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Article type : Original Article: Experimental Allergy and Immunology

Airway basophils are increased and activated in eosinophilic asthma

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Running title: Sputum basophils are increased in eosinophilic asthma

Key words: eosinophilic asthma, basophils, sputum, biomarker

This article has been accepted for publication and undergone full peer review but has not
been through the copyediting, typesetting, pagination and proofreading process, which may
lead to differences between this version and the Version of Record. Please cite this article as
doi: 10.1111/all.13197

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Abstract

Background. The impact of basophils on asthma pathogenesis remains largely unexplored, particularly in humans. Here, we evaluated the frequencies and activation status of basophils in the sputum of adult asthmatic patients and related our findings to other parameters of eosinophilic airway inflammation.

Methods. We enrolled 44 adult asthmatic patients who were being treated with inhaled corticosteroids (ICS). Analysis of the induced sputum, exhaled nitric oxide fraction (FeNO) measurement, and Asthma Control Test (ACT) were carried out together with standard blood and pulmonary function tests. The cellular composition of the sputum was examined by flow cytometry, and the phenotypes of blood and sputum basophils were compared.

Results. Basophils were increased in the sputum of asthmatic patients. The expression of CD203c on sputum basophils was significantly higher than that on blood basophils. The percentage of sputum basophils was positively correlated with those of eosinophils and mast cells; it was also correlated with that of blood eosinophils and FeNO. However, sputum basophils were not correlated with serum IgE, lung function, or the percentage of blood basophils. A receiver operating characteristics (ROC) curve showed the superiority of sputum basophils as a surrogate marker of the percentages of sputum eosinophils compared with absolute numbers of blood eosinophils and FeNO.

Conclusion. The number of activated basophils was increased in the sputum of patients with eosinophilic asthma and correlated with airway and blood eosinophils. Our observations suggest that sputum basophils may serve as a biomarker to monitor new therapeutic approaches for the treatment of eosinophilic asthma.

Introduction

Basophils are rare granulocytes accounting for less than 1% of leukocytes in the peripheral blood. Since basophils can rapidly release histamine, leukotrienes, and T helper 2 (Th2)-related-cytokines following IgE-dependent stimulation, they have been considered to be crucial effector cells in allergic diseases(1, 2, 3). Indeed, basophils were detected by immunochemistry in tissues from patients with

allergic rhinitis, various allergic skin diseases and asthma(4, 5, 6). Though the paucity of these cells has long hampered the study of their precise roles in diseases, recent studies have uncovered several aspects of basophil biology in health and disease. In mice, basophils induce IgE-mediated allergic chronic dermatitis and basophil depletion abrogates tissue eosinophil infiltration(7). In this model, basophils contribute to inflammation as initiators as well as effectors. Epithelium-derived cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which are key cytokines in the initiation and perpetuation of Th2 inflammation, activate basophils in an antigen-independent manner(8). These findings shed light on basophils' pivotal roles in the inflammations as innate immune cells. Moreover, basophil heterogeneity was reported in humans and mice(3, 9, 10). TSLP-elicited basophils display a different phenotype from classical IL-3-elicited basophils and are thought to be pathogenic in eosinophilic esophagitis and atopic dermatitis. However, the contribution of basophils to asthma pathogenesis is not well defined.

Eosinophilic inflammation is a typical feature of asthma. Airway eosinophils are related to the severity(11), rate of exacerbation(12, 13), and rapidity of lung function decline(14) in asthma patients. Though eosinophil accumulation in sputum is one of the predictors for good response to inhaled or oral corticosteroids(15, 16), some patients have severe and progressive disease in spite of standard therapy with inhaled corticosteroids (ICS). The Severe Asthma Research Program cohort and European U-BIOPRED adult severe asthma cohort revealed that the percentages of sputum eosinophils were higher in severe asthma groups compared with those in mild/moderate asthma groups despite similar levels of eosinophils in the blood(17, 18). More recently, novel biologicals targeting eosinophilic inflammation such as mAb to IL-5 succeeded in reducing the frequency of asthma exacerbation and the need for treatment with systemic glucocorticoids(19) (20). These findings indicate that airway eosinophilic inflammation, which is not well controlled by ICS, is a therapeutic target for severe refractory asthma.

We previously reported that lung basophils were increased in an allergic asthma mice model (21). Adaptive transfer of lung basophils from asthmatic mice to OVA-primed mice enhanced lung IL-4, IL-5, IL-13, and eotaxin production and promoted airway eosinophilic inflammation. Pulmonary basophils prolonged effector T cell survival and enhanced Th2 cytokine production. Other groups also reported the relationship between basophils and eosinophilic inflammation. In a papain-induced asthma model, basophils prolonged innate lymphocytes 2 survival and IL-5 production via IL-4, resulting in enhanced eosinophilic inflammation(22). In an IgE-dependent eosinophilic dermatitis model, basophil-derived IL-4 upregulates endothelial vascular cell adhesion molecule-1 (VCAM-1), allowing eosinophil recruitment to the target tissues(23). In humans, a positive correlation between the number of basophils and eosinophils in tissues was observed in eosinophilic esophagitis(24) and nasal polyposis without aspirin intolerance(25). However, basophil involvement in human eosinophilic asthma remains obscure.

To address the impact of basophils on adult asthma patients treated with ICS, we conducted a hospital-based observational study. Firstly, we examined whether basophils were increased in the sputum of asthmatic patients using flow cytometry. Secondly, to explore the roles of basophils in inflammatory sites, we tested whether airway basophils were activated compared with peripheral basophils. Thirdly, we analyzed the relationship between basophils and inflammatory cells including eosinophils in induced sputum from asthmatic patients.

Materials and methods

Subjects

Forty-four adult asthmatic patients were recruited from the outpatient clinic of Nagoya University Hospital from April 2014 to June 2015 (1st cohort). All patients had been diagnosed with asthma by pulmonologists and treated with ICS for more than 3 months. The patients were evaluated by spirometry analysis, asthma control test (ACT) score, exhaled nitric oxide fraction (FeNO) (NiOX MINO[®]; Aerocrine, Solna, Sweden), and blood tests. Exclusion criteria were current smoking,

COPD, autoimmune diseases, bacterial infection, and use of systemic corticosteroids and/or immunosuppressants in the preceding four weeks. For the extra analysis of surface markers of basophils, 14 other asthmatic patients participated (2nd cohort). The healthy controls reported no history of respiratory disease or smoking. All subjects signed informed consent forms approved by the Ethics Committee of our institution (number: 2012-0078).

Sputum collection and processing

Sputum samples were obtained using an established protocol. Briefly, the inhalation of nebulized 5% saline for 5 min was conducted up to five times. Subjects were instructed to rinse their mouths with tap water and blow their noses before sample collection to avoid contamination with saliva and postnasal drips. The samples were weighed, treated with two volumes of 0.05% dithiothreitol (DTT; Wako Pure Chemical Industries, Ltd, Osaka, Japan), and incubated for 30 min at 37°C. The suspension was then filtered through a 70µm nylon cell strainer to remove cell debris and mucus.

Blood collection and processing

Peripheral blood cells were obtained by whole blood lysing as reported previously(26). Briefly, 4 ml of whole blood was added to 20 ml of ACK lysing buffer and incubated for 5 min at room temperature and for 5 more min on ice. Then the cells were washed with PBS. This step was repeated up to three times in total until no more red blood cells were visible.

Flow cytometry analysis

After blocking with Human TruStain FcX™ (BioLegend, San Diego, CA), cells were stained with anti-CD14-Brilliant Violet 510, anti-CD16-PE/Cy7, anti-CD63-Pacific Blue, anti-CD66b-PerCP/Cy5.5, anti-CD117 (c-kit)-APC, anti-CD123 (IL-3R)-PerCP/Cy5.5, anti-CD172a (SIRPα)-PE/Cy7, anti-Siglec8-APC, and anti-TSLPR-PE/Cy7 (BioLegend, San Diego, CA), anti-CD45-APC-

H7 and anti-HLA-DR-FITC (BD Bioscience, San Jose, CA), anti-CD193 (CCR3)-PE and anti-FcεRIα (clone, AER-37) -PE (eBioscience, San Diego, CA), anti-CD203c-PE (Miltenyi Biotec, Bergisch Gladbach, Germany) and anti-ST2-FITC (MBL, Nagoya, Japan), and fixed. Cells were analyzed using FACS Canto (Becton, Dickinson and Co, Franklin Lakes, NJ) and data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR). Normalized expression of surface antigens on basophils was calculated as “positive mean fluorescent intensity (MFI)–negative MFI/2 x negative SD”.

Analysis of sputum inflammatory cells

The gating strategy for basophils and mast cells is illustrated in Fig. 1A. Eosinophils (CD45⁺ CD14⁻ CD66b⁺ CD16^{low} Siglec8⁺ CCR3⁺) and neutrophils (CD45⁺ CD14⁻ CD66b⁺ CD16^{high} Siglec8⁻ CCR3⁻) were identified by flow cytometry (Fig. S1A). The percentages of eosinophils and neutrophils were correlated with the percentages of these cells evaluated by microscopic examinations using Cytospin and Diff Quick staining (Sysmex, Kobe, Japan) (Fig. S1B).

Statistical analysis

Data were analyzed by SPSS for MS Windows, version 18.0 (SPSS, Inc, Chicago, IL). Results are given as mean values ± s.e.m. Mann-Whitney *U* test was used for two group comparisons. Wilcoxon signed-rank test was used for paired data. When more than two groups analyzed, ANOVA and Dunnett's multiple comparison test were used. Correlation was evaluated by the Spearman rank test. Differences were considered significant if *P* values were less than .05.

Results

1. Sputum basophils were increased in asthmatic patients.

We previously reported a gating strategy to identify, purify, and sort viable mucosal basophils without contamination with IgE-bearing dendritic cells(27, 28). Here we used the same gating strategy to detect basophils in the sputum of asthmatic patients. As depicted Fig. 1A, basophils identified as CD45⁺ FcεRI^{high} CD172a^{low} CD123⁺ c-kit⁻ cells comprised a homogeneous population of FSC^{low} SSC^{low} cells; they were smaller than the mast cells identified as CD45⁺ FcεRI^{high} CD172a^{low} CD123^{low} c-kit⁺.

To examine the implications of basophils in asthmatic patients, we analyzed the induced sputum from 44 asthmatic patients in the outpatient clinic of Nagoya University Hospital. The patients' characteristics are summarized in Table 1. Sixty-one percent were atopic. All patients had been on ICS but not on systemic steroids for at least two months. Sputum basophils were significantly increased in asthmatic patients compared with control subjects (Figure 1B). In addition, the percentages of eosinophils and mast cells as well as the number of CD45⁺ cells were increased in the sputum of asthmatic patients. There was no difference in the cellular composition of sputum between non-atopic patients and atopic patients (Figure 1C , Table S1). Forty-five percent of patients were eosinophilic asthma, which was defined as more than 2 % sputum eosinophils. As shown in Fig. 1C and Table S2, the numbers of sputum basophils and mast cells were significantly increased in eosinophilic asthma whereas neutrophils were reduced.

2. Airway basophils were activated.

Since immune cells are likely activated at inflammatory sites, we next compared CD203c expression on sputum and blood basophils. CD 203c is a transmembrane ectoenzyme known as ectonucleotide pyrophosphatase phosphodiesterase 3 (NPP3) that is widely used as activation marker for basophils(29). We hypothesized that airway basophils of asthmatics were more activated than circulating basophils. Indeed, as depicted Fig. 2A, CD203c expression on sputum basophils was significantly higher than

that on peripheral blood basophils. To rule out the effects of cell preparation processes using DTT or lysing buffer, peripheral whole blood cells or PBMCs were treated according to the same protocols and compared for CD203c expression. The CD203c expression on basophils was not affected by DTT or lysis buffer treatment (data not shown).

3. Comparison of the phenotypes of airway and blood basophils.

The difference in CD203c expression between local and circulating basophils prompted us to examine other activation or surface marker expressions on basophils. To address this question, we obtained induced sputum and peripheral blood samples from another group of asthmatic patients (2nd cohort, n=14) and analyzed some surface markers together with the first cohort. The patients characteristics of the second cohort are shown in Table S3. Several surface molecules were differentially expressed on blood versus sputum basophils. CD63 and IL-33R (ST2) expressions were higher on airway basophils, whereas FcεRI, IL-3R (CD123), SIRP-α (CD127a), and CD11b expressions were lower on airway than on blood basophils. TSLPR expression was similar on blood and sputum basophils (Fig.2B). The upregulation of CD63 expression and downregulation of IL-3R expression in activated basophils were previously reported(30, 31). Thus, we conclude that airway basophils were activated and displayed a different phenotype from that of circulating basophils.

4. Sputum basophils were correlated with eosinophilic inflammation.

The clinical significance of sputum basophils in asthmatic patients is illustrated in Table 2. As shown, the percentage of sputum basophils was positively correlated with those of sputum eosinophils and mast cells, with FeNO, and also with blood eosinophils (Table 2, Fig. S2). However, sputum basophils were correlated with neither ACT score, % FEV1, total IgE levels, or the percentages of blood basophils.

FeNO and blood eosinophils are considered to be good biomarkers of eosinophilic airway inflammation(32, 33) and are thought to be clinically useful to select patients for treatment with anti-IL-5 and anti-IgE. Our observations of a strong correlation between sputum basophils and sputum eosinophils prompted us to examine the utility of sputum basophils as a surrogate marker of

eosinophilic inflammation. Fig. 3 shows the receiver operating characteristics (ROC) curve for the cutoff value with sputum eosinophils $\geq 2\%$. The area under the curve (AUC) of the percentage of sputum basophils was higher than those of absolute number of blood eosinophils and FeNO. Moreover, we compared sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of those three parameters with various thresholds to diagnose eosinophilic inflammation. The percentage of the patients with eosinophilic inflammation that was defined as sputum eosinophils $\geq 2\%$ was 45%. As shown in Table 3, sputum basophils were superior to blood eosinophils and FeNO in terms of not only sensitivity, but also specificity, PPV, and NPV. These finding suggests that sputum basophils could be a good biomarker of eosinophilic inflammation.

Discussion

In this study, we showed increased numbers of sputum basophils in asthmatic patients compared with healthy control subjects. In earlier reports basophils were observed in lung tissues of patients with fatal asthma(6) as well as in the sputum or bronchoalveolar lavage fluid after allergen challenges of atopic asthmatic patients(34, 35, 36). Most recently, Gordon et al. reported that basophils were increased in the sputum of asthmatic patients and indicated that basophils and mast cells, but not innate lymphoid cells 2, are the main Th2 cytokine-producing cells in the sputum(37). In agreement with this study, we here report that basophils were increased in the sputum not only of allergic but also of non-allergic asthmatic patients treated with ICS during a non-exacerbated period. This population is a representative of the “real world patients” we usually see in the clinic. In addition, we examined the expression of some surface markers of sputum basophils and found that several of them were differentially expressed on sputum versus blood basophils. Siracusa et al. documented basophil heterogeneity in the mouse(9). IL-3- elicited basophils and TSLP- elicited basophils have different surface marker expressions and functions. IL-3- elicited basophils have higher expression of CD69, CD11b and CD62L compared with TSLP- elicited basophils and readily degranulate in response to Fc ϵ RI cross-linking. On the other hand, TSLP- elicited basophils have higher expression of CD123, IL-33R and IL-18R α and demonstrated an enhanced ability to produce cytokines and chemokines. In

humans, high IL-33R expression on basophils is observed in active inflammatory lesions of eosinophilic esophagitis and it was proposed that they were elicited by TSLP(24). On the contrary, IL-33R expression on basophils is increased if the cells are activated with IL-3(38). Thus, further studies are needed to delineate human basophil heterogeneity. In our observations, the expressions of FcεRI, IL-3R, SIRPα, and CD11b on basophils were decreased and the expression of IL-33R was increased together alongside the activation markers CD203c and CD63. We cannot conclude whether the airway basophils correspond to TSLP- elicited basophils or an activated form of circulating conventional basophils.

The percentages of basophils were positively correlated with the percentages of eosinophils in the sputum. We here showed that sputum basophils may be a marker of airway eosinophilic inflammation. The utility of blood eosinophils, FeNO, and serum periostin as surrogate markers of sputum eosinophils in asthma has been reported(32, 33). Among them, blood eosinophils are considered to be the best biomarker. Although we did not examine serum periostin, our data corresponds to the above observations. The AUC of blood eosinophils and FeNO for the diagnosis of eosinophilic inflammation were 0.765 and 0.709, respectively. However, the AUC of sputum basophils was 0.896. In addition, the specificity, PPV and NPV of sputum basophils were much higher than those of blood eosinophils and FeNO. Moreover, in terms of airway eosinophilic inflammation, sputum basophils may have an advantage over blood eosinophils as a surrogate marker. Indeed, according to previous studies, the number of blood eosinophils is similar in severe and mild asthma, whereas sputum eosinophil levels are different between the two groups(17, 18).

The strong relationship between sputum basophils and sputum eosinophils may suggest basophils as potential therapeutic targets. Indeed, omalizumab, a humanized monoclonal anti-IgE antibody known to reduce the frequency of acute exacerbations, utilization of ICS, and improve the quality of life(39), also reduces the number of circulating basophils as well as their FcεRI expression(40, 41). In addition, the effect of Omalizumab on FcεRI expression on basophils together with improvement of pulmonary function was observed even in non-atopic severe asthmatic patients(42). Our finding that

the increased airway basophils in non-atopic as well as in atopic asthma, may give us an explanation why omalizumab have some effect on severe asthma regardless of IgE levels. In animal models, basophil-derived IL-4 was involved with chronic allergic inflammations. It prolonged the survival of lung effector memory T cells and enhanced Th2 cytokine production(21). It also enhanced expression of CCL11, IL-5, IL-9, and IL-13 in ILC2(22). These Th2 cytokines and chemokines may attract eosinophils into the airway lumen and prolong survival of basophils/eosinophils. In addition, the upregulation of VCAM-1 via IL-4 produced by basophils promotes eosinophil recruitment to the target tissues(23). Collectively, these observations suggest that basophils may trigger and amplify eosinophilic inflammation. Moreover, in a recent study, the expression of IL-33R (ST2) in sputum cells from asthmatic patients was shown to be tightly correlated with expression of type-2 cytokines as well as mast cell and basophil protease genes. In addition, the alternative splicing of the IL-33 transcript, characterized by the deletion of exons 3 and 4, is detected in airway epithelial cells from stable asthmatic patients and correlated with the expression of Th2-related genes, mainly in basophils(37). These data indicate that tissue basophils may be activated via the IL-33/ST2 axis and aggravate Th2 inflammations. From these viewpoints, novel treatments such as a CRTH2 antagonist(43, 44) and anti IL-5R α (45) as well as biologics targeting epithelium-derived cytokines could be promising treatments for eosinophilic refractory asthma.

One limitation of this study was that the participants were only stable asthmatic patients who had not had exacerbations for at least two months. In this regard, we cannot conclude that airway basophils are crucial cells in severe refractory eosinophilic asthma. In addition, this may be the reason why the percentages of sputum basophils were not correlated with any clinical parameter. Thus, further studies are needed to elucidate the roles of basophils in severe asthma.

In summary, we identified basophils in the sputum of asthmatic patients who were treated with ICS but had eosinophilic inflammation. In these patients, airway basophils were activated and the percentage of sputum basophils was strongly correlated with sputum and blood eosinophils. Our observations suggest that basophils could be a good biomarker to monitor and perhaps also to treat patients with eosinophilic asthma.

Acknowledgments

We thank Mr. M. Tanaka for technical assistance to flow cytometry analysis, Dr. G. Delespesse for a critical reading of the manuscript and Ms. Katherine Ono for her language assistance. This work was supported by KAKEN (grant number: 15K09549) .

Author contributions

Y.S. and K.W. designed the experiments; Y.S., K.W., T.N. and S.I. collected clinical samples and performed the experiments; Y.S., K.W. and Y.H. analyzed the data and wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Legend to Figures

Figure 1: Basophils were observed in the sputum of asthmatic patients but not healthy subjects.

The induced sputum cells were analyzed by flow cytometry. (A) The gating strategy of sputum basophils in asthmatic patients and healthy subjects. (B) Inflammatory cells of the sputum in healthy subjects (n=7) and asthmatic patients (n=44). (C) The percentages of sputum basophils in non-atopic patients (n=17) and atopic patients (n=26). The percentages of sputum basophils in non-eosinophilic asthma (n=23) and eosinophilic asthma (n= 19). Eosinophilic asthma was defined as more than 2% sputum eosinophils. Data are expressed as mean and error bars indicate SEMs. *P<0.05 and **P<0.01 as determined by Mann-Whitney *U* test.

Figure 2: Basophils were activated and expressed different levels of surface markers in the airway.

The various surface markers expression on basophils in the blood and the sputum were examined. (A) The histogram shows CD203c expression levels on blood and sputum basophils. The CD203c expression was higher on sputum basophils than on blood basophils (n=31). (B) The expression levels of CD63 (n=11), ST2 (n=6), FcεRI (n=33), CD123 (n=33), CD172a (n=33), CD11b (n=11) and TSLPR (n=11) on basophils of blood and sputum. Data are expressed as standardized MFI (positive MFI-negative MFI/2 x negative SD), and error bars indicate SEMs. *P<0.05 and **P<0.01 as determined by Wilcoxon signed-rank test.

Figure 3: Receiver operating characteristics curve (ROC) analyses of the sensitivity and the specificity of the percentage of sputum basophils, the absolute number of blood eosinophils and FeNO for the diagnosis of eosinophilic inflammation (Sputum Eosinophils $\geq 2\%$) (1st cohort).

Sputum Baso, the percentage of sputum basophils; Blood Eos, the absolute number of blood eosinophils; AUC, area under the curve.

Supplementary Figures

Figure S1: Eosinophils and Neutrophils in induced sputum.

(A) The gating strategy of sputum eosinophils and neutrophils. (B) The percentages of eosinophils and neutrophils evaluated by flow cytometry were correlated with the percentages of those cells evaluated by microscopic examinations using Cytospin and Diff Quick staining.

Figure S2: Relationship between sputum basophils and sputum eosinophils, sputum mast cells, FeNO, or blood eosinophils.

The patients of 1st cohort were divided into four groups according to the percentages of sputum basophils: 0 (n=11), 0.01-<0.05 (n=15), 0.05-<0.1 (n=5), and 0.1 $\leq$ (n=13). Data are expressed as mean and error bars indicate SEMs. *P<0.05 and **P<0.01 as determined by Dunnett's multiple comparison test.

Table 1. Study populations (1st cohort)

Characteristics			
Subjects	44	(100%)	
Age (years)	61.5	± 15.3	
Male	18	(40.9%)	
BMI (kg/m ²)	25.3	± 5.1	
Ex-smokers	14	(31.8%)	
Pack-years	3.8	± 10.5	
Exacerbation within one year	10	(22.7%)	
ACT score	23.4	± 2.3	
FeNO (ppb)	37.8	± 22.5	
Atopy (positive IgE RAST)	27	(61.4%)	
Total serum IgE (IU/mL)	560.9	± 710.7	
Blood Basophils (%)	0.87	± 0.48	
Blood Basophils (/mm ³)	45.7	± 26.6	
Blood Eosinophils (%)	5.5	± 4.7	
Blood Eosinophils (/mm ³)	334.8	± 280.0	
Blood Neutrophils (%)	57.5	± 8.17	
Blood Neutrophils (/mm ³)	3641.4	± 1033.0	
FEV1 predicted %	83.6	± 19.0	
FEV1/FVC %	69.5	± 14.5	
Treatment characteristics			
ICS	44	(100%)	
LABA	40	(90.9%)	
LTRA	12	(27.3%)	
Theophylline	2	(4.5%)	
GINA Step 1*	0	(0%)	
GINA Step 2*	3	(6.8%)	
GINA Step 3*	25	(56.8%)	
GINA Step 4*	16	(36.4%)	
GINA Step 5*	0	(0%)	

Data presented as n (%) or mean ± SD unless otherwise noted.

BMI, Body mass index; FeNO, Fractional exhaled nitricoxide; ACT, Asthma Control Test; ICS, inhaled corticosteroids; LABA, long-acting beta 2-agonist; LTRA, Leukotrien receptor antagonists.

*Treatment characteristics based on 2015 GINA (Global Initiative for Asthma) guideline.

Atopy definition has at least one of the IgE RAST. (cat, dog, *Candida*, *Aspergillus*, *Alternaria*, moth, mite, house dust)

Table 2. Relationship between sputum basophils percentages with various clinical data in this study by Spearman correlation (1st cohort)

	r =	p =	n =
Age	-0.024	0.879	44
BMI	0.114	0.463	44
ACT score	-0.087	0.572	44
FeNO (ppb)	0.449	<0.05	44
FEV1%	0.025	0.871	44
%FEV1	-0.011	0.945	44
Total serum IgE (IU/mL)	0.088	0.571	44
Blood Eosinophils (%)	0.489	<0.05	44
Blood Eosinophils (/mm ³)	0.199	0.194	44
Blood Neutrophils (%)	0.124	0.424	44
Blood Neutrophils (/mm ³)	0.063	0.685	44
Blood Basophils (%)	0.203	0.186	44
Blood Basophils (/mm ³)	0.235	0.124	44
CD203c expression on blood Basophils	0.096	0.536	44
FcεRI expression on blood Basophils	0.165	0.286	44
Sputum Mast cells (%)	0.631	<0.001	44
Sputum Eosinophils (%)	0.765	<0.001	42
Sputum Neutrophils (%)	-0.386	<0.05	42

Spearman product-moment correlation coefficients for each data compared to sputum basophil percentage

Table 3. Sensitivity, specificity, PPV and NPV of different surrogate markers using alternative cut-points to diagnose eosinophilic airway inflammation (less than, more than or equal to 2% sputum eosinophils) (1st cohort)

	Threshold	Sensitivity	Specificity	PPV	NPV
Sputum Baso (%)	≥ 0.05%	78.9	87	83.4	83.3
Sputum Baso (%)	≥ 0.10%	63.2	95.7	92.4	75.9
Blood Eos (/mm ³)	≥ 250	78.9	69.2	68.2	80
Blood Eos (/mm ³)	≥ 350	47.4	65.2	52.9	60
Blood Eos (/mm ³)	≥ 450	26.3	91.3	71.4	60
FeNO (ppb)	≥ 30 ppb	68.4	52.2	54.2	66.7
FeNO (ppb)	≥ 40 ppb	57.9	73.9	64.7	68
FeNO (ppb)	≥ 50 ppb	42.1	91.3	80	65.6

NPV, negative predictive value; PPV, positive predictive value

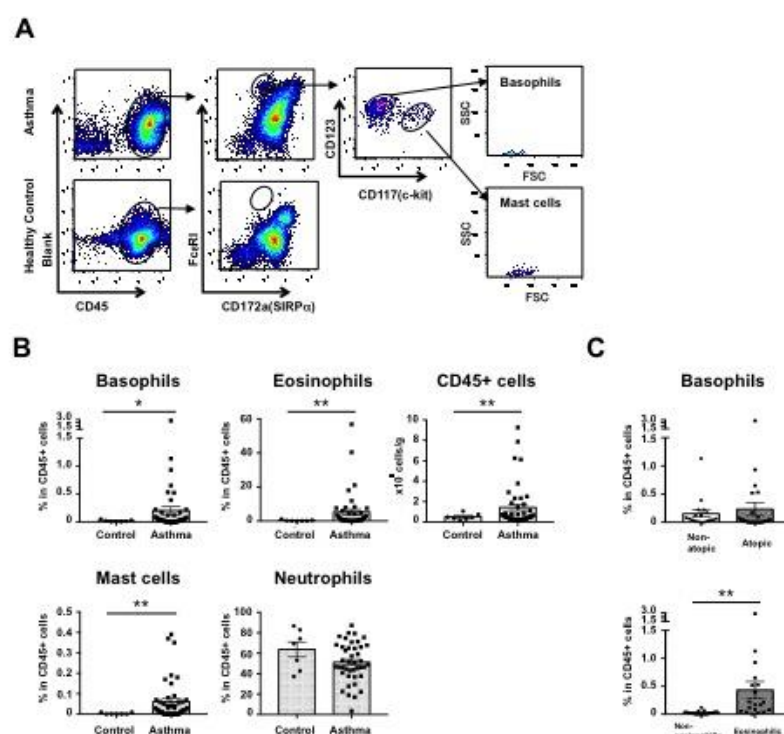


Figure 1

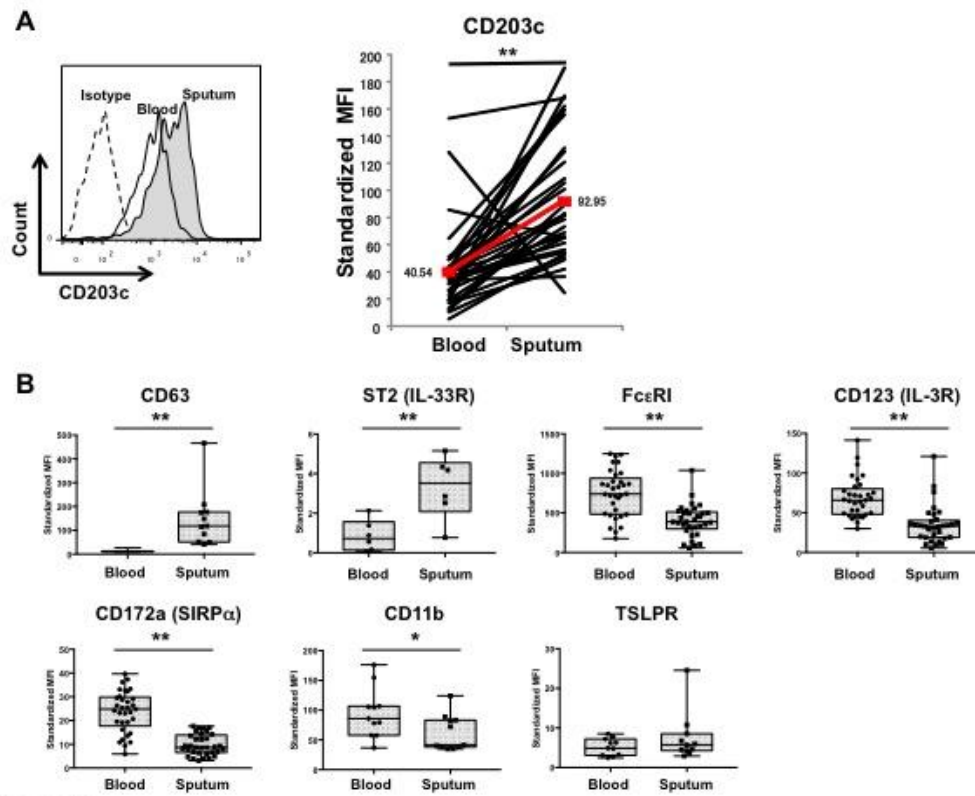


Figure 2

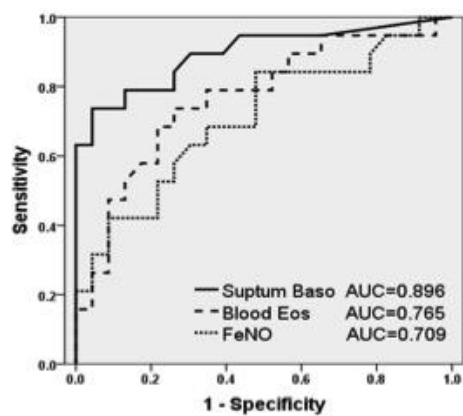


Figure 3