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

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Background: Clinical outcomes of patients with asthma are highly variable. Immunological biomarkers associated with asthma control have not been elucidated.

Objective: To identify the association between clinical control of asthma and serum immunological profiles of asthmatics and compare these profiles with those of healthy controls by using a multiplex assay.

Methods: Sera were obtained from 28 nonsmokers 18 to 55 years of age with moderate and severe persistent asthma. Patients were classified as having well-controlled (WC, $n = 14$) or poorly controlled (PC, $n = 14$) asthma based on their responses to the Asthma Control Questionnaire and Asthma Quality of Life Questionnaire. Sera from nonasthmatic control individuals (NAC, $n = 14$) were used for comparison. Levels of 50 analytes, including cytokines, chemokines, angiogenic, and growth factors, were determined, using a multiplex assay.

Results: Twelve of the 29 cytokines levels were significantly higher in patients with asthma than in NACs, but only interferon gamma levels were significantly lower in patients with asthma than in the NAC group. Among these, interleukin (IL)-3 and IL-18 levels were significantly higher in the PC group than the WC group. Five of the 12 tested chemokine levels were significantly higher in patients with asthma than in NACs. Five of six growth factor levels were significantly higher in patients with asthma than in NACs, and 3 were higher in PC than WC. Interleukin-18, fibroblast growth factor, hepatocyte growth factor, and stem cell growth factor-beta were positively correlated with poor asthma control and negatively with quality of life scores.

Conclusions: Increased serum levels of fibroblast growth factor, hepatocyte growth factor, and stem cell growth factor-beta might be useful biomarkers of asthma control status and targets of future asthma therapy.

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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% to 25% of patients with asthma are resistant to steroid treatment.² T helper (Th) 2

cells are the predominant inflammatory cell type in asthmatic airway biopsy and bronchoalveolar lavage (BAL), and studies found that these cells exhibit higher levels of interleukin (IL)-3, IL-4, IL-5, and IL-13 messenger RNA (mRNA) than cells from healthy controls. Reduced levels of the Th1 cytokines interferon (IFN)- α 2 and IFN- γ in peripheral blood mononuclear cells and BAL are associated with asthma exacerbations.^{3–5} However, as the disease becomes more severe, the T cell population becomes a mixed Th2/Th1 phenotype.^{6,7} Potential serum immunological biomarkers of asthma control have not been identified.

Previous studies evaluated cytokine profiles in asthma patient bronchoalveolar lavage fluid⁸ 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 al¹⁰ found no statistically significant differences between serum IL-5 levels in children with asthma with acute exacerbations and stable condition. However, IL-5 levels were higher than in normal controls. Joseph et al¹¹ found, in patients with mild and moderate persistent asthma, elevated serum IL-5 levels that were not affected by corticosteroid use. Because persistent asthma involves several factors including

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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 serum of patients with asthma, 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 patients with asthma. Patients were enrolled over a 19-month period in the general internal medicine clinic of Mount Sinai Hospital, located in East Harlem, New York. Additional details of the design of the parent study are reported elsewhere.^{13–17} This study was limited to nonsmoking patients with asthma 18 to 55 years of age, with mild persistent, moderate persistent, or severe disease.¹⁸ Nonasthmatic controls (NAC) were also recruited at Mount Sinai Hospital. Controls were nonsmokers older 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 by 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 and reliability, and it 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 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 7-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 patients with asthma classified as poorly controlled (PC), and 14 samples from patients classified as well con-

trolled (WC).^{13–17} For the purpose of this study, PC asthma was defined as an ACQ score greater than 4 and a Mini-AQLQ score of 2.5 or less, or history of 1 or more emergency department visits or hospitalizations for asthma during the prior year. Patients with ACQ scores between 1 and 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-plex and 27-plex panels of Bio-Plex human cytokine assays (Bio-Rad Laboratories, Irvine, CA) according to the manufacturer's instructions. Briefly, 50 μ L per well of capture antibody-coated beads was 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 was incubated with the beads for 30 minutes at room temperature with constant shaking at 10 g. 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 photomultiplier tube settings were used for 23-plex, and high photomultiplier tube settings were used for 27-plex. Detectable limits of analytes were variable and in the range of 0.1 to 7 pg/mL (see footnotes).

Statistical Analysis

Data were analyzed by using the SigmaStat statistical software package (SPSS Inc, Chicago, Illinois). Because the data distribution was not normal, the three groups were compared for each analyte by using the Kruskal-Wallis test followed by pairwise comparisons between groups using Student-Newman-Keuls correction. The χ^2 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

Table 1. Baseline Characteristics of Study Subjects

Characteristics	PC	WC	NACs	P
Demographics	14	14	14	
Number of subjects	37 ± 5	43 ± 9	42 ± 15	.28
Age, years (mean ± SD)				
Female, N (%)	13 (93)	11 (79)	10 (71)	.33
Race/ ethnicity, N (%)				.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)	
Asthma history				
Age of onset, years (mean ± SD)	14 ± 12	20 ± 16	NA	.31
Duration of asthma (mean ± SD)	23 ± 10	23 ± 15	NA	.89
PEF predicted, (mean ± SD)	294 ± 160	371 ± 61	NA	.1
ICS treatment, N (%)	11 (79)	12 (86)	NA	.62
Outpatient visits in past year (%)	12 (86)	9 (64)	NA	.19
Emergency department visits in the past year (%)	14 (100)	0	NA	.000
Hospitalized in the past year (%)	10 (71)	9 (64)	NA	.69
Ever intubated (%)	11 (79)	10 (71)	NA	.63
Required steroids in the past year (%)	2 (14)	0	NA	.14
ACQ scores	4.86	1.77	NA	<.001
AQLQ scores	2.75	4.8	NA	<.001
Indoor allergen sensitization (%)*				
<i>Dermatophagoides pteronyssinus</i> —dust mite, N (%)	4 (36)	5 (36)	NA	.97
<i>Dermatophagoides farinae</i> —dust mite, N (%)	3 (27)	5 (36)	NA	.65
Cat, N (%)	5 (46)	4 (29)	NA	.38
Mouse, N (%)	3 (27)	2 (14)	NA	.42
<i>Blattella germanica</i> —cockroach, N (%)	7 (64)	10 (71)	NA	.68
<i>Periplaneta americana</i> —cockroach, N (%)	2 (18)	3 (21)	NA	.84
<i>Aspergillus fumigatus</i> —mold, N (%)	3 (27)	1 (7)	NA	.17
<i>Alternaria alternata</i> —mold, N (%)	2 (18)	4 (29)	NA	.55
Sensitized to at least 1 allergen, N (%)	9 (82)	10 (71)	NA	.55

Abbreviations: PC, poorly controlled; WC, well controlled; NAC, nonasthmatic controls; ICS, inhaled corticosteroids; ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire.

* Patients were classified as sensitized if serum immunoglobulin E titers were greater than 0.35 kU/L.

not statistically different. All groups had a high percentage of females, PC (93%), WC (79%), and NAC (71%); however, no significant difference between groups was found. The high percentage of women with asthma in the study is consistent with the epidemiology of the 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 patients with asthma to be low-income Hispanic and African American individuals, which is consistent with epidemiology of inner-city patients with asthma. No whites were found in the PC group and no African Americans in the NAC group. Inhaled corticosteroids treatment in PC patients was 79%, in WC patients it was 86%, and other patients were on β_2 -agonists. The ACQ score ($P < .001$) and AQLQ score ($P < .001$) were significantly different between the 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, granulocyte-macrophage colony-stimulating factor, macrophage migration inhibitory factor, Tumor necrosis factor-related apoptosis-inducing ligand, and tumor necrosis factor alpha levels were significantly higher in sera from both PC and WC than from NAC (Table 2). Interferon gamma and IFN- α 2 levels were significantly lower in PC and WC sera than in NAC sera (Table 2, $P < .05$). However, no significant differences were seen between these cytokine levels in the PC and WC groups. Interleukin-3 and IL-18 serum levels were significantly higher only in the PC group ($P < .05$) and were significantly higher than in WC ($P < .05$; Table 2 and Fig 1A and B). Only IL-18 was positively correlated with the ACQ scores ($r = 0.530$; $P = .003$; Fig 1C) and negatively correlated with AQLQ ($r = -0.514$; $P = .005$; Fig 1D).

Table 2. Cytokine Levels Among Poorly Controlled, Well Controlled, and Control Groups Without Asthma

Cytokines	PC median (IQR)	WC median (IQR)	NAC median (IQR)	P-value		
				PC vs NAC	WC vs NAC	PC vs WC
IL-1 α	ND	ND	ND	–	–	–
IL-1 β	ND	ND	ND	–	–	–
IL-1ra	190 (128-382)	207 (149-281)	109 (102-144)	$P < .05$	$P < .05$	NS
IL-2	7 (6-15)	9 (6-11)	0	$P < .05$	$P < .05$	NS
IL-3	235 (203-311)	182 (149-222)	156 (142-182)	$P < .05$	NS	$P < .05$
IL-4	ND	ND	ND	–	–	–
IL-5	ND	ND	ND	–	–	–
IL-6	8 (6-10)	8 (5-10)	2 (2-3)	$P < .05$	$P < .05$	NS
IL-7	13 (10-16)	16 (11-19)	3 (2-4)	$P < .05$	$P < .05$	NS
IL-9	26 (12-69)	27 (14-58)	6 (4-11)	$P < .05$	$P < .05$	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)	$P < .05$	$P < .05$	NS
IL-17	18 (12-26)	17 (14-23)	0	$P < .05$	$P < .05$	NS
IL-18	83 (61-102)	43 (27-65)	42 (32-49)	$P < .05$	NS	$P < .05$
G-CSF	12 (11-19)	13 (10-16)	18 (15-20)	NS	NS	NS
GM-CSF	11 (5-29)	7 (3-18)	0	$P < .05$	$P < .05$	NS
IFN- α 2	↓ 45 (40-52)	↓ 42 (40-46)	51 (47-54)	$P < .05$	$P < .05$	NS
IFN- γ	↓ 56 (48-89)	↓ 56 (50-75)	107 (104-122)	$P < .05$	$P < .05$	NS
LIF	ND	ND	ND	–	–	–
M-CSF	ND	ND	ND	–	–	–
MIF	374 (257-746)	268 (198-351)	74 (38-122)	$P < .05$	$P < .05$	NS
SCF	91 (80-123)	92 (62-105)	109 (89-117)	NS	NS	NS
TNF- α	52 (38-80)	40 (35-57)	0	$P < .05$	$P < .05$	NS
TNF- β	ND	ND	ND	–	–	–
Trail	132 (89-169)	106 (66-154)	53 (36-76)	$P < .05$	$P < .05$	NS

Data are expressed as median in pg/mL with interquartile range in parentheses. One-way analysis of variance, with Student-Newman-Keuls correction for pairwise comparisons, was used to assess statistical significance between groups, and $P < .05$ was considered statistically significant.

Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- α 2 and γ , interferon alpha 2 and gamma; IL, interleukin; IL-1ra, interleukin 1 receptor antagonist; LIF, leukemia inhibitory factor; M-CSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; NAC, non-asthmatic controls; PC, poorly controlled; SCF, Stem cell factor; TNF- α , tumor necrosis factor- alpha; TNF- β , tumor necrosis factor-beta; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WC, well controlled; ↓ = values lower in patients with asthma compared with 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-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; TNF- α : 0.5.

Chemokine Levels in PC, WC, and NAC and Correlations With ACQ and AQLQ Scores

Of the 12 chemokines tested (Table 3), IL-8, macrophage chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and MCP-3 were significantly elevated in both PC and WC sera versus NAC. Interleukin-8 and MCP-1 levels were not detectable in NAC sera. Cutaneous T-cell-attracting chemokine (CTACK) levels were significantly higher in PC sera than in NACs. However, no significant differences were seen in IL-8, MCP-1, MIP-1 α , MIP-1 β , and MCP-3 and CTACK between PC and WC groups. Regulated on activation, normal T expressed and secreted levels in all groups were above the highest accurately measurable level (>2,330 pg/mL). No

significant correlation was evident 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. Platelet-derived growth factor and vascular endothelial growth factor (VEGF) were significantly higher in both PC and WC sera than in NAC sera ($P < .05$, Table 4), but PC and WC levels did not differ. Three growth factors, fibroblast growth factor (FGF-basic), hepatocyte growth factor (HGF), and stem cell growth factor-beta (SCGF- β), were significantly higher in PC than in WC and NAC groups ($P < .05$, Fig 2A). Fibroblast growth factor

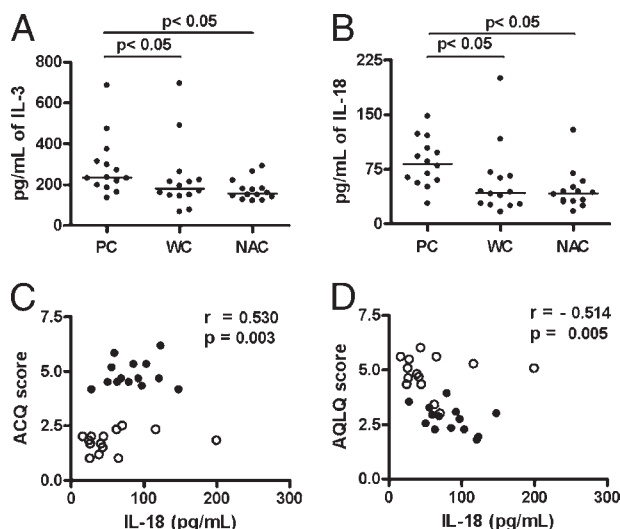


Figure 1. Serum cytokine levels of interleukin (IL)-3, IL-18, and correlation of IL-18 with asthma control and quality of life. Serum IL-3 and IL-18, levels were compared in poorly controlled (PC), well controlled (WC), and nonasthmatic controls (NAC) by multiplex assay (A, B). A one-way analysis of variance on ranks with Student-Newman-Keuls correction was used to calculate statistical significance between the three groups, and $P < .05$ was considered statistically significant. Spearman's rank correlation method was used to calculate the correlation coefficient 'r' value. A positive 'r' value shows an increase in cytokine level correlated with an increase in the Asthma Control Questionnaire (ACQ) score (poor control of asthma), and a negative value shows an increase in cytokine level correlated with a decrease in the Asthma Quality of Life Questionnaire (AQLQ) score (poor quality of life). A $P < .05$ value was considered significant. C, Correlation between serum IL-18 levels vs. ACQ score. D, Correlation between IL-18 levels and AQLQ score. Black circles, PC; white circles, WC.

($r = 0.602$, $P = .0007$), HGF ($r = 0.445$, $P = .017$), and SCGF- β ($r = 0.522$, $P = .004$) levels showed a positive correlation with ACQ scores (Fig 2B, D, and F). Similarly, FGF-basic ($r = -0.765$, $P < .0001$), HGF ($r = -0.538$, $P = .003$), and SCGF- β ($r = -0.577$, $P = .001$) showed a negative correlation with AQLQ scores (Fig 2C, 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 that reduction of asthma symptoms in response to specific pharmacological interventions, such as corticosteroids, varies widely between individuals is well known. Although the National Asthma Education and Prevention Program 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 Several different factors may influence asthma control, including inability to avoid indoor-outdoor allergens, smoking, viral infections, genetic makeup, treatment resistance, poor treatment adherence, and undertreatment. All these factors individually or in combination can influence the patients' serum immunological profile. Certain underlying defects in the innate immune systems of healthy individuals could make them susceptible to developing an asthmatic phenotype. Studies have shown impaired induction of virus-induced interferon secretion in patients with asthma, 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 patients with asthma as compared with NACs. 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 Because of the ensuing complexity of the disease, the mechanisms underlying asthma symptom control outcomes are not fully elucidated. Our study is the first to use a 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 to compare the results with those of NACs.

One potentially important finding is that IL-3 and IL-18 levels were significantly higher in the PC group than in the WC group. Interleukin-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} Interleukin-18 levels also showed a positive correlation with ACQ scores and a negative correlation with AQLQ scores in our study. Interleukin-18 is released from activated monocytes, macrophages, and 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 al³¹ demonstrated that IL-18 deficiency selectively enhances allergen-induced eosinophilia in mice. Other studies using murine models reported contradictory data. Hoshino et al³² demonstrated that in vivo administration of IL-18 induced immunoglobulin E production through Th2 cytokine induction and up-regulation of CD40 ligand expression on CD4+ T cells. These controversial findings may be attributable to the presence or absence of IFN- γ as previously reported.³³ Interleukin-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

Table 3. Chemokine Levels Among Poorly Controlled, Well Controlled, and Control Groups Without Asthma

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)	$P < .05$	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	$P < .05$	$P < .05$	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	$P < .05$	$P < .05$	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)	$P < .05$	$P < .05$	NS
MIP-1β	108 (63–165)	132 (96–165)	54 (42–84)	$P < .05$	$P < .05$	NS
MCP-3	33 (28–49)	29 (24–35)	6 (0–14)	$P < .05$	$P < .05$	NS
RANTES	>2,330	>2,330	>2,330	–	–	–
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 parentheses. One-way analysis of variance, with Student-Newman-Keuls correction for pairwise comparisons, was used to assess statistical significance between groups, and $P < .05$ was considered statistically significant.

Abbreviations: CTACK, cutaneous T-cell-attracting chemokine; GRO- α , growth regulated protein- α ; 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; PC, poorly controlled; RANTES, regulated on activation, normal T expressed and secreted; SDF-1 α , stromal cell-derived factor-1 alpha; WC = well controlled; NAC, nonasthmatic 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): 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.

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. Fibroblast growth factor 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, no reports have been made of a correlation between plasma FGF-basic levels and asthma. Hepatocyte growth factor is a pleiotropic biological compo-

nent 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 airway hyperresponsiveness and inflammatory cytokine levels.⁴⁴ Nomura et al⁴⁵ found higher levels of HGF in induced sputum of patients with asthma than in normal controls. However, HGF serum levels in well and poorly controlled asthma have not been previously reported. Stem cell growth factor is a cytokine of the C-type lectin family that supports hematopoietic stem cell/progenitor cell proliferation. Expression of SCGF- β messenger RNA is restricted to myeloid cells and fibroblasts. Stem cell growth factor

Table 4. Serum Growth Factors Levels Among Poorly Controlled, Well-Controlled, and Control Groups Without Asthma

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	$P < .05$	$P < .05$	$P < .05$
PDGF-BB	6,713 (5,890–9,701)	7,783 (4,831–8,752)	3,495 (3,005–4,082)	$P < .05$	$P < .05$	NS
VEGF	78 (54–159)	109 (75–138)	0	$P < .05$	$P < .05$	NS
HGF	1,312 (1,046–1,493)	894 (716–1,224)	771 (630–934)	$P < .05$	$P < .05$	$P < .05$
SCGF-β	84,965 (59,179–97,272)	55,278 (37,776–61,429)	38,530 (30,466–48,560)	$P < .05$	$P < .05$	$P < .05$

Data are expressed as median in pg/mL with interquartile range in parentheses. One-way analysis of variance, with Student-Newman-Keuls correction for pairwise comparisons, was used to assess statistical significance between groups, and $P < .05$ was considered statistically significant.

Abbreviations: β -NGF, nerve growth factor beta; FGF-basic, fibroblast growth factor; HGF, hepatocyte growth factor; NAC, nonasthmatic controls; PC, poorly controlled; PDGF-BB, platelet-derived growth factor; SCGF- β , stem cell growth factor beta; VEGF, vascular endothelial growth factor; WC, well controlled.

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.

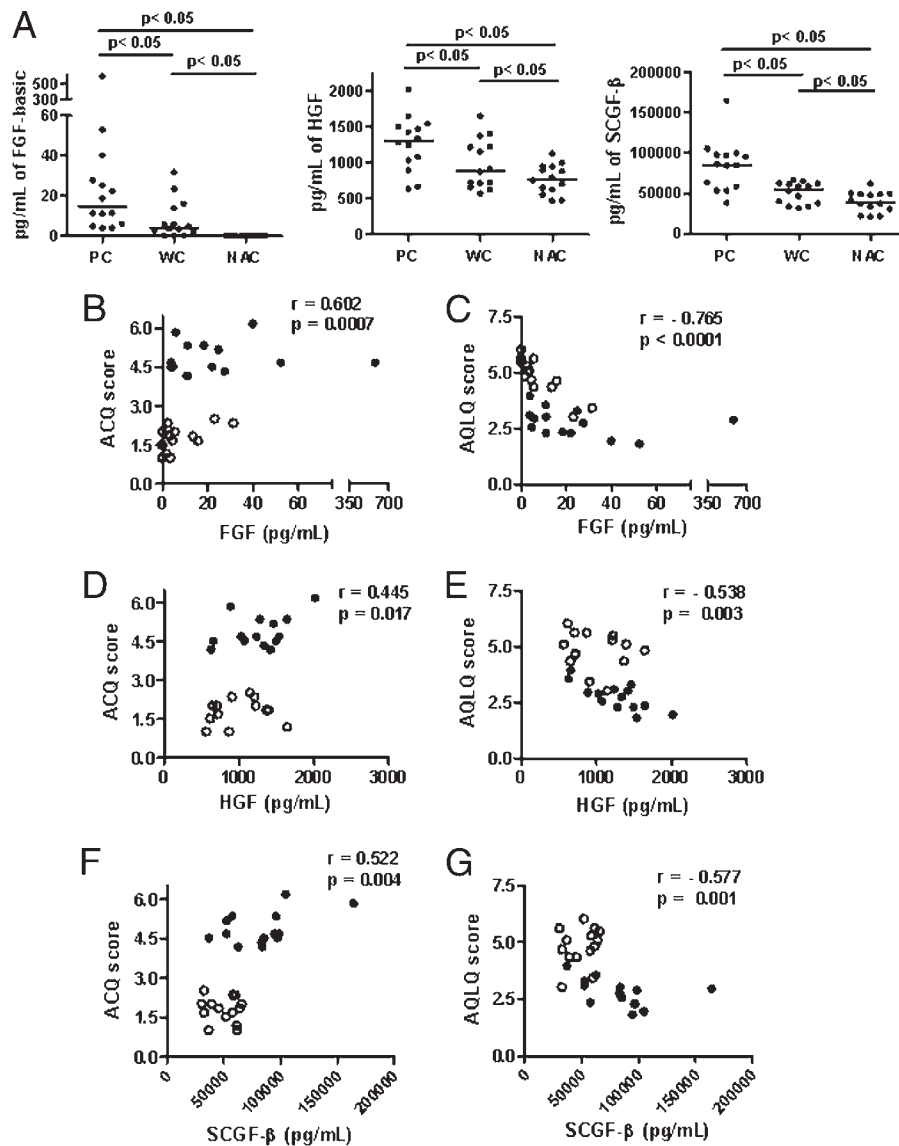


Figure 2. Comparison of serum growth factors fibroblast growth factor, hepatocyte growth factor, and stem cell growth factor- β levels in poorly controlled (PC), well-controlled (WC), and nonasthmatic control (NAC) groups and their correlation with the Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ) score. A, Serum growth factors fibroblast growth factor (FGF-basic), hepatocyte growth factor (HGF), and stem cell growth factor- β (SCGF- β) levels were compared in PC, WC, and NACs by multiplex assay. A one-way analysis of variance with Student-Newman-Keuls correction was used to calculate statistical significance between the three groups, and a $P < .05$ was considered statistically significant. Spearman's rank correlation method was used to calculate the correlation coefficient 'r' value. A $P < .05$ 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. Black circles, PC; and white circles, WC.

lacks colony-stimulating activity but has burst-promoting activity for human bone marrow erythroid progenitors, granulocyte-macrophage-promoting activity for granulocyte-macrophage progenitors, and colony-forming unit—granulocyte-macrophage—sustaining or recruiting effect during short-term liquid bone marrow cell culture.⁴⁶ No previous reports have been found of a role for SCGF- β in asthma. A recent study indicates that pathological mecha-

nisms 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 it might be associated with airway remodeling and inflammatory cell survival.^{48–50} Airway remodeling involves thickening of the airways because of increases in airway smooth muscles, myofibroblasts, collagen, and angiogenesis. Various cells, such as mast cells, eosinophils, macrophages,

Table 5. Adhesion Molecules and Receptor Levels Among Poorly Controlled, Well-Controlled, and Control Groups Without Asthma

Adhesion molecules and receptor	PC median (IQR)	WC median (IQR)	NAC median (IQR)	P-value		
				PC vs NAC	WC vs NAC	PC vs WC
VCAM-1	105,459 (91,186–267,674)	90,301 (66,182–141,906)	81,736 (54,050–97,599)	NS	NS	NS
ICAM-1	220,129 (103,039–565,058)	182,711 (78,336–279,389)	120,446 (73,567–193,757)	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 parentheses. One-way analysis of variance, with Student-Newman-Keuls correction for pairwise comparisons, was used to assess statistical significance between groups, and $P < .05$ was considered statistically significant.

Abbreviations: ICAM-1, intercellular adhesion molecule; IL-2ra, interleukin 2 receptor; NAC, nonasthmatic controls; PC, poorly controlled; VCAM-1, vascular cell adhesion molecule; WC, well controlled.

Detectable limits for 23-plex (Low PMT) in pg/mL were: ICAM-1, 1.8; IL-2ra, 0.6; VCAM-1, 1.4.

and epithelial cells, involved in airway inflammation secrete growth factors that are important for the proliferation of myofibroblasts that further secrete extracellular matrix components. The role of transforming growth factor β , platelet-derived growth factor, insulinlike growth factor-I, FGF-basic, and VEGF have been demonstrated in airway thickening and angiogenesis.^{38,45,48,49} Hoshino et al⁴⁸ also demonstrated that subepithelial layer thickening plays a role in promoting bronchial responsiveness. We did not test transforming growth factor- β and insulinlike growth factor-I; however, among the growth factors assessed, platelet-derived growth factor and VEGF were not significantly different in PCs and WCs; however, FGF-basic, HGF, and SCGF- β were important growth factors that were statistically higher in PC as compared with WC. The detection of statistically significant higher levels of growth factors and absence of proinflammatory cytokines in the serum of PC patients with asthma suggests that the current therapy may control inflammation; however, it is inadequate in suppressing airway remodeling. To generate biomarkers, the subjects were selected such that sharp contrast existed between PC and WC patients so as to avoid overlap between two groups. The bioplex technology is relatively new and expensive. To maintain cost-effectiveness, we used samples from well-defined groups, which limited the number of qualified patients from the parent study. Because of the small number of patients, the homogeneity in ethnicity was compromised, and therefore the PC group has no whites and NAC group has no blacks; this factor should be considered when interpreting the results of this study.

In conclusion, this study is the first attempt to use a 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. Interleukin-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 bronchoalveolar lavage fluid and sputum samples. This finding may provide a rationale for further identification of serum biomarkers associated with asthma control status.

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