EVALUATION OF SERUM INTERLEUKIN-6 AS A BIOMARKER OF CHILDHOOD ASTHMA SEVERITY

By

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ABSTRACT

Background: *Asthma is the most common chronic respiratory condition of childhood worldwide, with around 14% of children and young people are affected. Characteristic asthma features include wheeze, shortness of breath and cough, which are typically triggered by several possible stimuli. About 5–10% of patients with asthma suffer from poorly-controlled disease despite corticosteroid (CS) therapy. There is increased effort to identify biomarkers to define different phenotypes of asthma. IL-6 is known as an inflammatory cytokines and serum levels of IL-6 have been implicated as a biomarker for asthma especially in children.*

Aim of the study: *To investigate the role of IL-6 as a potential biomarker for asthma severity in children.*

Methods: *This study is a cross sectional study that carried out on 100 children (70 pediatric patients with bronchial asthma and 30 age- and sex-matched healthy control subjects) of the attendants to pediatric pulmonology clinic of Sayed Galal university hospital during the period from September 2022 to May 2023. Detailed history taking, thorough clinical examinations, Complete blood count (CBC), blood film and stool analysis, Serum interlukin-6 level using ELISA Technique was done to all patients and control groups. PRAM score and Pulmonary function tests were performed for all patients using Spirometry. Spirometry indices included FEV1, FVC, and FEV1/FVC.*

Results: *IL-6 was significantly higher among patients compared with controls, respectively. A positive correlation was found between serum IL6 an asthma severity. IL-6 was normal in all control subjects and elevated in 8.5%, 28.5%, 31.5% and 100% of patients with intermittent, mild persistent, moderate persistent and severe persistent asthma respectively.*

Conclusion: *IL-6 appears to be valuable for assessment of asthma severity in children.*

Keywords: *Bronchial Asthma, IL6, Biomarker, spirometry.*

INTRODUCTION

Asthma is a chronic respiratory disease characterized by episodes of wheezes, cough, and shortness of breath. Around 14% of children worldwide have a diagnosis of asthma, making it the most common chronic respiratory disease of childhood. **(Khaleva et al., 2023)**

There is no single 'gold-standard' test that can be used to accurately diagnose asthma. In practice, a diagnosis should be made based on characteristic symptom patterns, evidence of variability in airflow limitation in the presence of airway inflammation, response to treatment. Lung function tests can be used to aid the diagnosis of asthma in children over the age of 5 years. Peak expiratory flow (PEF) and spirometry are commonly used to assess airflow obstruction and reversibility. **(Zanobetti et al., 2022)**

In reference to asthma, an exacerbation is defined as an event characterized by change from the patient's previous status, including a progressive increase in relevant symptoms and a decrease in respiratory function. The most common causes of these exacerbations are exposure to external agents, such as indoor and outdoor allergens, air pollutants, and respiratory tract infections (primarily viral mainly human rhinovirus (HRV). **(Klain et al., 2022)**

Asthma inflammation is categorized as eosinophilic asthma when inflammatorytient has higher than 3% of eosinophils; neutrophilic asthma, when the sputum cells are predominantly neutrophils (i.e., more than 76%); mixed granulocytic, when an increase in the proportion of both types of the inflammatory cells are observed in the sputum sample and paucigranulocytic asthma, when none of the two inflammatory cells increase beyond a threshold level. **(Li Y-C et al., 2021)**

Depending on the type of immune cell responses implicated in disease pathogenesis, asthma endotypes are categorized as type 2 asthma, characterized predominantly by T helper type 2 (Th2) cell-mediated inflammations and non-type 2 asthma, associated with Th1 and/or Th17 cell inflammation. **(Wang et al., 2021)**

Structural cells such as epithelial and endothelial cells as well as the myocytes of airways secrete various mediators of the pathophysiology of asthma, some mediators as cytokines organize the inflammatory reaction in asthma. Cytokines regulate cell-

mediated immunity. **(Ren et al., 2023)**

The lung epithelial cells can also contribute to the type of immune response by secreting specific cytokines. One of the cytokines that is produced by lung epithelial cells is IL-6 and increased production of IL-6 by lung epithelial cells has been found in asthmatic patients relative to control subjects. **(Sze et al., 2020)**

Il-6 is a pleiotropic pro-inflammatory cytokine produced by different kinds of cells, including Lymphocytes and monocytes. IL-6 regulates host defense mechanisms, acute phase response, inflammation, and hematopoiesis. It also induces production of acute phase proteins, like C-reactive protein (CRP), amyloid protein, haptoglobin and hemopexin. **(Bateman et al., 2022)**

Serum IL-6 significantly increased with age and BMI percentile. IL-6 levels were also significantly higher in females versus males and significantly lower in black children. **(Reyes-Ange et al., 2022)**

Ethical consideration:

- An informed consent was obtained from parents or legal guardians before getting involved in the study.
- The study was done after approval of ethical committees of Pediatrics department and faculty of medicine for Al-Azhar University.
- The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
- All the data of patients and results of the study are confidential, and the patient has the right to keep it.
- The patient has the right to withdraw from the study at any time without giving any reasons.

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Sample size calculation:

The sample size was calculated using Power and Sample size software version 3 (epi info). The sample size was calculated using the following formula: **(Hoban et al., 2021)**

$$n = 2\left[\frac{\left(Z_{\alpha/2} + Z_{\beta}\right) * \sigma}{\mu_1 - \mu_2}\right]^2$$

By calculation, the sample size will be equal to 70 in total.

µ1: Mean change in IL-6 from baseline in diseased group.

µ2: mean change in IL-6 from baseline in controls.

Zα/2: This depends on level of significance, for 5% this is 1.96.

Zβ: This depends on power, for 80% this is 0.84.

Ó: standard deviation = 1.195.

Inclusion criteria:

- Children aged 5-16 years of both sexes.
- Proved asthma according to GINA 2022.

Exclusion Criteria:

Any of children with:

Malignant disease, Chronic allergy. Inflammatory diseases, Severe malnutrition, Immunodeficiency. Immunosuppressive, or antihistaminic drug intake.

PATIENTS AND METHODS

This case-control study was conducted on 100 children (70 pediatric patients with bronchial asthma and 30 age and sex-matched healthy control subjects). The patients were consecutively recruited from the pediatric pulmonology clinic at Sayed Galal university hospital during the period from September 2022 to May 2023 by sample random method.

Selected children were subjected to the following:

I. **Detailed history taking** (including Age of onset of asthma, asthma duration, asthma Drug therapy and Family history of asthma and other allergic diseases).

II. **Clinical examinations** (including Anthropometric measurements and Complete chest examination)

III. Lab evaluation including:

1. Complete blood count (CBC) and total differential WBCs: 2ml of venous blood are collected on EDTA (Ethylene diamine tetra acetic acid) tube whole blood for CBC on system ex xn350.
2. Stool analysis to exclude the presence of parasitic infestations.
3. Serum interlukin-6 level using ELISA Technique. The kit (Shanghai Sunred Biological Technology Co., Ltd, Shanghai China, Catalogue No. 201- 12-

0901) employs a double-antibody sandwich ELISA technique. The assay was carried out according to the manufacturer's instructions. The lower limits of detection were 0.5pg/ml.

IV. Pulmonary function tests were performed for each patient using Spirometry. Blue Cherry Screen spirometry will be done. This system will be calibrated for room temperature and pressure of saturated gas and volumes. Calibration will be performed on site before each testing session and according to the manufacturer's instructions. Spirometric indices included forced expiratory volume in the 1st second (FEV1), forced vital capacity (FVC), and FEV1/FVC were measured and will be expressed as percentage to predict values based on age, height, sex, and ethinicity.

Short-acting bronchodilator therapy was withheld for at least 8 hours and long-acting bronchodilator therapy for at least 24 hours.

Test validity: A valid test is composed of at least 3 acceptable maneuvers with consistent ("repeatable") results for both FVC and FEV1. Achieving repeatability during testing means that the difference between the largest and second largest values for both FVC and for FEV1 are within 0.15 L (150 ml). **(Weinberger et al., 2021)**

After pulmonary function test was done asthma severity was classified according to spirometry measures as shown in the following **(Figure 1)**.

Spirometry and Asthma, Patients 5 Years and Older

Asthma Severity	Spirometric Measurements			
	FEV_1 (Percentage Predicted), %	FEV_1: FVC (Absolute Ratios)[a] by Age		
		5–11 y	12–19 y	20–39 y
Normal	≥0.80	≥0.85	≥0.85	≥0.80
Mild persistent	≥0.80	0.80–0.84	≥0.85	≥0.80
Moderate persistent	0.60–0.79	0.75 ≤0.80	0.80 ≤0.85	0.75 ≤0.80
Severe persistent	<0.60	<0.75	<0.80	<0.75

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

PRAM score was done to all selected patients for assessment of asthma severity:

Emergent & Urgent Care Asthma Clinical Score (PRAM)*

Signs	0	1	2	3
Suprasternal Indrawing	absent		present	
Scalene Retractions	absent		present	
Wheezing	absent	expiratory only	inspiratory and expiratory	audible without stethoscope/ silent chest with minimal air entry
Air entry	normal	decreased at bases	widespread decrease	absent/ minimal
Oxygen saturation on room air	> 93%	90% - 93%	< 90%	

Severity Classification	PRAM CLINICAL Score
Mild	0 - 4
Moderate	5 - 8
Severe	9 - 12
Impending Respiratory Failure	12+ following lethargy, cyanosis,decreasing respiratory effort, and/or rising pCO_2

Figure (2): PRAM Score. ***(Martin et al., 2022)***

Statistical Analysis:

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data. One way ANOVA test was used to compare between more than two dependent groups of normally distributed variables. The Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in three groups for parametric and non-parametric variables respectively. Pearson's or Spearman's correlation coefficients were used for correlating normal and non-parametric variables respectively.

The (+) sign was considered as indication for direct correlation, also we consider values near to 1 as strong correlation & values near 0 as weak correlation. All statistical comparisons were three tailed with significance Level of P-value ≤ 0.05 indicates significant, p <0.001 indicates highly significant difference while, P> 0.05 indicates non-significant difference.

RESULTS

Our result will be demonstrated in the following Tables and figures.

Table (1): Demographic and clinical data of the studied groups

Variable	Asthma group (n = 70)	Control group (n = 30)	P-value
Age, years	11.07±2.9	10.3±2.2	.31
Male sex	14	8	.14
Weight, kg	32.07±7.9	28.5±4.3	.033
BMI	17.9±2.9	17.1±1.5	.449
Asthma onset	4.9±1.3	-	-
Asthma duration	6.9±2.09	-	-
Steroids	25	-	-

This table shows insignificant difference between the two studied groups regarding demographic and clinical data.

Table (2): Laboratory and pulmonary functions result in the studied case

Variable	Asthma group (n = 70)	Control group (n = 30)	P-value
FEV1/FVC	78.6±10.8	98.5±0.14	0.001
FVC	94.5±3.3	92.4±1.2	0.001
FEV1	76.7±13.4	91.1±1.2	0.002
Hb (gm/dL)	10.5±2.08	11.7±1.2	.21
WBC (1000/µL)	5626.8±2286.3	5638.6±2041.2	.827
Eosinophils (1000/µL)	466.4±134.3	343.01±67.54	0.001

BMI- Body Mass Index, FEV- Forced Expiratory Volume.

This table shows highly significant differences between asthmatic patient and control group regarding pulmonary function test and eosinophilia.

Table (3): Correlation between asthma severity and demographic and clinical data

Variable	Intermittent Asthma group (n =25)	Mild persistent asthma group (n = 20)	Moderate persistent asthma group (n = 9)	severe persistent asthma group (n =16)	P value	Correlation (r)
Age, years	8.7±2.4	10.5±2.3	13.2±1.09	14.1±1.03	0.1	+ve
Male sex	7	7	0	0	0.018	-
Weight, kg	26.6±6	28.7±5.4	40.2±5.06	40.1±2.4	.000	+ve
BMI	16.8±1.8	15.9±1.8	21.03±3.7	20.3±1.9	.000	+ve
Asthma onset years	4.8±1.3	5.1±1.4	4.8±1.1	5±1.2	0.428	-
Asthma duration years	3.8±2.4	5.04±2.3	8.3±1.8	9.1±1.4	0.001	+ve
Steroids	0	0	9	16	-	-ve

BMI- Body Mass Index, FEV- Forced Expiratory Volume.

This table shows +ve correlation between asthma severity and age, weight, body mass index, asthma duration.

Table (4): Correlation between asthma severity and lab and pulmonary function test

Variable	Intermittent Asthma group (n =25)	Mild persistent asthma group (n = 20)	Moderate persistent asthma group (n = 9)	Severe persistent asthma group (n =16)	P-value	Correlation (r)
FEV1/FVC	89.2±1.8	82.8±0.8	65.6±0.9	64.1±0.2	.000	-ve
FVC	97.8±1.6	94.8±1.08	94.3±2.5	90.2±0.87	.000	-ve
FEV1	89.2±1.8	82.8±0.8	62.9±2.5	57.8±0.7	.000	-ve
Hb (gm/dL)	10.9±2.5	10.2±1.3	9.9±2.1	10.4±1.9	.604	-
WBC (1000/µL)	6237.6±1963.4	4774.9±1641.3	7711.3±3225	4565±1865	.005	+ve
Eosinophils (1000/µL)	324.3±49.54	453.45±110.23	645.2±232.1	690.4±323.9	0.02	+ve
IL-6 *(pg./ml)*	39±18.21	93±56.91	95±46.16	272±116.35	.000	+ve

This is table shows +ve correlation between asthma severity and white blood cells, eosinophilia and interleukin-6 and -ve correlation between asthma severity and pulmonary function test.

Table (5): Serum IL-6 levels in patients and controls

Variable	Children with asthma	Controls	P value
IL-6 (pg/mL)			
Range	15-473.5	12-26.86	0.001
Median (IQR)	68(46.69, 164)	20(18.8, 23)	
Mean	115±110,48	20±3.77	

The table shows that median IL-6 was significantly elevated among patients compared with controls.

Table (6): Sensitivity and specificity of IL-6 as a Prediction of asthma severity

Variable	AUC	Cutoff	Sensitivity	Specificity
IL-6, pg/Ml	0.96	105 pg/mL	100%	83.3%

Interleuken-6 had AUC of 0.96, cut off level 105pg/ml and sensitivity 100% and specificity 83% in asthma severity.

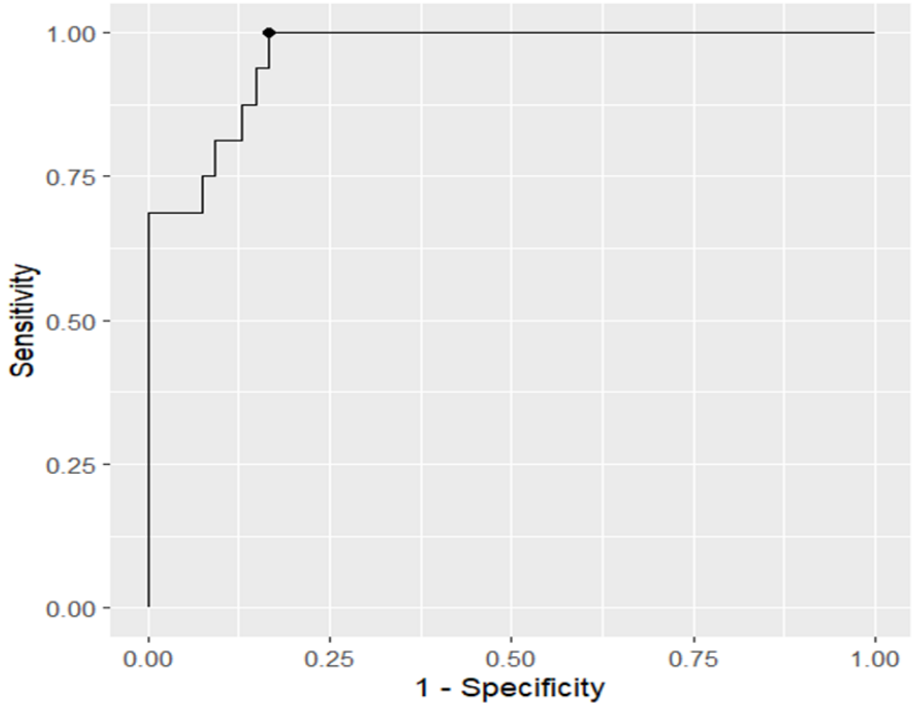

Figure (3): Roc Curve of IL-6

ROC curve shows that interleukin-6 has higher level sensitivity more than specificity among group of asthma severity.

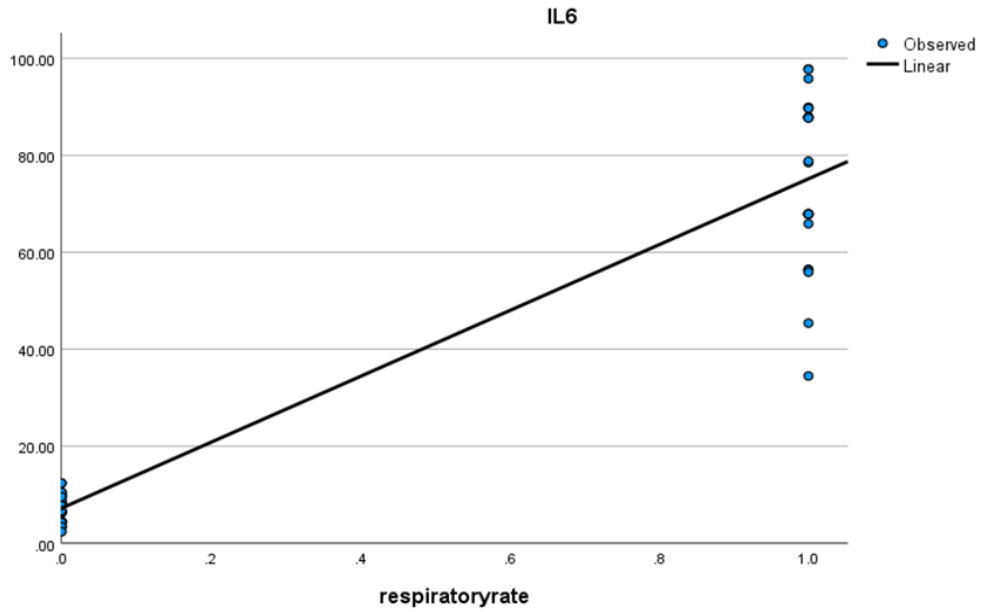

Figure (4): Regression blot curve for correlation between severity of asthma and IL-6

IL-6 showed a significant positive association with severe asthma (odds ratio = 1.033; 95% confidence interval = 1.019–1.048). This association remained after adjustment for age and weight (Adjusted odds ratio = 1.034; 95% confidence interval = 1.017–1.050).

DISCUSSION

IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis, acts as a pro-inflammatory mediator and acute-phase response inducer but has also been reported to have anti-inflammatory properties. While largely associated with T-cells and macrophages, it is increasingly apparent that the airway epithelium is a major source of IL-6 in the lungs. As such, IL-6 has strong biological plausibility as a major, pivotal cellular signaling modality in asthma. **(Kaur et al., 2020)**

In disease, circulating IL-6 is elevated in asthmatic patients and elevated in bronchoalveolar lavage fluid (BALF) of patients in whom asthma is clinically active and in patients with intrinsic asthma. **(Walsemann et al., 2023)**

In our study we observed significant correlations between serum IL-6 level and body mass index, this agreed with **Jackson et al. 2020**.

We found associations between serum IL-6 and other baseline laboratory measures .IL-6 was significantly correlated with total white blood cells and total blood eosinophils.

Our study showed that interleuken-6 was high in asthmatic patient. The level was postivly correlated with asthma severity and iterleuken-6 has –ve correlation with central airway function test such as PEFR and FEV1/FVC this in agree with **Chen et al., 2022** where he found +ve correlation between asthma severity and interleukin-6.

Childhood asthma is a heterogenous condition with multiple and phenotypes despite the use of treatments targeting type 2 inflammations, a subset of children continues to have asthma exacerbations. A cross-sectional analysis of adults enrolled into the National Institutes of Health/National Heart demonstrated that high plasma level of interleukin-6, reduced lung function, and greater asthma severity, dependent of body mass index (BMI). **(Chen et al., 2022)**

Prospective analysis of cohort study also found a strong association between plasma IL-6 and an increased rate of asthma exacerbations over a 3-year period. **(Ross et al., 2020)**

Very few studies exist evaluating peripheral blood IL-6 as a biomarker for asthma morbidity and severity in children/adolescents. **(Ross et al. 2020)**

Jackson et al. demonstrated that children with higher IL-6 had risk of asthma exacerbations during a 1-year longitudinal period. **(Jackson et al. 2020)**

In our study we found that serum IL6 was correlated with severity of asthma, using regression plot curve, which correlates between respiratory rate and IL6.

The mechanism through which peripheral blood IL-6 plays a role in asthma pathogenesis is largely unknown, although multiple processes have been suggested. **(Pijnenburg et al., 2020)**

Using ROC curve in our study, which showed that IL6 has higher levels of sensitivity more than specificity in the presence of tachypnea among bronchial asthma pediatric patients.

Our study emphasized the significant association between

IL-6 and the probability of experiencing an asthma exacerbation treated with systemic corticosteroids. A similar significant relationship was observed for number of exacerbations in Zoratti EM et al study which was published in 2015.

CONCLUSION

IL6 is a valid and predictive factor for bronchial asthma severity among children.

RECOMMENDATION

Longer follow-up is recommended to fully realize the impact of IL6, further studies are recommended to highlight the testing of IL6.

LIMITATIONS

- The small sample size, which may restrict the generalizability of the findings.
- Pulmonary function test assessment in early pediatric age was challenging which can lead to limit highly accurate values.
- There are several other limitations that are commonly associated with case-control studies. Confounding Variables: It can be challenging to account for all possible confounding variables, which are extraneous factors that may affect the correlation. Hence, we recommend that future research endeavors focus on larger sample sizes to enhance the robustness of the results and provide more comprehensive insights.

REFERENCES

1. **Bateman ED, Price DB, Wang H-C, Khattab A, Schonffeldt P, Catanzariti A, et al. (2022):** Short-acting β2-agonist prescriptions are associated with poor clinical outcomes of asthma: the multi-country, cross-sectional SABINA III study. European Respiratory Journal;59(5).
2. **Chen W, Chen S, Yan C, Zhang Y, Zhang R, Chen M, et al. (2022):** Allergen protease-activated stress granule assembly and gasdermin D fragmentation control interleukin-33 secretion. Nature Immunology; 23(7):1021-30.
3. **Hoban MT, Lipson SK, Zhou S, Eisenberg D, et al. (2021):** More than inconvenienced: The unique needs of US college students during the COVID-19 pandemic. Health Education & Behavior;48(1):14-9.
4. **Klain A, Dinardo G, Salvatori A, Indolfi C, Contieri M, Brindisi G, et al. (2022):** An Overview on the Primary Factors That Contribute to Non-Allergic Asthma in Children. Journal of Clinical Medicine.11(21):6567.
5. **Khaleva E, Rattu A, Brightling C,**

Bush A, Bossios A, Bourdin A, et al. (2023): Development of Core Outcome Measures sets for paediatric and adult Severe Asthma (COMSA). European Respiratory Journal.61(4).

6. **Lederer AM, Hoban MT, Lipson SK, Zhou S, Eisenberg D, et al. (2021):** More than inconvenienced: The unique needs of US college students during the COVID-19 pandemic. Health Education & Behavior;48(1):14-9.
7. **Kaur S, Bansal Y, Kumar R, Bansal G. A. (2020):** panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. Bioorganic & medicinal chemistry;28(5):115327.
8. **Li Y-C, Hsu H-HL, Chun Y, Chiu P-H, Arditi Z, Claudio L, et al. (2021):** Machine learning–driven identification of early-life air toxic combinations associated with childhood asthma outcomes. The Journal of Clinical Investigation. 131(22).
9. **Martin J, Townshend J, Brodlie M, et al. (2022):** Diagnosis and management of asthma in children. BMJ Paediatrics Open;6(1).
10. **Jackson DJ, Bacharier LB, Calatroni A, Gill MA, Hu J, Liu AH, et al. (2020):** Serum IL-6: A biomarker in childhood asthma? Journal of Allergy and Clinical Immunology; 145(6):1701-4. e3.
11. **Pijnenburg MW, Fleming L. (2020):** Advances in understanding and reducing the burden of severe asthma in children. The Lancet Respiratory Medicine; 8(10):1032-44.
12. **Reyes-Angel J, Kaviany P, Rastogi D, Forno E. (2022):** Obesity-related asthma in children and adolescents. The Lancet Child & Adolescent Health.
13. **Ren M, Li L, Jia J, Wei B, et al. (2023):** Association between PLA2G4A and P2RX7 genes and eosinophilic phenotype and environment with pediatric asthma. Gene;857:147182.
14. **Ross KR, Gupta R, DeBoer MD, Zein J, Phillips BR, Mauger DT, et al. (2020):** Severe asthma during childhood and adolescence: a longitudinal study. Journal of Allergy and Clinical Immunology; 145(1):140-6. e9.
15. **Sze E, Bhalla A, Nair P, et al. (2020):** Mechanisms and therapeutic strategies for non-T2 asthma. Allergy;75(2):311-25.
16. **Walsemann T, Böttger M, Traidl S, Schwager C, Gülsen A, Freimooser S, et al. ((2023):** Specific IgE against the house dust mite allergens Der p 5, 20 and 21 influences the phenotype and severity of atopic diseases. Allergy;78(3):731-42.
17. **Wang S, Wuniqiemu T, Tang W, Teng F, Bian Q, Yi L, et al. (2021):** Luteolin inhibits autophagy in allergic asthma by activating PI3K/Akt/mTOR signaling and inhibiting Beclin-1-PI3KC3 complex. International immunopharmacology; 94:107460.
18. **Weinberger SJ, Cowan KJ, Robinson KJ, Pellegrino CA, Frankowski BL, Chmielewski MV, et al. (2021):** A primary care learning collaborative to improve office systems and clinical

management of pediatric asthma. Journal of Asthma;58(3):395-404.

19. **Zanobetti A, Ryan PH, Coull B, Brokamp C, Datta S, Blossom J, et al. (2022):** Childhood asthma incidence, early and persistent wheeze, and neighborhood socioeconomic factors in the ECHO/CREW consortium. JAMA pediatrics. 176(8):759-67.