometimes conflicting results of such studies indicate the need for comparative and intense search for the proper biomarker that would better help in the assessment of asthma control in a particular asthma phenotype.

The task of achieving control of the disease is difficult in patients with SRA. Although sputum induction is a semi-invasive and time consuming procedure it is generally well-tolerated and has been the procedure of choice for measuring biomarkers in patients with asthma. The absence of repeatability data on IL-13 represents a potential limitation of this exploratory study and needs to be addressed in future longitudinal studies. It seems that sputum IL-13 is a valuable biomarker in the assessment of asthma control in mild to moderate asthma but more importantly in SRA in which a more vigilant evaluation of the patient is usually pursued.

In conclusion IL-13 levels in sputum supernatant are significantly higher in patients with not well controlled asthma compared to those with well controlled asthma and in patients with SRA compared to those with mild to moderate asthma. The diagnostic performance of sputum IL-13 is superior to both sputum eosinophils and FeNO levels

for the identification of well-controlled asthma, and accordingly IL-13 levels in sputum could serve as a useful biomarker for asthma control assessment.

**Conflict of interest statements:** No conflicts exist for the specified authors

### **Contributors**

KK is the guarantor of the content of the manuscript, including the data and analysis. SL, KK and PB were involved in the study conception and design. ZT, EK, AP, LA, AIP and AP were involved in the recruitment of patients, samples handling and data collection. SP, NK, PB, SL, and KK reviewed the manuscript and provided important scientific input.

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**Table 1.** Demographic characteristics of study participants.

| Variables                                 | Total<br>(n=170) | Mild-<br>Moderate<br>(n=123) | SRA<br>(n=47)  | P value          |
|-------------------------------------------|------------------|------------------------------|----------------|------------------|
| Age (years)                               | 53 [42-62]       | 51[41-62]                    | 55[46-62]      | 0.205            |
| Gender (female, %)                        | 117 (68.8%)      | 84 (68.3%)                   | 33 (70.2%)     | 0.324            |
| Smokers (current)                         | 41 (24.1%)       | 37 (30.1%)                   | 4 (8.5%) *     | 0.003            |
| Atopy                                     | 90 (52.9%)       | 64 (52.0%)                   | 26 (55.3%)     | 0.340            |
| Duration (years)                          | 23[15-34]        | 23[13-32]                    | 30[22-41]      |                  |
| BMI (kg/m <sup>2</sup> )                  | 27.6±4.7         | 27.8±4.8                     | 27.2±4.6       | 0.621            |
| Well- controlled<br>asthma (ACT≥20,<br>%) | 139 (81.7%)      | 114 (92.3%)                  | 25 (53.2%) *   | <b>&lt;0.001</b> |
| ACT                                       | 23[21-24]        | 23[22-24]                    | 20[18-23]      | <b>&lt;0.001</b> |
| FEV <sub>1</sub> (% pred.)                | 75 [64-91]       | 81[72-96]                    | 60[50-65]      | <b>&lt;0.001</b> |
| FEV <sub>1</sub> /FVC ratio               | 69±10            | 72±9                         | 61±8           | <b>&lt;0.001</b> |
| FeNO (ppb)                                | 22 [16 – 35]     | 21 [14 – 28]                 | 25 [20 – 63] * | <b>0.001</b>     |
| Sputum eosinophils<br>(%)                 | 3 [0– 8]         | 2 [0 – 7]                    | 4.0 (2 – 15) * | <b>0.008</b>     |
| Sputum neutrophils<br>(%)                 | 32 [20 – 44]     | 32 [20 – 40]                 | 41 (22 – 52) * | <b>0.023</b>     |
| Treatment                                 |                  |                              |                |                  |
| High dose ICS                             | 52 (30.6%)       | 5 (4.1%)                     | 47 (100%)      | ND               |
| LABA                                      | 147 (86.4%)      | 100 (81.3%)                  | 47 (100%)      | ND               |
| LTRA                                      | 29 (17.1%)       | 13 (10.6%)                   | 16 (34.0%)     | ND               |
| Oral corticosteroids                      | 24 (14%)         | 0                            | 24 (51%)       | ND               |
| Omalizumab                                | 11(6.5%)         | 0                            | 11(23%)        | ND               |

Normally distributed data are presented as mean±SD; skewed data are presented as median (interquartile ranges); and categorical data as n (%). Abbreviations: ACT: Asthma Control Test; BMI: Body Mass Index; FeNO: Fraction of exhaled nitric oxide; FEV<sub>1</sub>: forced expiratory volume in one second; FVC=Forced vital capacity; SRA: Severe Refractory Asthma; ICS: inhaled corticosteroids; LABA=Long acting  $\beta_2$  agonists; LTRA= Leukotriene receptor antagonists; ND=Not done; P value refers to comparisons between mild to moderate and SRA\*statistically significant difference compared to mild to moderate asthma. Bold letters indicate statistical significance.

**Table 2.** Study inflammatory variables according to asthma control & severity.

| <b>Inflammatory Variables</b> | <b>Well controlled<br/>(n=139)<br/>ACT <math>\geq</math>20<br/>23 [22-24]</b> | <b>Not well controlled<br/>(n=31)<br/>ACT &lt;20<br/>18[17-19]</b> | <b>SRA<br/>(n=47)</b> | <b>Mild to Moderate<br/>(n=123)</b> | <b>P value</b>                            |
|-------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------|-----------------------|-------------------------------------|-------------------------------------------|
| <b>IL-13 (pg/ml)</b>          | 78 [66-102]                                                                   | 213[180-265]                                                       | 156[80-245]           | 78[66-103]                          | * <b>&lt;0.001</b><br>** <b>&lt;0.001</b> |
| <b>Sputum Eosinophils %</b>   | 2[0-7]                                                                        | 6[1.5-17]                                                          | 4[2-15]               | 2[0-7]                              | * <b>0.007</b><br>** <b>0.008</b>         |
| <b>FeNO (ppb)</b>             | 21[14-28]                                                                     | 45[19-67]                                                          | 25 [20-63]            | 21[15-28]                           | * <b>&lt;0.001</b><br>** <b>0.001</b>     |

Skewed data are presented as median (interquartile ranges); Abbreviations: ACT: Asthma Control Test; FeNO: Fraction of exhaled nitric oxide; IL-13: Interleukin-13; SRA: Severe Refractory Asthma;

\*P value refers to comparisons between well controlled and not well controlled asthmatics, \*\*P value refers to comparisons between mild to moderate and SRA

\*statistically significant difference compared to well controlled asthma and \*\* statistical significance compared to mild to moderate asthma. Bold letters indicate statistical significance.

**Table 3.** Diagnostic performance characteristics of IL-13, FeNO and sputum eosinophils for the identification of well-controlled (ACT20) asthma in the whole study population as well as in the subgroups based on the underlying severity.

| All (n=170)                     | Cut-point | Sensitivity | Specificity | PPV | NPV | AUC (95% CI)      | p-value          |
|---------------------------------|-----------|-------------|-------------|-----|-----|-------------------|------------------|
| IL-13pg/ml                      | <156      | 94          | 81          | 95  | 76  | 0.92<br>0.87-0.95 | <b>&lt;0.001</b> |
| FeNO ppb                        | <43       | 87          | 55          | 89  | 50  | 0.71<br>0.64-0.78 | <b>&lt;0.001</b> |
| Sputum Eosinophils%             | <4        | 69          | 61          | 88  | 32  | 0.65<br>0.58-0.72 | <b>0.008</b>     |
| <b>Mild to moderate (n=123)</b> |           |             |             |     |     |                   |                  |
| IL-13 pg/ml                     | <117      | 88          | 67          | 97  | 32  | 0.80<br>0.72-0.86 | <b>0.008</b>     |
| FeNO ppb                        | <43       | 89          | 44          | 94  | 25  | 0.64<br>0.55-0.72 | 0.160            |
| Sputum Eosinophils%             | <3        | 63          | 55          | 94  | 11  | 0.58<br>0.5-0.67  | 0.402            |
| <b>SRA (n=47)</b>               |           |             |             |     |     |                   |                  |
| IL-13 pg/ml                     | <156      | 92          | 95          | 95  | 91  | 0.98<br>0.89-0.99 | <b>&lt;0.001</b> |
| FeNO ppb                        | <31       | 76          | 63          | 70  | 70  | 0.69<br>0.53-0.81 | <b>0.016</b>     |
| Sputum Eosinophils%             | <4        | 68          | 68          | 70  | 65  | 0.64<br>0.49-0.78 | <b>0.048</b>     |

Data for the optimum cut-points based on maximum AUCs from ROC analyses are given. Abbreviations: AUC: area under the curve; BHR: bronchial hyperresponsiveness; CI: confidence intervals; IL-13: Interleukin-13; FeNO: fraction of exhaled nitric oxide; NPV: negative predictive value; PPV: positive predictive value; Sens: sensitivity; Spec: specificity. Bold letters indicate statistical significance.

## FIGURE LEGENDS

### Figure 1

(A) IL-13 values [pg/ml] in patients with well controlled and not well controlled asthma.  $p < 0.001$  in favor of not well controlled asthma. (B) IL-13 values [pg/ml] in patients with mild to moderate asthma [MMA] and Severe Refractory Asthma [SRA].  $p < 0.001$  in favor of SRA. Individual patients have also been plotted according to the presence of high ( $\geq 3\%$  - solid symbols) or low ( $< 3\%$  - open symbols) sputum eosinophils. Bars represent median and interquartile ranges. For data see table 2.

### Figure 2

Receiver operating characteristics (ROC) curves for IL-13, FeNO and % sputum eosinophils for the identification of patients with well-controlled asthma in the whole study population. For data see table 3.

### Figure 3

3A) Receiver operating characteristics (ROC) curves for IL-13, FeNO and % sputum eosinophils for the identification of patients with well-controlled asthma in mild to moderate asthmatics. For data see table 3.

3B) Receiver operating characteristics (ROC) curves for IL-13, FeNO and % sputum eosinophils for the identification of patients with well-controlled asthma in Severe Refractory Asthma [SRA]. For data see table 3.

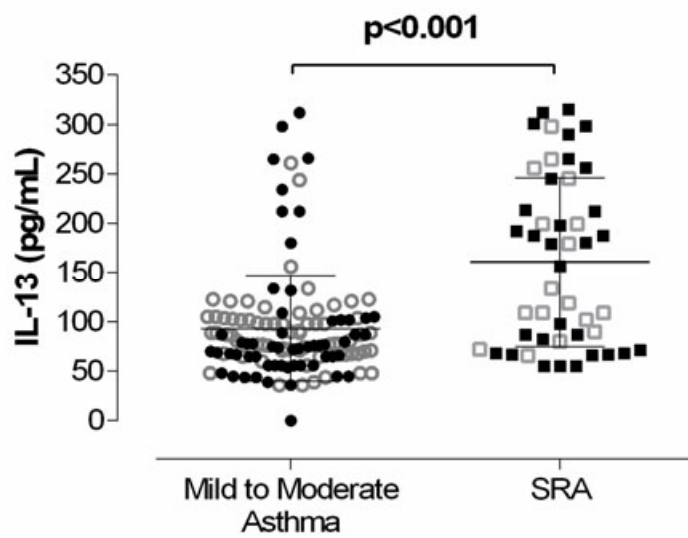
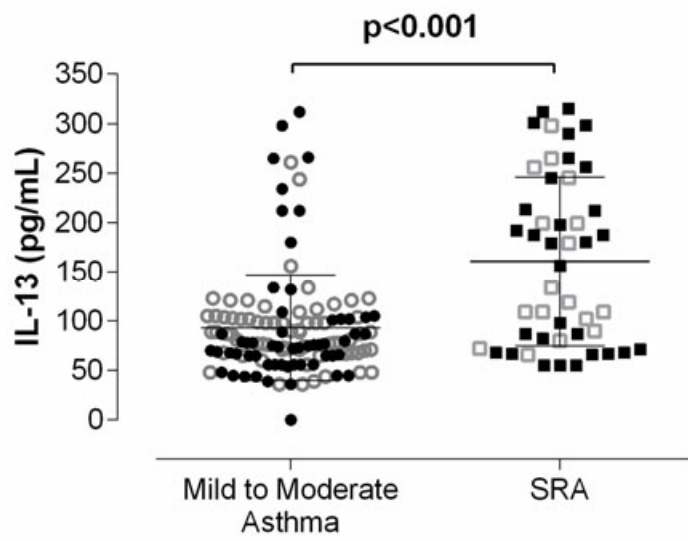


Figure 2

