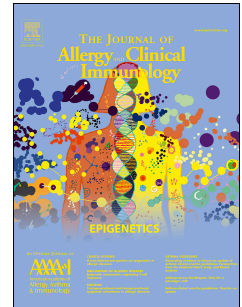


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Serum IL-6: A Biomarker in Childhood Asthma?

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Key Words:

Asthma, children, IL-6, exacerbations, obesity

Capsule Summary: Serum IL-6 was associated with asthma exacerbation risk but not with symptoms or lung function in urban children; further studies to evaluate the role of IL-6 in the life course of asthma are needed.

To the Editor:

Peripheral blood IL-6 was recently identified as a potential biomarker in adult asthma. Peters et al. associated systemic inflammation, as demonstrated by elevated IL-6 levels, with other markers of metabolic dysfunction and greater asthma severity in both lean and obese adults in the Severe Asthma Research Program.(1) Individuals with elevated IL-6 levels had both reduced lung function and enhanced exacerbation risk. Further, epithelial IL-6 trans-signaling was recently identified as a potential mechanism underlying a phenotype of asthma with enhanced airway inflammation.(2) However, IL-6 has not been evaluated as a biomarker in children with asthma, and it is currently unknown whether systemic inflammation and metabolic dysfunction play a role in asthma severity and exacerbation risk in children and adolescents.

The Asthma Phenotypes in the Inner City (APIC) study, performed within the NIH/NIAID-sponsored Inner City Asthma Consortium, enrolled 717 participants 6-17 years of age and managed their asthma for 1 year according to a guideline-based algorithm as previously described.(3) The overall goal of APIC was to identify factors that differentiated difficult-to-control vs. easy-to-control asthma in urban children. A total of 702 children had baseline serum samples available for IL-6 measurement and were included in this *post hoc* analysis. Serum IL-6 was measured with a high sensitivity assay with a lower limit of detection of 0.18 pg/ml (Millipore Milliplex MAP kit HSTCMAG-28SK). To compare the relationships of serum IL-6 with demographics and other baseline blood biomarkers, Kruskal Wallis Rank Test and trend test were used for categorical variables, and Pearson correlations were used for continuous variables. Asthma characteristics and outcomes were estimated by mixed models for longitudinal data, odds ratio and rate ratio for binary and count exacerbation outcomes respectively.

The distribution of serum IL-6 levels is displayed in Figure E1. The median value was 0.85 pg/ml (IQR 0.39-1.65 pg/ml). A relatively small proportion of children (8.4%) had IL-6 levels above the cut-off for "IL-6 high" of 3.1 pg/ml identified by Peters et al in adults. Considering this and the lack of normative IL-6 values for children, we evaluated IL-6 as a continuous variable rather than a dichotomous (high/low) variable. Serum IL-6 significantly increased with age and BMI percentile (Table E1). IL-6 levels were also significantly higher in females versus males and significantly lower in black children versus other study participants (Table E1).

We examined associations between serum IL-6 and other baseline laboratory measures (Table 1). IL-6 was significantly correlated with total white blood cells (R 0.18, 95% confidence interval (CI) (0.11, 0.25); $p < 0.01$), total blood neutrophils (0.20 95% CI (0.12, 0.27); $p < 0.01$), and serum C-reactive protein (CRP) (0.10 95% CI (0.03, 0.18); $p < 0.01$). In contrast, IL-6 was not associated with any markers of type-2 inflammation, including total blood eosinophils, total IgE, or number of allergen sensitizations (Table 1).

While the observed associations of IL-6 with BMI percentiles and markers of metabolic dysfunction were similar to that seen previously in adults, the relationships between IL-6 and measures of asthma severity were not. At study baseline, IL-6 was not significantly associated

with the Composite Asthma Severity Index (CASI),(4) asthma treatment step, symptom days in the past 14 days, lung function, or exhaled nitric oxide (Table 2). Similarly, during the one-year longitudinal characterization period when study participants were managed by standardized guidelines-based asthma and rhinitis management, baseline IL-6 was not associated with these same measures of asthma severity and control or with previously described asthma phenotypes.(5) However, a significant association was observed between baseline IL-6 and the probability of experiencing an asthma exacerbation treated with systemic corticosteroids during the 1-year study. The odds of experiencing at least 1 exacerbation during the study increased by 24% for each quartile increase in serum IL-6 (unadjusted Odds Ratio (OR) 1.24 (95% CI 1.02, 1.51), $p=0.03$; adjusted OR 1.32 (1.08, 1.62), $p<0.01$). A similar significant relationship was observed for number of exacerbations during the study (adjusted Risk Ratio 1.27 (1.07, 1.50), $p<0.01$; Table 2). After adjusting for other asthma exacerbation risk factors, baseline IL-6 remained a significant predictor of subsequent asthma exacerbation risk (Table E2).

In this study of urban children with persistent asthma over a wide range of disease severity, we observed significant correlations between serum IL-6 and BMI and markers of metabolic dysfunction similar to what has previously been reported in adults. However, despite the very large proportion of our study population with obesity (BMI $>95^{\text{th}}$ ile), we observed fewer asthmatic children with IL-6 levels >3.1 pg/ml compared to what was previously reported for asthmatic adults.(1) Furthermore, we did not observe associations between IL-6 and markers of asthma severity such as the CASI, lung function, and symptoms. Interestingly, we did observe an increased risk of asthma exacerbations, a major source of morbidity and mortality, as serum IL-6 levels increased.

These findings suggest differential impact of systemic inflammation, as reflected by serum IL-6 levels, on asthma in children/adolescents versus adults. It is interesting that IL-6 was associated with greater exacerbation risk in children, but not lung function or other markers of severity as previously reported in adults. Given the association between recurrent exacerbations and loss of lung function over time,(6, 7) it is interesting to speculate that the children with higher serum IL-6 and more frequent exacerbations could eventually develop the more severe phenotype seen in adults with high IL-6 levels.

IL-6 was not associated with any markers of type 2 inflammation in our study, but was independently associated with increased exacerbation risk regardless of T2 biomarker status in the cohort. Further studies are needed to identify mechanisms that may link systemic inflammation as assessed by serum IL-6 and propensity to asthma exacerbation.

Strengths of our study include a large sample of very well characterized children managed with a standardized, guideline-based approach. Furthermore, APIC included primarily minority children who are often underrepresented in clinical studies. Limitations include lack of normative IL-6 values in children, utilization of serum rather than plasma and a different assay for the measurement of IL-6 compared to the study by Peters et al.(1), making direct comparisons with the adult data difficult. By analyzing relationships with IL-6 as a continuous

variable, we confirmed the expected relationships between IL-6 and measures of metabolic dysfunction, suggesting this was a valid approach.

In summary, in contrast to prior work in adults with asthma, serum IL-6 levels were not associated with measures of disease severity or control such as symptoms and lung function. However, we observed a significant association between IL-6 and risk of asthma exacerbations. Further studies such as interventional trials with anti-IL6 therapy are needed to define the potential role of IL-6 and systemic inflammation in the life course of asthma.

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Table 1. Associations of Serum IL-6 and Other Biomarkers at Baseline

<i>Characteristic</i>	<i>N</i>	<i>Correlation (95% CI)</i>	<i>P-value</i>	<i>Correlation (95% CI)</i>	<i>P-value</i>
Total White Blood Cell	692	0.18 (0.11, 0.25)	<0.01	0.14 (0.07, 0.22)	<0.01
Total Monocyte - log10	691	-0.01 (-0.09, 0.06)	0.71	-0.01 (-0.09, 0.06)	0.74
Total Neutrophils - per ul (log10)	690	0.20 (0.12, 0.27)	<0.01	0.14 (0.07, 0.22)	<0.01
Total Eosinophils - per ul (log10)	690	-0.01 (-0.08, 0.07)	0.85	0.01 (-0.06, 0.09)	0.71
Total IgE (kUA/L) - log10	694	0.01 (-0.06, 0.09)	0.71	0.02 (-0.05, 0.09)	0.60
CRP ng/mL - log10	699	0.10 (0.03, 0.18)	<0.01	0.06 (-0.02, 0.13)	0.14
Blood pressure - Systolic	697	0.05 (-0.03, 0.12)	0.21	-0.01 (-0.08, 0.07)	0.87
Blood pressure - Diastolic	697	0.06 (-0.02, 0.13)	0.13	0.04 (-0.04, 0.11)	0.35
Number of Allergen Sensitizations	699	-0.01 (-0.08, 0.07)	0.82	0.01 (-0.07, 0.08)	0.87

[†] Pearson Correlation

[‡] Models adjusted for gender, race, age, and BMI

Table 2. Associations Between Serum IL-6 and Asthma Characteristics

		Unadjusted		Adjusted [‡]	
Characteristic	N	Statistic (95% CI)	P-value	Statistic (95% CI)	P-value
Cross-sectional Estimate					
CASI Score (0-20) at Screening	698	0.15 (-0.15, 0.44)	0.32	0.17 (-0.13, 0.47)	0.27
Asthma Treatment Step at Screening	699	0.03 (-0.15, 0.21)	0.78	0.01 (-0.18, 0.19)	0.92
Max Symptom Days in last 14 Days at Screening	699	0.30 (-0.07, 0.68)	0.11	0.31 (-0.07, 0.70)	0.11
FEV1 (% predicted) at Screening	699	-0.04 (-1.63, 1.54)	0.96	-0.31 (-1.93, 1.30)	0.70
FEV1/FVC at Screening	688	0.10 (-0.80, 1.00)	0.82	0.14 (-0.76, 1.04)	0.76
FEV1 Bronchodilator Response	689	-0.87 (-2.17, 0.42)	0.19	-0.64 (-1.96, 0.68)	0.34
eNO (ppb) at Baseline - log10	641	0.02 (-0.01, 0.05)	0.24	0.02 (-0.01, 0.05)	0.28
Longitudinal Estimate					
CASI Score (0-20)	699	0.02 (-0.16, 0.20)	0.80	0.06 (-0.12, 0.24)	0.50
Asthma Treatment Step	699	-0.01 (-0.18, 0.16)	0.94	0.00 (-0.17, 0.18)	0.96
Max Symptom Days in last 14 Days	699	0.01 (-0.13, 0.15)	0.89	-0.00 (-0.15, 0.14)	0.98
FEV1 (% predicted)	699	0.22 (-0.91, 1.35)	0.71	-0.04 (-1.18, 1.10)	0.94
FEV1/FVC	698	0.31 (-0.33, 0.95)	0.34	0.23 (-0.40, 0.86)	0.48
eNO (ppb) - log10	681	0.01 (-0.02, 0.04)	0.43	0.00 (-0.02, 0.03)	0.75
Odds Ratio					
Exacerbation between Baseline and end of study*	699	1.24 (1.02, 1.51)	0.03	1.32 (1.08, 1.62)	<0.01
Asthma Treatment Class (Difficult vs. Easy) [†]	473	0.97 (0.78, 1.22)	0.81	0.97 (0.77, 1.23)	0.81
Rate Ratio					
Exacerbation Count between Baseline and end of study	699	1.22 (1.04, 1.44)	0.02	1.27 (1.07, 1.50)	<0.01

*The probability of having at least one exacerbation increases significantly with one interquartile range increase in IL-6

[†]The probability of being in the Easy-to-Control Asthma group decreases with an increase in IL-6

[‡]Models adjusted for gender, race, age, and BMI. Visit was additionally adjusted for the longitudinal model

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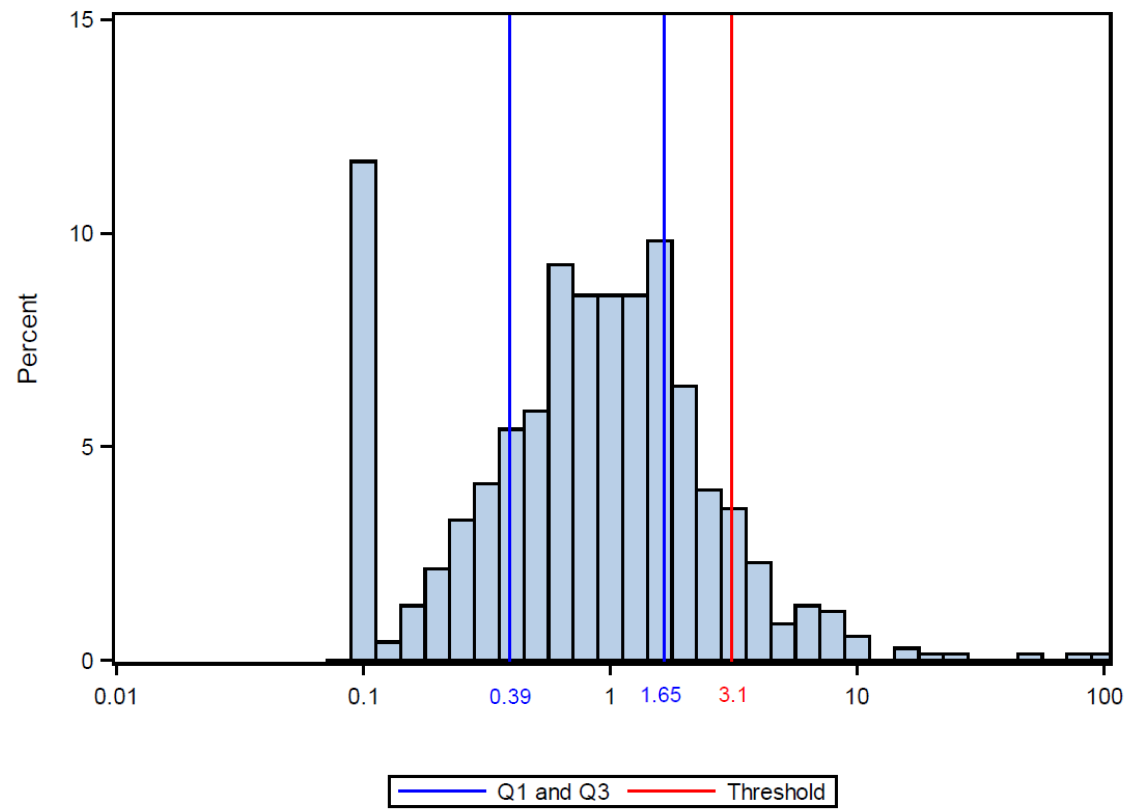


Table E1. Serum IL-6 and Baseline Demographics

<i>Characteristic</i>	<i>Overall</i>	<i>N</i>	<i>Median [Q1, Q3]</i>	<i>P-value</i>
Age [†]	6-8	206	0.67 [0.26, 1.29]	<0.01
	9-11	218	0.97 [0.43, 1.70]	
	12-14	181	0.97 [0.46, 1.83]	
	15-17	97	0.87 [0.46, 1.73]	
Gender*	Male	404	0.75 [0.36, 1.56]	0.03
	Female	298	0.94 [0.43, 1.75]	
Race*	Black	451	0.75 [0.33, 1.59]	<0.01
	Other	248	1.05 [0.49, 1.69]	
BMI [†]	<85	345	0.75 [0.34, 1.44]	<0.01
	85-95	116	0.83 [0.33, 1.83]	
	>95	241	1.04 [0.49, 1.73]	

*Kruskal Wallis Rank test

[†]Trend test

Table E2. Multivariable Models Assessing Associations Between IL-6 and Risk of Exacerbation and Exacerbation Count Adjusting for other Risk Factors for Exacerbation

<i>Characteristic</i>	<i>Exacerbation*</i>		<i>Exacerbation Count</i>	
	<i>Odds Ratio (95% CI)</i>	<i>P-value</i>	<i>Rate Ratio (95% CI)</i>	<i>P-value</i>
IL-6	1.24 (1.02, 1.51)	0.03	1.21 (1.02, 1.43)	0.03
IL6+Demographics(age, BMI, gender, and race)	1.31 (1.07, 1.61)	0.01	1.24 (1.08, 1.43)	<0.01
IL6+Demographics+WBC+Neutrophil+CRP	1.26 (1.02, 1.55)	0.03	1.20 (1.03, 1.39)	0.02
IL6+Demographics+WBC+Neutrophil+CRP+Total IgE	1.26 (1.02, 1.55)	0.03	1.17 (1.01, 1.35)	0.03
IL6+Demographics+WBC+Neutrophil+CRP+Total IgE+Eosinophil	1.26 (1.02, 1.55)	0.03	1.17 (1.01, 1.35)	0.03
Stepwise model selection	1.31 (1.07, 1.61)	<0.01 [†]	1.21 (1.02, 1.44)	0.03 [‡]

*The probability of having at least one exacerbation

[†] Age, Total IgE, and IL-6 were used in the model

[‡] Age, Neutrophil, Total IgE, and IL-6 were used in the model

Online Repository Figure Legend

Figure E1. Distribution of serum IL6 (N=702) with 25th and 75th percentiles (blue lines) and threshold for high and low IL6 (red line). 0.18 pg/ml is the lower limit of detection.