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Detection of immunological biomarkers correlated with asthma control and quality of life measurements in sera from chronic asthmatic patients

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Keywords

Asthma control; multiplex assay; cytokines; growth factors

Introduction

Asthma is a chronic disease with a prevalence of 7% in US adults (1). It is a multifactorial disease in which patients exhibit various degrees of asthma control and responses to therapy. Management of asthma involves the use of β_2 -agonists, often in combination with corticosteroids for patients with persistent symptoms. However, 10-25% of asthmatics are resistant to steroid treatment (2). Th2 cells are the predominant inflammatory cell type in asthmatic airways biopsy and bronchoalveolar lavage (BAL) and studies found that these cells exhibit higher levels of IL-3, IL-4, IL-5 and IL-13 mRNA than cells from healthy controls. Reduced levels of the Th1 cytokines IFN- α 2 and IFN- γ in PBMCs and BAL are associated with asthma exacerbations (3-5). However, as the disease becomes more severe

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the T cell population becomes a mixed Th2/ Th1 phenotype (6;7). Potential serum immunological biomarkers of asthma control have yet not been identified.

Previous studies evaluated cytokine profiles in asthma patient bronchoalveolar lavage fluid (8) or induced sputum (9). Bronchoalveolar lavage is an invasive and time consuming technique, and induced sputum measurements can lack reproducibility. Few studies have reported serum cytokine levels. Huang et al. (10) found no statistically significant differences between serum IL-5 levels in asthmatic children with acute exacerbations and stable condition. However IL-5 levels were higher than normal controls. Joseph et al. (11) found elevated serum IL-5 levels in mild and moderate persistent asthmatics which were not affected by corticosteroid use. Since persistent asthma involves several factors including airway inflammation and airway remodeling, the overall immunological profile tends to be a complex amalgam of cytokines, chemokines and growth factors (12). A more sophisticated approach such as the multiplex assay that allows detection of multiple analytes in a single well of a 96-well plate might be a suitable approach.

This study is the first attempt to determine protein profiles of cytokines, chemokines, growth factors, adhesion molecules and cytokine receptors in asthmatic patients' serum using the multiplex assay in which 50 different analytes were analyzed simultaneously, correlated with measures of asthma control and quality of life.

Methods

Patient Selection

This study used samples from a subgroup of patients enrolled in a prospective cohort study of inner-city asthmatics. Patients were enrolled over a 19 month period in the general internal medicine clinic of Mount Sinai Hospital, located in East Harlem, NY. Additional details of the design of the parent study are reported elsewhere (13-17). This study was limited to non smoking asthmatics 18 to 55 years of age, with mild persistent, moderate persistent or severe disease (18). Non-asthmatic controls (NAC) were also recruited at Mount Sinai Hospital. Controls were non-smokers greater than 18 years of age with a history and spirometry test to rule out physician diagnosis of asthma.

As part of the interview of patients with asthma, we collected data on sociodemographic characteristics, asthma history, self-management behaviors, and asthma treatment. Asthma control was assessed using the Asthma Control Questionnaire (ACQ) (19), a validated instrument that includes information about wheezing, shortness of breath, awakening during the night, limitation of daily activities, severity of morning symptoms, and bronchodilator use. All questions were scored on a 7-point Likert scale with higher scores indicating worse asthma control. This instrument has been shown to have good responsiveness, reliability, and is one of the most commonly used methods of evaluating asthma control (20). Additional measures of health care services used at baseline included frequency of emergency department (ED) visits and hospitalizations for asthma during the previous year.

Asthma-related quality of life was assessed with the Mini-Asthma Quality of Life Questionnaire (AQLQ) (21). The Mini-AQLQ is a 15-item questionnaire that assesses

respondents' asthma-related quality of life in four domains: symptoms, emotional function, environmental stimuli, and activity limitation. Responses for these items were recorded on a seven point Likert scale (1 = all of the time to 7 = none of the time). The validity and reliability of the instrument has been demonstrated and this is one of the most frequently used instruments to measure asthma related quality of life (22).

From the study cohort, we randomly selected 14 samples from asthmatics classified as poorly controlled (PC), and 14 samples from patients classified as well controlled (WC) (13-17). For the purpose of this study, PC asthma was defined as an ACQ score >4 and Mini-AQLQ score ≤ 2.5 and/or history of ≥ 1 ED visit or hospitalization for asthma during the prior year. Patients with ACQ scores between 1- 2.5 were categorized as WC asthmatics. Additionally, sera of 14 NACs were analyzed. The study protocol was approved by the Mount Sinai School of Medicine Institutional Review Board, and all patients gave informed consent.

Multiplex assay for detection of cytokines, chemokines, growth factors and adhesion molecules

Serum protein levels of cytokines, chemokines, growth factors and adhesion molecules were evaluated using 23 and 27-Plex panels of Bio-Plex human cytokine assays (Bio-Rad laboratories, CA, USA) according to the manufacturer's instructions. Briefly, 50 μ l per well of capture antibody coated beads were added to a 96 well filter plate. The plate was washed between steps with a vacuum manifold and 50 μ l per well of serum samples at a dilution of 1:4 or standards were incubated with the beads for 30 mins at room temperature with constant shaking at 300rpm. Thereafter the plate was washed as in the earlier step and 25 μ l per well of detection antibody was added and incubated with shaking for 30 minutes at room temperature. After washing, streptavidin-phycoerythrin was added and incubated for 10 minutes at room temperature with shaking. The plate was washed and 125 μ l of Bio-Plex assay buffer was added. After rapid shaking for 30 seconds the plate was read using the Bio-Plex 200 Suspension Array System (Bio-Rad). Concentration of various analytes was calculated using the Bio-Plex Manager software version 4.1 (Bio-Rad). Low PMT settings were used for 23-plex and high PMT settings were used for 27-plex. Detectable limits of analytes were variable and in the range of 0.1-7pg/mL.

Statistical Analysis—Data were analyzed by using the SigmaStat statistical software package (SPSS Inc, Chicago, Ill). As the data distribution was not normal, the three groups were compared for each analyte using the Kruskal-Wallis test followed by pairwise comparisons between groups using Student-Newman-Keuls correction. The Chi-square and t-test were used for demographic data analysis. The relationship between the expression of different analytes and asthma control score and quality of life measurements were assessed with the Spearman's Rank correlation coefficient using SAS statistical software (SAS, Cary, NC).

Results

Patient characteristics

Baseline characteristics of the study cohort are described in Table 1. The mean age of the patients in three groups was not statistically different. All groups had a high percentage of females, PC (93%), WC (79%) and NAC (71%); however there was no significant difference between groups. High percentage of women asthmatics in the study is consistent with the epidemiology of urban asthma population. The study population was predominantly Hispanic, with PC (57%), WC (57%) and NAC (50%). Although the number of patients in this study is small to investigate any statistical correlations regarding ethnicity, the parent study showed a high percentage of asthmatics to be low-income Hispanics and African Americans which is consistent with epidemiology of inner-city asthmatics. There were no Whites in the PC group and no Blacks in the NAC group. ICS treatment in PC patients was 79%, in WC patients was 86%, and other patients were on β 2-agonists. ACQ score ($p < 0.001$) and AQLQ score ($p < 0.001$) were significantly different among PC and WC groups.

Cytokine levels in PC, WC and NAC sera and correlations with ACQ and AQLQ scores

Eighteen of 29 cytokines tested were detectable. The levels of 15 cytokines differed significantly between groups. Among these, IL-1ra, IL-2, IL-6, IL-7, IL-9, IL-17, GM-CSF, MIF, Trail and TNF- α levels were significantly higher in sera from both PC and WC than NAC (Table 2). IFN- γ and IFN- α 2 levels were significantly lower in PC and WC sera than NAC sera (Table 2, $p < 0.05$). However there were no significant differences between these cytokine levels in the PC and WC groups. IL-3 and IL-18 serum levels were significantly higher only in the PC group ($p < 0.05$), and were significantly higher than in WC ($p < 0.05$ Table 2 and Figure 1 A and B). Only IL-18 was positively correlated with the ACQ scores ($r = 0.530$; $p = 0.003$; Figure 1C) and negatively correlated with AQLQ ($r = -0.514$; $p = 0.005$; Figure 1D).

Chemokine levels in PC, WC and NAC and correlations with ACQ and AQLQ scores

Of the 12 chemokines tested (Table 3), IL-8, MCP-1, MIP-1 α , MIP-1 β and MCP-3 were significantly elevated in both PC and WC sera vs. NAC. IL-8 and MCP-1 levels were not detectable in NAC sera. CTACK levels were significantly higher in PC sera than in NAC. However there were no significant differences in IL-8, MCP-1, MIP-1 α , MIP-1 β and MCP-3 and CTACK between PC and WC groups. RANTES levels in all groups were above the highest accurately measurable level (>2330 pg/mL). There was no significant correlation between IL-8, MCP-1, MIP-1 α , MIP-1 β , MCP-3 and CTACK levels and ACQ or AQLQ scores.

Growth factor levels in PC, WC and NAC sera, and correlation with ACQ and AQLQ scores

Five growth factors were tested. PDGF-BB and VEGF were significantly higher in both PC and WC sera than NAC sera ($p < 0.05$, Table 4), but PC and WC levels did not differ. Three growth factors- FGF-basic, Hepatocyte Growth Factor (HGF) and Stem Cell Growth Factor- β (SCGF- β) were significantly higher in PC than WC and NAC groups ($p < 0.05$, Fig 3A). FGF-basic ($r = 0.602$, $p = 0.0007$), HGF ($r = 0.445$, $p = 0.017$) and SCGF- β ($r =$

0.522, $p = 0.004$) levels showed a positive correlation with ACQ scores (Fig. 3B, D and F). Similarly, FGF-basic ($r = -0.765$, $p < 0.0001$), HGF ($r = -0.538$, $p = 0.003$) and SCGF- β ($r = -0.577$, $p = 0.001$) showed a negative correlation with AQLQ scores (Fig. 3C, E and G).

Adhesion molecules and IL-2ra receptor levels in PC, WC and NAC sera

No significant differences were found in levels of the two adhesion molecules VCAM-1, and ICAM-1 and IL-2ra receptor between groups (Table 5).

Discussion

Asthma is a heterogeneous disease, and it is well known that reduction of asthma symptoms in response to specific pharmacological interventions, such as corticosteroids, varies widely between individuals. Although the National Asthma Education and Prevention Program (NAEPP) guideline-based asthma controller therapy has been in place for 20 years, clinical outcomes remain suboptimal. Nearly 1.8 million asthma patients were treated in emergency departments in 2005 (23) and asthma accounts for 10.1 million missed work days annually (24). There are several different factors that may influence asthma control including inability to avoid indoor-outdoor allergens, smoking, viral infections, genetic makeup, treatment resistance, poor treatment adherence, and under treatment. All these factors individually or in combination can influence the patients' serum immunological profile. Certain underlying defects in the innate immune system of the healthy individuals could make them susceptible to developing an asthmatic phenotype. Studies have shown impaired induction of virus-induced interferon secretion in asthmatics which could be correlated with severity of virus-induced asthma exacerbations (25). Our observation in the current study is in agreement with this study. In the current study we observed a statistically significant decrease in IFN- $\alpha 2$ and IFN- γ levels in asthmatics as compared to non asthmatic controls. Several previous studies investigated possible correlations between IL-5 and other cytokines and asthma control status but found no association between asthma exacerbation and IL-5 levels (10). In our study IL-4 and IL-5 were not detectable and IL-13 although detectable was not different among the 3 groups. The Th1/Th2 imbalance at the cytokine level leads to airway tissue damage prompting the immune system to respond with a repair process, airway remodeling- a hallmark feature of asthma, which involves several other cytokines and growth factors (26). Due to the ensuing complexity of the disease the mechanisms underlying asthma symptom control outcomes are not fully elucidated. Our study is the first to use multiplex technique to determine an array of cytokines, chemokines, growth factors, adhesion molecules and receptors in sera from patients with poorly and well controlled asthma and compare the results to non asthmatic controls.

One potentially important finding is that IL-3 and IL-18 levels were significantly higher in the PC group than in the WC group. IL-3, a cytokine secreted by lymphocytes and basophils, plays an important role in the maturation and priming of basophils, a pathway associated with asthma exacerbations (27;28). IL-18 levels also showed a positive correlation with ACQ scores, and a negative correlation with AQLQ scores in our study. IL-18 is released from activated monocytes, macrophages, airway epithelial cells and has been described as an interferon-gamma-inducing factor (29;30). Therefore IL-18 might be

expected to suppress Th2 cytokine secretions. However, Kodama et al. demonstrated that IL-18 deficiency selectively enhances allergen-induced eosinophilia in mice (31). Other studies using murine models reported contradictory data. Hoshino et al. (32) demonstrated that *in vivo* administration of IL-18 induced IgE production through Th2 cytokine induction and up-regulation of CD40 ligand expression on CD4+ T cells. These controversial findings may be due to the presence or absence of IFN- γ as previously reported (33). IL-18 in the absence of Th1 cytokines skews the response to Th2 type (34-36). Our study found that higher levels of IL-18 in serum from PC was also associated with decreased IFN- γ and elevated IL-3, levels. This combined cytokine profile may prove to be an important marker of PC, however, further investigation is required.

In this study, three growth factors, FGF-basic, HGF and SCGF- β were significantly higher in PC than WC and NAC sera and showed a positive correlation with poor asthma control and poor quality of life scores. FGF-basic is a pleiotropic polypeptide involved in angiogenesis, tissue repair and inflammatory processes and is produced by fibroblasts, endothelial cells, macrophages, T-lymphocytes and mast cells (37-41). Increased levels of FGF-basic have been found in BAL fluids and airway tissue of subjects with bronchial asthma (38;42), however, there are no reports of a correlation between plasma FGF-basic levels and asthma. HGF is a pleiotropic biological component that plays a vital role in angiogenesis, cell-proliferation, anti-apoptosis and anti-fibrosis. It is known to play a role in oncogenesis and cancer metastasis (43). The role of HGF in asthma is controversial. Administration of exogenous HGF in a murine asthma model significantly diminished AHR and inflammatory cytokine levels (44). Nomura et al. (45) found higher levels of HGF in induced sputum of asthmatic patients than normal controls. However HGF serum levels in well and poorly controlled asthma have not been previously reported. SCGF is a cytokine of the C-type lectin family which supports hematopoietic stem/progenitor cell proliferation. Expression of SCGF- β mRNA is restricted to myeloid cells and fibroblasts. SCGF lacks colony-stimulating activity, but has burst-promoting activity for human bone marrow erythroid progenitors, granulocyte/macrophage-promoting activity for GM progenitors and colony forming unit –GM –sustaining or recruiting effect during short-term liquid BM cell culture (46). There have been no previous reports of a role for SCGF- β in asthma. A recent study indicates that pathological mechanisms of asthma involve more than just Th1/Th2 inflammation (47). The role of growth factors in asthma pathological mechanisms has not been extensively studied, but might be associated with airway remodeling and inflammatory cell survival (48-50). Airway remodeling involves thickening of the airways due to increase in airway smooth muscles, myofibroblasts, collagen and angiogenesis. Various cells such as mast cells, eosinophils, macrophages and epithelial cells involved in airway inflammation secrete growth factors which are important for the proliferation of myofibroblasts that further secrete extracellular matrix components. The role of transforming growth factor β (TGF- β), PDGF, insulin like growth factor (IGF)-I, FGF-basic and VEGF have been demonstrated in airway thickening and angiogenesis (38;45;48;49). Hoshino et al. also demonstrated that subepithelial layer thickening plays a role in promoting bronchial responsiveness (48). We did not test (TGF- β) and (IGF)-I but among the growth factors assessed PDGF and VEGF were not significantly different in PC and WC however, FGF-basic, HGF and SCGF- β were important growth factors that were statistically higher in PC

as compared to WC. The detection of statistically significant higher levels of growth factors and absence of proinflammatory cytokines in the serum of PC asthmatics suggests that the current therapy may control inflammation however is inadequate in suppressing airway remodeling. To generate biomarkers, the subjects were selected such that there was sharp contrast between poor controlled and well controlled patients so as to avoid overlap between two groups. The bioplex technology is relatively new and expensive. To maintain the cost-effectiveness we used samples from well defined groups which limited the number of qualified patients from the parent study. Due to the small number of patients, the homogeneity in ethnicity is compromised and PC group has no whites and NAC group has no blacks, this factor should be considered while interpreting the results of this study.

In conclusion, this study is the first attempt to use broad array of immunological and growth factor measures to identify possible candidates as markers of asthma control status. The use of sera and multiplex assay deserves additional application. IL-18, FGF-basic, HGF and SCGF- β levels were significantly elevated in the poorly controlled group in our study. Our study has provided important candidates for future studies which may include comparison of BALF and sputum samples. This finding may provide a rationale for further identification of serum biomarkers associated with asthma control status.

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Figure 1. Serum cytokine Levels of IL-3, IL-18, and correlation of IL-18 with asthma control and quality of life

Serum IL-3 and IL-18, levels were compared in poorly controlled (PC), well controlled (WC) and non-asthmatic controls (NAC) by multiplex assay (A-B). A One Way Anova on ranks with Student-Newman-Keuls correction was used to calculate statistical significance between the three groups and a $p < 0.05$ was considered statistically significant. Spearman's Rank correlation method was used to calculate the correlation coefficient 'r' value. A positive 'r' value shows increase in cytokine level correlated with increase in ACQ score (poor control of asthma) and a negative value shows increase in cytokine level correlated with decrease in AQLQ score (poor quality of life). A $p < 0.05$ value was considered significant. C) Correlation between serum IL-18 levels vs. ACQ score. D) Correlation between IL-18 levels and AQLQ score. ● - PC and ○- WC.

Figure 2. Comparison of serum growth factors FGF-basic, HGF and SCGF- β levels in PC, WC and NAC groups and their correlation with ACQ and AQLQ score

A) Serum growth factors FGF-basic, HGF and SCGF- β levels were compared in poorly controlled (PC), well controlled (WC) and non-asthmatic controls (NAC) by multiplex assay. A One Way Anova with Student-Newman-Keuls correction was used to calculate statistical significance between the three groups and a $p < 0.05$ was considered statistically significant. Spearman's Rank correlation method was used to calculate the correlation coefficient 'r' value. A $p < 0.05$ value was considered significant. B) Correlation between FGF-basic values and ACQ score. C) Correlation between FGF-basic value and AQLQ

score. D) Correlation between HGF value and ACQ score. E) Correlation between HGF value and AQLQ score. F) Correlation between SCGF- β value and ACQ score. G) Correlation between SCGF- β and AQLQ score. ● - PC and ○- WC.

Table 1

Baseline characteristics of study subjects

Characteristics	PC	WC	NACs	P value
<u>Demographics</u>	<u>14</u>	<u>14</u>	<u>14</u>	
Number of subjects	37 ± 5	43 ± 9	42 ± 15	0.28
Age, years (mean ± SD)				
Female, N (%)	13 (93)	11 (79)	10 (71)	0.33
Race/ ethnicity, N (%)				0.69
White	0	2 (14)	4 (29)	
Black	5 (36)	3 (21)	0	
Hispanic	8 (57)	8 (57)	7 (50)	
Others	1 (7)	1 (7)	3 (21)	
<u>Asthma history</u>				
Age of onset, years (mean ± SD)	<u>14 ± 12</u>	<u>20 ± 16</u>	<u>NA</u>	<u>0.31</u>
Duration of asthma (mean ± SD)	<u>23 ± 10</u>	<u>23 ± 15</u>	<u>NA</u>	<u>0.89</u>
PEF predicted, (mean ± SD)	<u>294 ± 160</u>	<u>371 ± 61</u>	<u>NA</u>	<u>0.1</u>
ICS treatment, N (%)	11 (79)	12 (86)	NA	0.62
Outpatient visits in past year (%)	<u>12 (86)</u>	<u>9 (64)</u>	<u>NA</u>	<u>0.19</u>
ED visits in the past year (%)	<u>14 (100)</u>	<u>0</u>	<u>NA</u>	<u>.000</u>
Hospitalized in the past year (%)	<u>10 (71)</u>	<u>9 (64)</u>	<u>NA</u>	<u>0.69</u>
Ever intubated (%)	<u>11 (79)</u>	<u>10 (71)</u>	<u>NA</u>	<u>0.63</u>
Required steroids in the past year (%)	<u>2 (14)</u>	<u>0</u>	<u>NA</u>	<u>0.14</u>
ACQ scores	4.86	1.77	NA	<0.001
AQLQ scores	2.75	4.8	NA	<0.001
<u>Indoor allergen sensitization (%) *</u>				
<i>Dermatophagoides pteronyssinus</i> - dust mite, N (%)	<u>4 (36)</u>	<u>5 (36)</u>	<u>NA</u>	<u>0.97</u>
<i>Dermatophagoides farinae</i> - dust mite, N (%)	<u>3 (27)</u>	<u>5 (36)</u>	<u>NA</u>	<u>0.65</u>
Cat, N (%)	<u>5 (46)</u>	<u>4 (29)</u>	<u>NA</u>	<u>0.38</u>
Mouse, N (%)	<u>3 (27)</u>	<u>2 (14)</u>	<u>NA</u>	<u>0.42</u>
<i>Blattella germanica</i> - cockroach, N (%)	<u>7 (64)</u>	<u>10 (71)</u>	<u>NA</u>	<u>0.68</u>
<i>Periplaneta americana</i> - cockroach, N (%)	<u>2 (18)</u>	<u>3 (21)</u>	<u>NA</u>	<u>0.84</u>
<i>Aspergillus fumigatus</i> - mold, N (%)	<u>3 (27)</u>	<u>1 (7)</u>	<u>NA</u>	<u>0.17</u>
<i>Alternaria alternata</i> - mold, N (%)	<u>2 (18)</u>	<u>4 (29)</u>	<u>NA</u>	<u>0.55</u>
Sensitized to at least 1 allergen, N (%)	<u>9 (82)</u>	<u>10 (71)</u>	<u>NA</u>	<u>0.55</u>

PC = Poorly Controlled, WC = Well Controlled, NAC = Non-Asthmatic Controls, ICS = Inhaled Corticosteroids, ACQ = Asthma Control Questionnaire and AQLQ = Asthma Quality of Life Questionnaire.

* Patients were classified as sensitized if serum IgE titers were > 0.35kU/L.

Table 2

Cytokine Levels Among Poorly Controlled, Well Controlled and Non- Asthmatic Control groups

Cytokines	PC Median (IQR)	WC Median (IQR)	NAC Median (IQR)	P value		
				PC vs NAC	WC vs NAC	PC vs WC
IL-1α	ND	ND	ND	–	–	–
IL-1β	ND	ND	ND	–	–	–
IL-1ra	190 (128-382)	207 (149-281)	109 (102-144)	p < 0.05	p < 0.05	NS
IL-2	7 (6-15)	9 (6-11)	0	p < 0.05	p < 0.05	NS
IL-3	235 (203-311)	182 (149-222)	156 (142-182)	p < 0.05	NS	p < 0.05
IL-4	ND	ND	ND	–	–	–
IL-5	ND	ND	ND	–	–	–
IL-6	8 (6-10)	8 (5-10)	2 (2-3)	p < 0.05	p < 0.05	NS
IL-7	13 (10-16)	16 (11-19)	3 (2-4)	p < 0.05	p < 0.05	NS
IL-9	26 (12-69)	27 (14-58)	6 (4-11)	p < 0.05	p < 0.05	NS
IL-10	ND	ND	ND	–	–	–
IL-12 (p40)	ND	ND	ND	–	–	–
IL-12 (p70)	ND	ND	ND	–	–	–
IL-13	7 (6-7)	8 (5-15)	7 (5-13)	NS	NS	NS
IL-15	ND	ND	ND	–	–	–
IL-16	390 (319-434)	365 (189-410)	189 (137-223)	p < 0.05	p < 0.05	NS
IL-17	18 (12-26)	17 (14-23)	0	p < 0.05	p < 0.05	NS
IL-18	83 (61-102)	43 (27-65)	42 (32-49)	p < 0.05	NS	p < 0.05
G-CSF	12 (11-19)	13 (10-16)	18 (15-20)	NS	NS	NS
GM-CSF	11 (5-29)	7 (3-18)	0	p < 0.05	p < 0.05	NS
IFN-α2	↓ 45 (40-52)	↓ 42 (40-46)	51 (47-54)	p < 0.05	p < 0.05	NS
IFN-γ	↓ 56 (48-89)	↓ 56 (50-75)	107 (104-122)	p < 0.05	p < 0.05	NS
LIF	ND	ND	ND	–	–	–
M-CSF	ND	ND	ND	–	–	–
MIF	374 (257-746)	268 (198-351)	74 (38-122)	p < 0.05	p < 0.05	NS
SCF	91 (80-123)	92 (62-105)	109 (89-117)	NS	NS	NS
TNF-α	52 (38-80)	40 (35-57)	0	p < 0.05	p < 0.05	NS
TNF-β	ND	ND	ND	–	–	–
Trail	132 (89-169)	106 (66-154)	53 (36-76)	p < 0.05	p < 0.05	NS

Data are expressed as median in pg/mL with interquartile range in paranthesis. One Way analysis of variance, with Student-Newman-Keuls correction for pair-wise comparisons, was used to assess statistical significance between groups and p < 0.05 was considered statistically significant. IL: Interleukin, IL-1ra: Interleukin 1 receptor antagonist, G-CSF: Granulocyte colony-stimulating factor, GM-CSF: Granulocyte macrophage colony-stimulating factor, IFN- α 2 and γ : Interferon alpha 2 and gamma, LIF: Leukemia inhibitory factor, M-CSF: Macrophage colony-stimulating factor, MIF: Macrophage migration inhibitory factor, SCF: Stem cell factor, TNF- α :Tumor necrosis factor- alpha, TNF- β :Tumor necrosis factor- beta, Trail: tumor necrosis factor-related apoptosis-inducing ligand. PC = Poorly Controlled, WC = Well Controlled, NAC = Non-Asthmatic Controls ↓ = values lower in asthmatics compared to NAC. Detectable limits for 23-plex (Low PMT) in pg/mL were: IL-1 α : 1.3, IL-3: 1.9, IL-12(p40): 1.6, IL-16: 0.9, IL-18:1.8, IFN- α 2:0.3, LIF: 1.5, M-CSF: 2.1, MIF: 1.6, SCF: 1.7, TNF- β : 1.6, Trail: 2.6 and for 27-plex (high PMT) were: IL-1 β : 0.3, IL-1ra: 0.1, IL-2: 0.1, IL-4: 0.1, IL-5: 0.2, IL-6: 0.2, IL-7: 0.3, IL-9: 0.1, IL-10: 0.2, IL-12(p70): 0.3, IL-13: 0.1, IL-15: 0.1, IL-17: 0.2, G-CSF: 0.2, GM-CSF: 0.1, IFN- γ : 0.1 and TNF- α : 0.5.

Table 3

Chemokine Levels Among Poorly Controlled, Well Controlled and Non-Asthmatic Control groups

Chemokines	PC Median (IQR)	WC Median (IQR)	NAC Median (IQR)	P value		
				PC vs NAC	WC vs NAC	PC vs WC
CTACK	594 (490-700)	478 (391-681)	439 (366-532)	p < 0.05	NS	NS
Eotaxin	56 (32-107)	83 (63-105)	91 (34-139)	NS	NS	NS
GRO-α	369 (9102-730)	136 (79-195)	102 (67-157)	NS	NS	NS
IL-8	6 (5-13)	8 (5-17)	0	p < 0.05	p < 0.05	NS
IP-10	356 (261-974)	280 (189-465)	352 (231-619)	NS	NS	NS
MCP-1 (MCAF)	41 (31-47)	60 (36-81)	0	p < 0.05	p < 0.05	NS
MIG	354 (259-470)	396 (269-482)	263 (249-468)	NS	NS	NS
MIP-1α	4 (3-8)	4 (3-7)	8 (8-9)	p < 0.05	p < 0.05	NS
MIP-1β	108 (63-165)	132 (96-165)	54 (42-84)	p < 0.05	p < 0.05	NS
MCP-3	33 (28-49)	29 (24-35)	6 (0-14)	p < 0.05	p < 0.05	NS
RANTES	>2330	>2330	>2330	–	–	–
SDF-1α	745 (503-1089)	409 (325-784)	470 (381-556)	NS	NS	NS

Data are expressed as median in pg/mL with interquartile range in paranthesis. One Way analysis of variance, with Student-Newman-Keuls correction for pair-wise comparisons, was used to assess statistical significance between groups and p < 0.05 was considered statistically significant. CTACK: Cutaneous T-cell attracting chemokine, GRO- α : Growth regulated protein- alpha; IL: Interleukin, IP-10: Interferon gamma inducible Protein-10, MCP-1 (MCAF) and 3: Macrophage chemoattractant protein-1 (monocyte chemotactic and activating factor) and 3, MIG: Monokine induced interferon gamma, MIP-1 α and β : Macrophage inflammatory protein-1 alpha and beta, RANTES: Regulated on activation, normal T expressed and secreted, SDF-1 α : Stromal cell derived factor-1 alpha. PC = Poorly Controlled, WC = Well Controlled, NAC = Non-Asthmatic Controls. Detectable limits for 23-plex (Low PMT) in pg/mL were: CTACK: 2.1, GRO- α : 1.7, MIG: 0.2, MCP-3: 1.1, SDF-1 α : 2.4 and for 27-plex (high PMT) were: Eotaxin: 0.1, IL-8: 0.1, IP-10: 0.4, MCP-1: 0.2, MIP-1 α : 0.1, MIP-1 β : 0.2 and RANTES: 0.1.

Table 4

Serum Growth Factors Levels Among Poorly Controlled, Well Controlled and Non- Asthmatic Control groups

Growth factors	PC Median (IQR)	WC Median (IQR)	NAC Median (IQR)	P value		
				PC vs NAC	WC vs NAC	PC vs WC
β-NGF	ND	ND	ND	–	–	–
FGF-basic	15 (7-27)	4 (2-12)	0	p < 0.05	p < 0.05	p < 0.05
PDGF-BB	6713 (5890-9701)	7783 (4831-8752)	3495 (3005-4082)	p < 0.05	p < 0.05	NS
VEGF	78 (54-159)	109 (75-138)	0	p < 0.05	p < 0.05	NS
HGF	1312 (1046-1493)	894 (716-1224)	771 (630-934)	p < 0.05	p < 0.05	p < 0.05
SCGF-β	84965 (59179-97272)	55278 (37776-61429)	38530 (30466-48560)	p < 0.05	p < 0.05	p < 0.05

Data are expressed as median in pg/mL with interquartile range in paranthesis. One Way analysis of variance, with Student-Newman-Keuls correction for pair-wise comparisons, was used to assess statistical significance between groups and p < 0.05 was considered statistically significant. β-NGF: Nerve growth factor beta, FGF-basic: fibroblast growth factor, PDGF-BB: Platelet derived growth factor, VEGF: Vascular endothelial growth factor, HGF: Hepatocyte growth factor, SCGF-β: Stem cell growth Factor beta. PC = Poorly Controlled, WC = Well Controlled, NAC = Non-Asthmatic Controls. Detectable limits for 23-plex (Low PMT) in pg/mL were; β-NGF: 1.1, HGF: 2.1, SCGF-β: 7 and for 27-plex (high PMT) were; FGF-basic: 0.1, PDGF-BB: 0.2 and VEGF: 0.2.

Table 5

Adhesion Molecules And Receptor Levels Among Poorly Controlled, Well Controlled and Non- Asthmatic Control groups

Adhesion molecules & receptor	PC Median (IQR)	WC Median (IQR)	NAC Median (IQR)	P value		
				PC vs NAC	WC vs NAC	PC vs WC
VCAM-1	105459 (91186-267674)	90301 (66182-141906)	81736 (54050-97599)	NS	NS	NS
ICAM-1	220129 (103039-565058)	182711 (78336-279389)	120446 (73567-193757)	NS	NS	NS
IL-2ra	93 (82-141)	70 (58-99)	98 (86-110)	NS	NS	NS

Data are expressed as median in pg/mL with interquartile range in paranthesis. One Way analysis of variance, with Student-Newman-Keuls correction for pair-wise comparisons, was used to assess statistical significance between groups and $p < 0.05$ was considered statistically significant. VCAM-1: Vascular cell adhesion molecule, ICAM-1: Intercellular adhesion molecule, IL-2ra: Interleukin 2 receptor. PC = Poorly Controlled, WC = Well Controlled, NAC = Non-Asthmatic Controls. Detectable limits for 23-plex (Low PMT) in pg/mL were; VCAM-1: 1.4, ICAM-1: 1.8 and IL-2ra: 0.6.