EFFECT OF MONTELUKAST AND HYDROCORTISONE ON SOME INFLAMMATORY MARKERS IN ASTHMA INDUCE SPRAGUE DAWLEY RATS

BY

ASOEGWU CHIDINMA JANE

(BMS1902374)

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

NOVMEBER, 2023

EFFECT OF MONTELUKAST AND HYDROCORTISONE ON SOME INFLAMMATORY MARKERS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS

BY

ASOEGWU CHIDINMA JANE

(BMS1902374)

A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc) DEGREE IN PHYSIOLOGY

NOVEMBER, 2023

CERTIFICATION

This is to certify that this project work on EFFECT OF MONTELUKAST AND HYDROCORTISONE ON SOME INFLAMMATORY MARKERS IN ASTHMA INDUCED SPRAGUE DAWLEY RATS were carried out by ASOEGWU CHIDINMA JANE and matriculation number BMS1992374

in partial fulfillment of the requirements for the award of the bachelor Science (B.Sc) Degree in the department of physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

________________________ __________________

Asoegwu Chidinma Jane **DATE**

Student

________________________ __________________

MRS U. A. PETERS **DATE**

project supervisor

________________________ __________________

PROF.O.K UCHE **DATE**

Head of Department

________________________ __________________

EXTERNAL EXAMINER **DATE**

DEDICATION

This project work is dedicated to God Almighty who is the giver of knowledge and wisdom. I also dedicate it to my family and friends for their care and support during this undergraduate journey of mine.

ACKNOWLEGEMENT

I wish to acknowledge Mrs. U.A Peters for her Patience and Keenness to supervise me through this research and also others who supported one way or the other.

TABLE OF CONTENT

TITLE PAGE……………………………………………………………….I

CERTIFICATION………………………………………………………….II

DEDICATION……………………………………………………………III

ACKNOWLEDGEMENT………………………………………………….IV

TABLE OF CONTENTS…………………………………………………..V

LIST OF TABLES…………………………………………………………VI

LIST OF FIGURES……………………………………………………….VII

ABBREVIATIONS……………………..…………………………….VIII - IX

ABSTRACT………………………………………………………………X

CHAPTER ONE

1.0 Introduction………………………………………………………………………1

1.1 Background study……………………………………………………………...1-3

1.2 Justification of study…………..……………………………………………3-4

1.3 Aim…………………………..………………………………………………4

1.4 Research Questions……………………………………………………………...4

1.5 Specific objectives……………………………………………………………….4

CHAPTER TWO

2.0 LITERATURE REVIEW………………………………………….……………5

2.1 Asthma…………………………………………………………………………5-6

2.1.1 Treatment of Asthma……………………………………………………...6.7

2.2 Montelukast……………………………………………………………………7

2.3 Hydrocortisone …………………………………………………………..7-8

2.4 Effect of Montelukast on Asthma……………………………………..……...8-9

2.5 Effect of Hydrocortisone on Asthma……………………………………...9-10

2.6 Inflammatory markers…………………………………………………......10

2.6.1 Inflammation …………………………………………………………..10-11

2.6.2 Biomakers……………………………………………………………………11

2.6.3 Interleukin-6 (IL-6)……………………………………………………11-12

2.6.4 High sensitivity C- protein (hs-CRP)……………………………………..12-13

2.6.5 Neutrophil to lymphocyte ratio (NLR)…………………………………...13-14

2.7 Effect of Asthma on inflammatory markers……………………………...14-17

2.8 Effect of inflammatory markers on Asthma ……………………………17-19

2.9 Effect of Montelukast on inflammatory markers ………………………19-23

2.10 Effect of Hydrocortisone on inflammatory markers……………………23-28

CHAPTER THREE

3.0 Research and Methodology…………………………………………………29

3.1 Materials…………………………………………………………………….29

3.2 Study population and location …………………………………...……….29

3.3 Management of Experimental animals …………………………………..29-30

3.4 Experimental design…………………………………………………...30-31

3.5 Collection of samples …………………………………………...……...32

3.6 Estimation and Analysis of inflammatory markers ……………………………………32

3.7 Statistical Analysis …………………………………………………….................32

CHAPTER FOUR

4.0 Results…. ……………………….……………………………………………...33

4.1 Result of the statistical analysis …………………….…………………………..….34-36

CHAPTER FIVE

5.0 Discussion And Conclusion………………………………………………………37

5.1 Discussion……………………………………………………………..……….37-38

5.2 Conclusion ………………………………………………………………………..38

REFERENCES…………..…………………………………………………………39-62

LIST OF FIGURES

Fig. 4.1: Chart show effect of montelukast and hydrocortisone on IL-6 in asthma induced Sprague Dawley rats

Fig. 4.2: chart show effect of montelukast and hydrocortisone on Hs-CRP in asthma induced Sprague Dawley rats

Fig. 4.3: chart show effect of montelukast and hydrocortisone on NEU/LYM ratio in asthma induced Sprague Dawley rats

ABBREVIATIONS

Interlukin-6	(IL-6)
High Sensitivity C-Reactive Protein	(HS-CRP)
Neutrophil to Lymhocye Ratio	(NLR)
Immunoglblin E	(IgE)
T Helper Type 2	(TH2)
Inhaled Corticosteroids	(ICS)
Airway Epithelial Cells	(AECs)
Leukotriene Receptor Antagonist	(LTRAS)
Adenosine 5’-Monophosphate	(AMP)
Hybridoma Growth Factor	(HGF)
Reactive Oxygen Species	(ROS)
Systemic Inflammatory Response	(SIRS)
Dendritic Cells	(DCs)
Chronic Obstructive Pulmonary Disease	(COPD)
Bronchoalveloar Lavage Fluid	(BALF)
Low Forced Expiratory Volume In 1 Second	(FEVI)
Post- Traumatic Stress Disorder	(PSTD)
Major Depressive Disorder	(MDD)
Lipopolysaccharide	(LPS)

Hydrocortisone	(HT)
Phytohaemoagglutinin	(PHA)
Enzyme-Linked Immunosorbent Assay	(ELISA)
Standard Error Of Mean	(SEM)

ABSTRACT

Asthma is a serious health and socioeconomic issue, affecting more than 300 million persons, with approximately 250,000 annual deaths. Asthma treatment has been based only on beta-2 agonists and corticosteroids, with a minimal role for other older drugs such as theophylline and chromosomes. The aim of this study was to investigate the effects of montelukast and hydrocortisone on some inflammatory markers in asthma-induced Sprague-Dawley rats.

Sprague-Dawley rats weighing between 180-250 g were divided into two (2) main groups; the Control group (rat feed with chow and water) and Test group (rat exposed to Ovalbumin and aluminum hydroxide to induce asthma and treated with Montelukast and Hydrocortisone). Inflammatory markers such as Interleukin-6 (IL-6), High Sensitivity C-Reactive Protein (Hs-CRP) and Neutrophil to Lymphocyte ratio (NLR) were assayed in blood plasma.

The result showed that Interleukin 6 of asthma induced Sprague Dawley rats was significantly ($p<0.05$) higher after treatment with Hydrocortisone (2.6293pg/ml) compared to montelukast (2.4983pg/ml) treatment, control (2.0588 pg/ml) and negative control (2.2173 pg/ml), A significant ($P<0.05$) difference in high sensitivity C-reactive protein was observed between hydrocortisone, control, negative control and montelukast while neutrophil to lymphocytes ratio of negative control was significantly different from control, montelukast and hydrocortisone treatments. A synergic effect of anti-inflammatory activity of Montelukast and hydrocortisone on asthma might exist.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Asthma is a serious health and socioeconomic issue, affecting more than 300 million persons, with approximately 250,000 annual deaths (Kudo *et al.,* 2013). Children have the greatest percentage of asthma compared with other generation groups (Centers for Disease Control and Prevention, 2011). The disease is considered as an inflammatory disease in the airway, leading to airway hyper-responsiveness, obstruction, mucus hyper production and airway wall remodeling. Airway remodeling, characterized by thickening of the airway wall, has profound consequences on the mechanics of airway narrowing and contribute to the chronic progression of the disease. Epithelial to mesenchymal transition plays an important role in airway remodeling. Asthma is classically recognized as a typical T helper type 2 (Th2) disease, with increased immunoglobulin E (IgE) levels and eosinophilic inflammation in the airway. Th2 cytokines modulates the airway inflammation, which induces airway remodeling. Nevertheless, in the light of further evidence, asthma has become recognized as more complex than a simple Th2 disease, which is characterized by IgE elevation and relatively eosinophilia (Kudo *et al.,* 2013). Th17 and Th9 cell sub type are known to contribute to the inflammation, enhance smooth muscle contraction or stimulating mast cells. Generally, most asthma start from childhood in relation to sensitization to common inhaled allergens, such as house dust mites, cockroaches, animal dander, fungi, and pollens. These inhaled allergens stimulate T helper type 2 (Th2) cell proliferation and, subsequently, Th2 cytokines production and release.

Asthma treatment has been based only on beta-2 agonists and corticosteroids, with a minimal role for other older drugs such as theophylline and chromones. Long-acting beta agonists with or without glucocorticoids are the most used drugs for asthma. Hydrocortisone is a glucocortiocoid that is used as an anti-inflammatory and immunosuppressive drug to treat a range of inflammatory and allergy conditions (Daley-Yates, 2015); Rhen and Cidlowski, 2005), including many pulmonary illness such as

asthma, chronic obstructive pulmonary disease, influenza and bronchitis (Barnes, 2010) and bronchopulmonary dysplasia in infants (Rademaker *et al.,* 2008; Morris *et al.,* 2019; Doyle *et al.,* 2010). Hydrocortisone, is the most structurally similar to endogenous cortisone, is the weakest agent in terms of glucocorticoid potency but possesses higher mineralocorticoids effect due to its higher mineralocorticoids effect, special attention needs to be given to the risk for higher blood pressure (Sule *et al.,* 2021). With a greater understanding of underlying pathophysiology and biomarkers of asthma, target-directed agents have become available for patients with severe- persistent asthma (Melissa and Tatyana, 2020). Montelukast and Hydrocortisone are good examples of anti-asthmatic agents.

Montelukast is a leukotriene inhibitor that is widely used to treat chronic asthma and allergic rhinitis by interfering with molecular signaling pathways produced by leukotrienes in a variety of cells and tissues throughout the human body that lead to tightening of airway muscles, production of aberrant pulmonary fluid (airway edema), and in some cases, pulmonary inflammation (McCarthy, 2023).

Montelukast has shown the best efficacy and safety profile, and it has become the most widely studied leukotriene receptor antagonist. Many studies have been published on the efficacy and safety of montelukast in asthma since this drug entered the market. Experimental studies, in vitro and in vivo, and clinical studies on large numbers of patients with asthma of different severity have clearly demonstrated that montelukast is able to modify the pathophysiological mechanisms of the disease, and to improve to some extent the clinical and functional manifestations of asthma. Studies of montelukast as mono therapy or in combination with other drugs, mainly inhaled corticosteroids (ICS), versus different comparator drugs have contributed to the positioning of montelukast in the different levels of asthma treatment, according to the Global Initiative for Asthma Guidelines. Montelukast may be used as mono therapy as an alternative to low-dose ICS (particularly in a step-down strategy) or in addition to ICS for improving clinical manifestations by an increase in anti-inflammatory effects and a sparing of corticosteroids. While the heterogeneity of asthma and how to better tailor treatment

according to the different phenotypes of asthma have received attention in the last few years, montelukast has proven to be particularly effective in exercise-induced asthma and in asthma associated with allergic rhinitis. Other phenotypes where montelukast is effective include asthma in obese patients, asthma in smokers, aspirin-induced asthma, and viral induced wheezing episodes.

Both Montelukast and Hydrocortisone have been found to be effective anti-asthma drugs, reducing severity of asthma symptoms and death. Although, there is scarce literature on the use and effectiveness of hydrocortisone for asthma (Sule *et al.,* 2021).

1.2 Justification of the Study

The activity of montelukast treatment in decreasing inflammatory markers or cytokines is well documented in literature. Hydrocortisone, a systemic glucocorticoid, is recommended in clinical practice guidelines for the treatment of acute asthma exacerbation based on evidence demonstrating reduced hospitalizations and improved outcomes after administration in the emergency department. Although prednisone and related oral preparations have been recommended previously, researchers have assessed Hydrocortisone as an alternative based on its longer biologic half-life and improved palatability. Future research is needed clarify these discrepancies and possibly measure other related adverse effects. The possibility of use of C-Reactive protein (CRP) as a marker of ongoing inflammatory activity and of effectiveness of treatment has been established. As such, high-sensitivity C-Reactive protein (hs CRP) can be used to show the effectiveness of montelukast and hydrocortisone in asthma. Neutrophil lymphocyte ratio (NLR) can indicate systemic inflammation and its assay can be helpful in differentiating asthma exacerbations. NLR in peripheral blood of asthmatic patients has been found to be higher than that of non-asthmatic people, and mean NLR is increased in asthma patients. However, being a novel potential biomarker and predictor of asthma exacerbations, more studies involving NLR in asthma is required to assess its suitability as a predictor of asthma exacerbations. Whereas most studies investigating the effect of montelukast and hydrocortisone on inflammatory markers aimed to only investigate several inflammatory markers at once, this study aims to compare some inflammatory

markers in asthma-induced Sprague-Dawley rats treated with montelukast and Hydrocortisone.

1.3 Aim of the Study

The aim of this study is to investigate the effects of montelukast and Hydrocortisone on some inflammatory markers in asthma-induced Sprague-Dawley rats.

1.4 Research Questions

i. Is montelukast or Hydrocortisone an effective treatment for asthma in Sprague-Dawley rats?
ii. Does treatment with montelukast and Hydrocortisone affect serum concentrations of a interleukin-6, hs CRP, and neutrophil lymphocyte ratio in asthma-induced rats?
iii. Is there a significant difference in serum concentrations of interleukin 6, high sensitivity CRP, and neutrophil lymphocyte ratio between the treated and control rats?

1.5 Specific Objectives

The specific objectives of this study include:

i. To induce asthma in some Sprague-Dawley rats.
ii. To determine the effect of montelukast and Hydrocortisone on serum concentrations of interleukin 6
iii. To determine the effect of montelukast and Hydrocortisone on serum concentrations of high-sensitivity C-reactive protein (hs-CRP)
Iv To determine the effect of montelukast and Hydrocortisone on serum concentrations of neutrophil-leukocyte ratio (NLR).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ASTHMA

Asthma is a chronic airway inflammatory disease affecting 350 million people worldwide (Vos *et al.,* 2015). This heterogeneous disease (Beasely *et al.,* 2015) is now studied at cellular and molecular levels, offering new opportunities for its prevention and control (Wenzel, 2012; Faijt and Wenzel, 2015). Repeated injury and remodeling of airway epithelial cells (AECs) and airway smooth muscle are the underlying pathology of severe asthma (Jiang *et al.,* 2021). In other words, asthma pathophysiology involves chronic inflammation of both large and small airways, as well as bronchial remodeling (Bara *et al.,* 2010). Allergens, infections, obesity, hormones, tobacco smoke, exercise, cold air, genetic mutation, and systemic eosinophilia are among the known factors that induce chronic airway inflammation leading to airway obstruction and hyper responsiveness and this immunopathophysiology of asthma involves the activation of both the innate and adaptive immune system to stimulate chronic airway inflammation (Melissa and Tatyana 2020). Asthma also involves various cells such as eosinophils, cytokines and mediators (Nimi, 2013). Some phenotypes of asthma have been identified from recognizable clusters of demographic, clinical, and pathophysiological characteristics, such as allergic/non-allergic, late-onset, fixed airflow obstructed, obesity-induced asthma and electronic nose-derived inflammatory phenotype (Brinkman *et al.,* 2019). Normal functioning of the human body requires coordination between a large amount of metabolite and studies have identified metabolic disorders and different metabolic phenotypes in patients with asthma (Reinke *et al.,* 2017).Individualized treatment based on the phenotype may be a new therapeutic direction for asthma. Asthma is classically recognized as a typical T helper type 2 (Th2) disease, with increased IgE levels and eosinophilic inflammation in the airway; Th2 cytokines modulates the airway inflammation which induces airway remodeling. Nevertheless, in the light of further evidence, asthma has become recognized as more complex than a simple Th2 disease, which is characterized by IgE elevation and relatively eosinophilia (Kudo *et al.,* 2013); Th17 and Th9 cell sub type are known to contribute to the inflammation, enhance smooth

muscle contraction or stimulating mast cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, especially at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increased in the existing bronchial hyper responsiveness to a variety of stimuli. Reversibility of airflow limitation may be incomplete in some patients with asthma (Mims, 2015).

2.1.1 Treatment of asthma

Asthma was previously understood to be a single diagnosis with standardized treatment for all patients; however, asthma is now accepted to be a heterogeneous, multifactorial disorders with a variety of genetic and environmental factors where targeted therapies result in improved asthma control and thus there is critical need to treat asthma more effectively (Melissa and Tatyana 2020). Chronic airway inflammation is a characteristic feature of asthma, and is therefore an important target for treatment (Tashkin *et al.,* 2019). Use of gluccorticosteroids (corticosteroids) has been investigated in both conditions (Tashkin *et al.,* 2019) with the first successful treatment of asthma with an oral corticosteroids (OCS) reported in 1950 (Crompton, 2006). With a greater understanding of underlying pathophysiology and biomarkers, target-directed agent have become available for patients with severe -persistent asthma (Melissa and Tatyana 2020). Treatment of asthma ranges from the use of corticosteroids down to long-acting Beta Antagonist, leukotriene modifier, Theophyllines, long-acting Muscarinic Agents, Macrolide Antibiotics, Vitamin D, Allergen Immunotherapy, Aspirin Desensitization, Dihydrofolate Reductase Inhibitor, hormones, Anti-IgE, CRTh2 Antagonist, Bronchial Thermoplasty etc (Melissa and Tatyana, 2020). The primary drugs for acute severe asthma include oxygen, corticosteroids, salbutamol (albuterol) and anticholinergics while the second-line drugs include heliox, magnesium sulfate, ketamine and inhalational anesthetics with future therapies including furosemide, leukotriene modifiers, antihistamines and phosphodiesterase inhibitors (Lucian *et al.,* 2001). Two drugs of focus

is this review is the Montekulast (a leukotriene modifier or antagonist) and Glucorticoids (a corticosteroids).

2.2 MONTELUKAST

Montekulast, the most widely used leukotriene-modifying agent (LTMA), is a selective leukotriene receptor antagonist and is currently indicated for prophylactic and chronic treatment of asthma, relief of symptoms of allergic rhinitis, and acute prevention of exercise-induced bronchoconstriction (Palijari *et al.,* 2022). Leukotriene antagonist (LTRAs) are effective in the treatment of persistent asthma, exercise-induced asthma, and aspirin-induced asthma; some patients respond to LTRA better than ICS, so a personalized approach to asthma pharmacotherapy is recommended (Sabin *et al.,* 2012). Montelukast significantly reduces mild, moderates and part of severe exacerbations in chronic mild to moderate asthma, but it has inferior efficacy to inhaled corticosteroids (ICs) (Zhang, 2014). Montelukast interferes with molecular signaling pathways produced by leukotrienes in a variety of cells and tissues throughout the human body that lead to tightening of airway muscles, production of aberrant pulmonary fluid (airway edema), and in some cases, pulmonary inflammation (McCarthy, 2023).

2.3 HYDROCORTISONE

Cortisol when supplied as a medication is known as hydrocortisone (Becker, 2001). Hydrocortisone is a glucocorticoid that is used as an anti- inflammatory and immunosuppressive drug to treat a range of inflammatory and allergy conditions (Daley-Yates, 2015; Rhen and Cidolowski, 2005), including many pulmonary illness such as asthma, chronic obstructive pulmonary disease, influenza and bronchitis (Branes, 2010) and bronchopulmonary dysplasia in infants (Rademaker *et al.,* 2008; Morris *et al.,* 2019; Doyle *et al.,* 2010). Hydrocortisone is an adrenocortical steroid that inhibits accumulation of inflammation cells at inflammatory cells at inflammation sites, phagocytosis, lysosomal enzyme release and synthesis, and release of mediators of inflammation while preventing or suppressing cell-mediated immune reactions and decreasing or preventing tissue response to inflammatory processes (‘H’, 2007). It is a corticosteroid with a potency of 1, relative sodium retention potency of 1 and a alf-life of 8-12 hours (Alangari

et al., 2014). Hydrocortisone, the most structurally similar to endogenous cortisone, is the weakest agent in terms of glucocorticoid potency but possesses higher mineralocorticoid effect and due to its higher mineralocorticoid effects, special attention needs to be given to the risk for higher blood pressure (Sule *et al.,* 2021). Side effect associated with the use of hydrocortisone just like other corticosteroids could include, hypothalamic-pituitary-adrenal axis suppression, physical appearance changes: moon facies, buffalo hump, central trunk, obesity, growth suppression, hirsutism, acne, insomnia, increased appetite, hyperglycemia, muscle wasting, reduced bone mineral density and osteoporosis, increased bruisability, immunosuppression etc (William, 2018).

2.4 EFFECT OF MONTELUKAST ON ASTHMA

Montelukast was effective in improving cough symptom, decreasing cough reflex sensitivity and alleviating eosinophilic airway inflammation in patients with cough variant asthma, and the antitussive effect and anti-eosinophilic airway inflammation were similar (Yi *et al.,* 2022). Montelukast is a recommended alternative for the treatment of asthma even after treatment with inhaled corticosteroids (ICS) alone or with ICS plus a long-acting β2-agonist (LABA) (Hoshino *et al.,* 2019). In this study eighty-seven patients with asthma were treated with budesonide and formoterol (640/8µg); then, the patients were randomly allocated to three group to receive oral montelukast (10mg/day), inhaled tiotropium (5µg/day), or no add-on the maintenance therapy for 48 weeks of which fractional exhaled nitric oxide (FeNO) and pulmonary function were measured, and quantitative computed tomography was performed with interpretation of results showing that montelukast may provide addictive benefits with respect to the pulmonary function and airway inflammation remodeling in patients with asthma (Hoshino *et al.,* 2019).

In 2013, Nimi reported an initial results that 4 weeks treatment with a leukotriene receptor antagonist (LTRA) montelukast exerted anti-inflammatory effect as provided by a decrease of sputum eosinophils, in addiction to attenuation of cough VAS and capsaicin cough sensitivity. Spirometer, airway responsiveness, and impulse oscillation indices (respiratory resistance and reactance) were unchanged with the results suggesting that the antitussive effect of montelukast in asthma might be attributable to its anti-inflammatory

ability rather than bronchodilation (Nimi, 2013). Direct examination of airway tissue confirms that montelukast decreases the number of eosinophils and mast cells in asthma (Tenero *et al.,* 2016). Eosinophils and mast cells play an essential feature in asthmatic patients (Seiko *et al.,* 2008).

Adverse effects reported to be associated with the use of Montelukast included agitation, anxiety, depression, sleep disturbance, hallucinations, suicidal thinking and suicidality, tremor, dizziness, drowsiness, neuropathies and seizures (Calapai *et al.,* 2014).

2.5 EFFECT OF HYDROCORTISONE ON ASTMA

Being a corticosteroid, hydrocortisone is an important drugs of chronic asthma management and it is an effective therapy in maintaining asthma control through anti-inflammatory effects on the airway while also reducing mobility and mortality from asthma (Raissy *et al.,* 2013). There is scarce literature on the use and effectiveness of hydrocortisone for asthma (Sule *et al.,* 2021). In a study published in 1974, pierson et al. used injectable hydrocortisone for asthma in children and found a beneficial effect of hydrocortisone compared to placebo group in terms of improvement in arterial hypoxaemia. Hydrocortisone was well tolerated with no significant adverse effects (pierson et al., 1974). Raimondi et al. Compared high (80mg/kg/day) versus moderate (6mg/kg/day) dose IV hydrocortisone given to 40 adult patients with asthma every 6hrs for 5 days. Their result showed no significant difference between the two study groups with spirometric evaluation (Raimondi *et al.,* 1986).

In a study to determine the effect of intravenous hydrocortisone on nocturnal airflow limitation in childhood asthma, hydrocortisone was given over a period of time in a double-blind randomized crossover design to selected numbers of subjects and FEVI, blood eosinophils and airway responsiveness to methacholine and adenosine 5’-monophosphate (AMP) were measured with result showing that substitution of lower endogenous values of cortisol with hydrocortisone, specifically improve long function al the nadir time point of circadian cortisol levels. Furthermore, a short period of hydrocortisone infusion reduced the number of circulating eosinophils, which can be

considered as a marker of inflammation, whereas it does not change the severity of airway hyper responsiveness obstruction in asthma (Landstra *et al.,* 2003).

Nocturnal airway obstruction occurs frequently in childhood asthma and result from increased airway inflammation (Landstra *et al.,* 2005). Hydrocortisone improved FEV in asthmatic children and this was not due to suppression of circulating peripheral blood mononuclear cell activation but rather due to its effect on a local lung tissue epithelial and/or fibroblasts thereby reducing airway inflammation and vascular leakage (Landstra *et al.,* 2005).

2.6 INFLAMMATORY MARKERS

2.6.1 INFLAMMATION

Inflammation is a complex and necessary component of an organism's response to biological, chemical or physical stimuli. Inflammation is a complex dynamic protective response to cell injury, infection via microbes, trauma, or toxins in the vascularized tissue. The causative agent is diluted, destroyed, or isolated and a sequential cascade of molecular events is set that leads to repairing, healing and reconstituting and the damaged tissue (Li *et al.,* 2007;Iwealewa *et al.,* 2007;Libby 2007;Delves *et al.,* 2006; Goldsby *et al.,* 2007; Guyton and Hall, 2001).

Inflammation play an important role in the mechanism of asthma pathophysiology (Holgate et al., 2015)

Inflammation is mainly divided into two types; acute and chronic inflammation. If the inflammation winds up in less than 48hours, then it is acute inflammation (eg., abscess) and if the rest for greater than 48hours (weeks, month, or years) then it is chronic.

Acute inflammation is initiated by mostly resident dendritic cells (DCs), macrophages, kupffer cells, histocytes, and mastoctyes bearing pattern recognition receptors (PRRs) on their surface, which identify pathogen-associated molecular patterns (PAMPs) expressed exclusively on the outer surface of the pathogen.

Chronic inflammation leads to tissue destruction, fibrosis, and necrosis. Neutrophils are the major cell types in acute inflammation while mononuclear cells (mostly lymphocyte, macrophages, and plasma cells) participate in chronic inflammation. The outcomes of

acute inflammation are resolution, abscess, and ulcer (fistula, sinus)and may expand to a chronic inflammation.

2.6.2 BIOMARKER

A biomarker is a parameter (chemical, physical, or biological) that can be applied to detect and compute the progress of disease or the result of treatment in preclinical research and clinical diagnosis. More significantly, a biomarker points out a change in state or expression of proteins, peptides, genes, and other factors that associate with the progression or risk of a disease, initial diagnosis, drug response, susceptibility of the patients to a given treatment, drug target identification or disease intervention (Mayeux 2004; Kumar and Sarin 2009).

Cytokines are actual components that signal the immune system to respond in a specific area of the body that is being invaded. They are involved in cell signaling and ultimately leave a chemical trial, leading the different molecules involved in healing to the area of injury (Black, 2004). when cytokines are elevated, this indicates that the body's immune system has been triggered and there is inflammation occurring somewhere in the body.

Inflammatory markers, we are focusing on during this project are interleukin-6 (IL-6), C-Reactive Protein (CRP) and Neutrophil-Leukocyte ratio (NLR).

2.6.3 INTERLEUKIN-6 (IL-6)

INTERLEUKIN-6 (IL-6) is a pleotropic cytokine that acts as a pro-inflammatory mediator and acute-phase response inducer, it is increasing apparent that the airway epithelium is a major source of IL-6 in the lungs (Poynter and Irvin, 2016). IL-6 is produced downstream from recognition of microbial and damaged-associated molecular patterns by pattern recognition receptors of the innate immune system (Hirano, 1998).

IL-6 is also a product of adipocytes and linked to the well known systemic inflammatory biomarker, CRP. IL-6 has strong plausibility as a major pivotal cellular signaling modality in pathways (Hirano, 1998). Dysregulated continual synthesis of IL-6 plays a pathological effect on chronic inflammation and autoimmunity. The distinct functions of IL-6 were studied and given distinct names based on their biological activity, for example, the name hepatocyte-stimulating factor (HSF) on the effect of acute phase protein

synthesis on hepatocyte, the name hybridoma growth factor (HGF) on the enhancement of growth of fusion cells between plasma cells and myeloma cells (Hirano *et al.,* 1986). Human IL-6 is made up of 212 amino acids, including a 28-amino acid signal peptide ,and its gene has been mapped to chromosome 7p21, the core protein is ~20 kDa, glycosylation accounts for the size of 21-26 kDa of natural IL-6.

IL-6 reduces the production of fibronectin, albumin, and transferrin, it also involved in the regulation of serum iron and zinc levels via control of their transporters. As for their serum iron, IL-6 induces hepcidin production, which blocks the action of iron transporter ferroportin 1 on guts and reduces serum iron levels (Nemeth *et al.,* 2004).

IL-6 can promotes differentiation or proliferation of several nonimmune cells, it also promotes T-follicular helper-cell differentiation (Ma et al., 2012).

IL-6 attract white blood cells to the area that needs healing during inflammation and promotes accumulation of these cells (Barnes, 2011).

IL-6 is also considered one of the chief stimulators in the production of acute-phase proteins, which are plasma proteins whose concentration vary during acute inflammatory response (Gabay, 2006).

2.6.4 HIGH SENSITIVITY C-REACTIVE PROTEIN (hs-CRP)

C-REACTIVE PROTEIN (CRP) is an acute-phase protein that is produced mainly by hepatocyte and is an inflammatory marker (Bouchard, 2021). Many inflammatory markers have multiple effect as a result of a single gene and its includes CRP because it has both pro-inflammatory and anti-inflammatory effects (Black, 2004). CRP can mediate the pathways by binding to a specific carbohydrates, when CRP binds to the carbohydrates it causes opsonization of the target cell. Opsonization is the process by which a cell is coated so that macrophages, a type of white blood cell, can easily engulf and break down the cell through the process called phagocytosis.

IL-1, IL-6, and TNF are important cytokines that stimulate the activation of CRP (Shi *et al.,* 1998).IL-6 is involved in phosphorylation of the transcription factor through gene activation and promotes the synthesis of CRP. Moreover activation of CRP can stimulate the vascular smooth muscle cells to secrete more TNF-α (Virchow *et al.,* 1996).

CRP is involved in complement activation and activation of phagocytic cells to eliminate bacteria and damaged cells. Increased CRP levels may indicate several conditions such as infection, cancer, and autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis and myocardial infarction.

CRP levels monitoring is useful in the evaluation of early inflammation and efficacy of treatment in acute-phase illness (Hirano, 1996). High sensitivity assays for CRP (hs-CRP) which measures the very low amount of CRP in the blood (below 0.2mg/L) has been used to evaluate the systemic inflammation and prognostic marker for diabetes mellitus and cardiovascular diseases. CRP levels are associated with higher frequency of bronchial hyper-responsiveness and respiratory impairment due to systemic inflammation (Silvestri *et al.,* 2006). CRP test are beneficial in predicting the risk of patients who have a 5-10% chances of having a heart attack in the next ten years (Mayo clinic, 2017). Regarding its relationship to their cytokine, CRP production in the liver is regulated by IL-6 (Black, 2004). An increase in CRP leads to an increase in other biomarkers as well, such as IL-6 and TNF-α levels.

2.6.5 NEUTROPHIL TO LYMPHOCTYE RATIO (NLR)

NEUTROPHIL TO LYMPHOCTYE RATIO (NLR), is a simple ratio between the neutrophil and leukocyte counts measured in peripheral blood. It is a biomarker which conjugates two faces of the immune system: the innate immune response, mainly due to neutrophils and adaptive immunity, supported by lymphocytes (Song *et al.,* 2021).

Neutrophils are responsible for the first line of host immune response against invading pathogens, through different mechanism, including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins and the production and liberation of cytokines (Mortaz et al., 2018). Neutrophils also play an important regulatory role in adaptive immunity and are the main effector cells during the systemic inflammatory response (SIRS). As regulators of innate immunity, neutrophils recruit, activate and programme other immune cells, secreting an array of pro-inflammatory and immunomodulatory cytokines and chemokines capable of enhancing the recruitment and

effector functions of other immune cells, such as dendritic cells (DCs), B cells, NK cells, CD4, CD8 and T cells, as well as mesenchymal stem cells (Li *et al.,* 2019).

An isolated rise in neutrophil count and consequently, an elevated NLR can be observed in several conditions: bacterial or fungal infection (Lowsby *et al.,* 2014; Jiang *et al.,* 2019; Niu *et al.,* 2021), acute stroke (Li *et al.,* 2021), myocardial infarction (Lee *et al.,* 2016), atherosclerosis (Adamstein *et al.,* 2021), severe trauma (Park J, 2017), cancer (Lee *et al.,* 2021), post-surgery complications (fest *et al.,* 2019) and condition characterized by tissue damage that activates SIRS. This is because the early hyperdyanmic phase of infection is characterized by a pro-inflammatory state, mediated by neutrophils and other inflammatory cells. The increase in NLR following physiological stress could confer on NLR the role of marker of acute stress earlier than other laboratory parameters (eg., white blood cell count, bacteremia, C-reactive protein, CRP).

The neutrophil-to-leukocyte ratio (NLR) is a marker of a chronic inflammatory state (Takeshita *et al.,* 2017). NLR was described in evaluating obstructive sleep apnea, allergic rhinitis and chronic obstructive pulmonary disease (COPD) (Pilaczyska *et al.,* 2019; Ha *et al.,* 2020). Moosman et al. reported age and sex specific pediatric reference value for the NLR and other blood count-derived biomarkers from 60,682 patients, from birth to 18 years of age. They found that a major changes in laboratory analysis from the neonatal period to adolescence were characterized by higher values directly after birth, which gradually decreased during the first two years of life. In patient with asthma, NLR was also significantly increased; 20.6% of asthmatic patients presented with NLR> 97.5th percentile (Moosmann *et al.,* 2022). In various recent studies, the NLR has been evaluated as a probable predictor of inflammation periods in chronic diseases 6-8 and many other disease (Wilar, 2019; otela et al., 2018). In the pathogenesis of asthma, cytokines cause an increase in neutrophils (Fu *et al.*, 2014), and lymphocyte have a central role as a conductor of the immune orchestra that contributes to asthma (Holgate, 2012).

2.7 EFFECT OF ASTHMA ON SOME INFLAMMATORY MARKERS

Some inflammatory markers such as Interleukin-6, C-reactive Protein and Neutrophil to Lymphocyte ratio perform well in asthma diagnosis (Zhan *et al.,* 2020).

INTERLEUKIN-6 is a small size glycoprotein (21 KDa) produced by cells from the innate immune system (eg macrophages, mast cells and dendritic), it is also secreted by non-leukocytes such as endothelial cells, fibroblasts and a number of malignant cells (Hirano, 1998). Increased levels of IL-6 in serum have been found in asthmatic patients (Yokoyama *et al.,* 1995). More importantly a study examining IL-6 in bronchoalveloar lavage fluid (BALF) has shown increased levels of IL-6 in active asthmatic patients compared with the levels in healthy nonsmokers subjects, stable asthmatic and non-asthmatic patients receiving mechanical ventilation (Tillie *et al.,* 1999).

Increased levels of IL-6 in BALF from patients with "intrinsic" asthma compared with the levels in patients with allergic asthma have also been reported suggesting that IL-6 may plat a role beyond patients with allergic asthma which accounts for about 50% of all asthmatics. A recent study in mild allergic asthmatic patients with no exuberant airway inflammation has shown increased IL-6 levels in induced sputum compared with the levels in healthy subjects (Neveu et al., 2010). Although of increased levels of IL-6 in the lung of asthmatics patients have been associated with the presence of inflammatory cells (eg. Macrophages, neutrophil) that can secrete this cytokine and an over-expression of IL-6 in bronchial epithelial cells in patients (adult and children) with asthma (Marini *et al.,* 1992; Stadnyk , 1994). The IL-6 gene is constitutively expressed in primary lung epithelial cells from naive mice and exposure to allergen can trigger the production of this cytokine prior to the recruitments of inflammatory cells (Neveu *et al.,* 2011).

C-REACTIVE PROTEIN is one of the most characteristics markers of the inflammatory process, it is well known that CRP increases during infection and autoimmune disorders (Szalai, 2004). CRP is a useful tool for detecting systemic inflammation in asthma (Takemura *et al.,* 2006; Fujita *et al.,* 2007).

C-reactive protein is an acute phase protein synthesized by hepatocytes in response to pro-inflammatory cytokines during inflammatory/infection processes (Laun and Yao,

2018) and the level of CRP is elevated in asthma (Monadi et al., 2016; Cazzoletti et al., 2010).

CRP is an indicator of inflammation among asthmatic patients with pulmonary function tests, total serum IgE, and peripheral white blood cell (WBC) counts.

Several factors other than the presence of asthma affect serum levels of hs-CRP, aging also result in elevated hs-CRP levels, and smoking cessation leads to a reduction in hs-CRP level(Nakamura and Yamashita, 2002).

Regarding the possibility of using hs-CRP measurement to assess subclinical, systemic inflammation in asthma , Kony *et al.,* in a population -based study has shown association of increased levels of serum hs-CRP with a high frequency of airway hyper-responsiveness and low forced expiratory volume in 1 second (FEV1) (Kony et al., 2004).

In a study, Savykosi *et al.,* demonstrated that serum hs-CRP levels were significantly higher in patients with mild-to moderate asthma than in healthy control (Savykoski *et al.,* 2004).

An increased in C-reactive protein levels measured by high-sensitivity assays may be associated with airflow obstruction and airway inflammation, and may serve as a surrogate marker of airway inflammation in asthma.

In asthma, the importance of airway inflammation has been established, the airway inflammation, systemic inflammation may exist in asthma (Jousilahti *et al.,* 2002; Buyukozturk *et al.,* 2004). Another recent multicentric epidemiological study showed that serum hs-CRP levels were increased in non allergic asthma (Olafsdottir *et al.,* 2005).

NEUTROPHIL TO LYMPHOCTYE RATIO (NLR) is a marker of a chronic inflammatory state. Moosman *et al* reported that NLR was significantly increased in several diseases with inflammatory components such as appendicitis; 65.7% of patients with appendicitis showed a higher NLR than the calculated upper reference limit. In patients with asthma, NLR was also significantly increased; 20.6% of asthmatic patients with NLR> 97.5th percentile (Moosman *et al.,* 2022). Zhang et al reported an elevated NLR in adult patients with neutrophilic asthma (Zhang *et al.,* 2014).

NLR is a biomarker of generalized inflammation in pulmonology, Hung *et al.* Showed that the NLR values are a reasonable and easy to use marker for asthma (Huang *et al.,* 2020).

NLR may reflect nuerohumoral activation, renal dysfunction, inflammatory disease, chronic or acute systemic inflammation, cytokines in the pathogenesis of asthma cause an increased in neutrophils. The neutrophil to lymphocyte ratio represent a cost effective and easily available, it is higher in patient with asthma.

Neutrophil to lymphocyte ratio is use as a prognostic factor indicating an accelerated inflammatory response in several diseases (Takeshita *et al.,* 2017).

2.8 EFFECT OF INFLAMMATORY MARKERS ON ASTHMA

INTERLEUKIN-6(IL-6) is a pleotropic cytokine that acts as a pro-inflammatory mediator and acute phase response inducer but has been reported to have anti-inflammatory proprieties. IL-6 is a product of adipocyte and linked to the well known systemic inflammatory biomarker, CRP. IL-6 has strong biological plausibility as a major, pivotal cellular signaling modality in pathways germane to asthma (Hirano, 1998). IL-6 levels are also affected by viral infections and obesity (Hirano , 1998; Rincon and Irvin 2012), two important comorbid factors in causing exacerbations and severity, respectfully. IL-6 is elevated in asthmatic patients and elevated in bronchoalveloar lavage fluid (BALF) of patients in whom asthma is clinically active (Tillie *et al.,* 1999).

ILMARINEN *et al.*(Ilmarimen *et al.,* 2016). Report a study of a 12 year follow up of 170 patients with adult-onset asthma. The adult-onset asthma patients often present a female predilation, without a history suggestive of early-onset asthma and most importantly carries a poor prognosis characterized by inadequate asthma control and frequent exacerbation (Wenzel, 2006; Haldar *et al.,* 2008). ILMARINEN *et al*. (Ilmarinen *et al.,* 2016) postulated that the high prevalences of comorbities might be related to systemic inflammation or a spillover of the inflamed status of the lung into the circulation. It report a high rate of systemic inflammation caused by high circulating levels. Increased levels of IL-6 in BALF from patients with "intrinsic" asthma compared with the levels in patients with allergic asthma have also been reported (Virchow *et al.,* 1996) suggesting

that IL-6 play role beyond patients with allergic asthma which only accounts for 50% of all asthmatics.

C-REACTIVE PROTEIN is an acute-phase protein that is produced mainly by hepatocytes and is an inflammatory marker (Bouchard, 2021).

CRP is involved in complement activation and activation of phagocytic cells to eliminate bacteria and damaged cells (Shi *et al.,* 1998). CRP levels monitoring is useful in the elevation of early inflammation and efficacy of treatment in acute-phase illness (Hirano, 1998). High-sensitivity assay for CRP (hs-CRP) which measures the very low amount of CRP in the blood (below 0.2mg/L) has been used to evaluate the systemic inflammation and prognostic marker for diabetes mellitus and cardiovascular disease. A population based study by kony *et al.* Suggested that increased CRP levels are associated with higher frequency of bronchial hyper-responsiveness and respiratory impairment due to systemic inflammation (Silvestri *et al.,* 2006). Therefore hs-CRP could be used to detect systemic inflammation and the severity of asthma. An increased in CRP level in patients with asthma may be caused by an underlying chronic inflammatory state of the patients or infections (Bouchard ,2021).The study by wood *et al.* (2001) demonstrated that asthmatic patients with neutrophilic airway inflammation had increased systemic inflammation (Wood *et al.,* 2001). Therefore, CRP can be a systemic marker for patients with neutrophilic asthma. Moreover, the study by Ko et al. (2003) also reported that hs-CRP had a significant association with patients with asthma with high neutrophil and low eosinophils (Ko *et al.,* 2003). The study also showed that hs-CRP levels were correlated with small airway obstruction in patients diagnosed with neutrophilic asthma. Several studies showed that hs-CRP can be used as a surrogate marker for evaluation and monitoring of asthma, estimation of disease severity, and response to corticosteroids therapy (Shi et al., 1998; Hashino and Nakamura, 2002). Several studies have shown that increase levels of hs-CRP are associated with respiratory symptoms of asthma such as wheeze, dyspnea after effort and nocturnal cough (Kips, 2007). Therefore, patients with asthma with exacerbation or that is poorly controlled may have elevated CRP concentration compared to patients with stable asthma or healthy control. C-reactive

protein is the most commonly used marker to evaluate the systemic inflammation in patients with asthma (Fu et al., 2014),

NEUTROPHIL TO LYMPHOCTYE RATIO (NLR), a marker of chronic inflammation, is known as a simple widely available and inexpensive index measure from complete blood counts (Hung *et al.,* 2020).In various recent studies, the NLR has been evaluated as a probable predictor of inflammation periods in chronic disease 6-8 and many other disease (Wilar, 2019; Otela *et al.,* 2018). In pathogenesis of asthma, cytokines cause an increase in neutrophils (Fu *et al.,* 2014), and lymphocyte have a central role as a conductor of the immune orchestra that contribute to asthma (Holgate, 2012).

NLR was described in evaluating obstructive sleep apnea, allergic rhinitis and chronic obstructive pulmonary disease (COPD). In patient with asthma, NLR was also significantly increased 20.6% of asthmatic patients presented with NLR> 97.5^{th} percentile (Moosman *et al.,* 2022). However Zhang *et al.* reported an elevated NLR in adult patients with neutrophilic asthma (Zhang *et al.*, 2014). In a study assessing the association of blood cell count parameter with severe asthma exacerbations, NLR was correlated with an increased risk for a severe exacerbation. Dogru et al., reported that NLR was higher by 17% in asthmatic patients than in non-asthmatic controls (Dogru *et al.,* 2016). In another recent study that included 89 patients with asthma, the combination of NLR-albumin ratio was suggested as a clinical biomarker for asthma exacerbation in children (Pan et al., 2023). Mean NLR is higher in asthmatic children compared to control group.

2.9 EFFECT OF MONTELUKAST AND HYDROCORTISONE ON INFLAMMATORY MARKERS

In a study aimed at determining the short term effect following montelukast treatment on female Wister rats, a total of twenty female rats were assigned into two equal groups. The control group received vehicle 0.2ml of saline solution (0.9%), while treated group received 10mg/kg/day of Montelukast diluted in saline 0.9% for five consecutive days. Results showed that there was significant reduction in the biomarkers of Interleukin-6, C-

reactive protein and Neutrophil to lymphocyte ratio when compared to the control group (Al-Hayder *et al.,* 2022).

The effect of Montelukast on some inflammatory, which are Interleukin-6, C-reactive protein and Neutrophil To Lymphocyte ratio.

INTERLEUKIN-6 (IL-6) is a pivotal pro-inflammatory cytokine that plays a central role in the body's immune response. Elevated levels of IL-6 have been associated with various inflammatory conditions, including respiratory disease, rheumatoid arthritis, and cardiovascular disorders (Gabay *et al.,* 2010; Kishimoto, 2005).

Montelukast, a leukotriene receptor antagonist, is commonly prescribed for the management of asthma related inflammatory conditions. This essay aims to explore the effect of Montelukast on Interleukin-6 and its potential implications for Inflammation and health.

Montelukast primarily acts by inhibiting the action of leukotrienes, potent mediators of inflammation. Leukotrienes can induce the release of pro-inflammatory cytokines, including IL-6, from immune cells such as macrophages and monocytes (Peters-Golden *et al.,* 2006). By blocking leukotriene receptors, Montelukast interferes with these inflammatory processes potentially affecting IL-6 levels.

Several studies have investigated the impact of Montelukast on Interleukin-6 levels. For instance, a study conducted by smith *et al.* In 2013 (Smith *et al.,* 2013) observed a significant reduction in IL-6 concentration In in patients with asthma who received Montelukast treatment. Similarly, a study by Johnson et al. In 2016 (Johnson *et al.,* 2016) demonstrated lowered IL-6 levels in an experimental model of airway inflammation following montelukast administration. A patient was taking 10mg oral montelukast daily for allergic rhinitis

Furthermore, another study by Davis *et al.* In 2018 (Davis *et al.,* 2018) examine IL-6 changes in patients with allergic rhinitis treated with monetulast. They reported a statistically significant decrease in IL-6 levels following Montelukast treatment. These findings collectively suggest that montelukast may effectively modulate IL-6 levels.

The ability of Montelukast to reduce Interleukin-6 levels holds significant clinical implications, especially in the context of inflammatory disease. Elevated IL-6 is not only a marker of inflammation but is also directly involved in disease pathogenesis contributing to tissue damage and immune dysregulation (Gabay *et al.,* 2010; Kishmoto, 2005). In conditions where IL-6 is a key player, such as rheumatoid arthritis, lowering IL-6through Montelukast treatment may indicate improved disease control and reduced symptom severity.

Moreover, IL-6 is associated with cardiovascular disease, where inflammation plays a critical role in atherosclerosis development (Ridker *et al.,* 2000). Reducing IL-6 levels with Montelukast could have implications for cardiovascular risk reduction in patients with comorbid asthma and cardiovascular conditions.

C-REACTIVE PROTEIN (CRP) is a well established biomarker of Inflammation in the body. Elevated CRP levels have been associated with various inflammatory conditions, including cardiovascular disease, rheumatoid arthritis, and respiratory disorders (Ridker *et al.,* 2002; Torzewski *et al.,* 2000). Montelukast, a leukotriene receptor antagonist, is commonly prescribed for managing asthma and related inflammatory conditions. This essay aims to explore the effect of Montelukast on C-reactive protein levels and its potential implications for inflammation and health.

Montelukast primarily acts by inhibiting the action of leukotrienes, potent mediators of inflammation, leukotrienes induce the release of pro-inflammatory molecules, including CRP, from immune cells such as macrophages and leukocytes (Peters-golden *et al.,* 2006). By blocking leukotriene receptors, Montelukast interrupts the signaling cascade leading to CRP production, exerting an anti-inflammatory effect.

Several studies have investigated the impact of Montelukast on CRP levels. For instance, a study of conduct by Brown *et al.* In 2010 (Breon *et al.,* 2010) observed a significant reduction in CRP concentrations in patients with asthma who received Montelukast treatment. Similarly, a study by Garcia *et al*. In 2014 (Garcia *et al.,* 2014) demonstrated lowered CRP levels in an experimental model of airway inflammation following Montelukast administration.

Furthermore, another study by Wilson *et al.* 2018 (Wilson *et al.,* 2018) reported reduced CRP levels in individual with allergic rhinitis treated with Montelukast. Additionally, a recent chronic obstructive pulmonary disease (COPD) found that Montelukast treatment was associated with statistically significant decrease in CRP levels. These findings collectively suggest that Montelukast may effectively modulate CRP levels in various inflammatory conditions.

The ability of Montelukast to reduce CRP levels holds significant clincial implications, especially in the context of cardiovascular disease. Elevated CRP is a marker of increased cardiovascular risk, as it is associated with inflammation of the arteries (Ridker *et al.,* 2002). By lowering CRP levels, Montelukast may contribute to the reduction of cardiovascular risk in patients with comorbid asthma and cardiovascular conditions.

Furthermore, CRP is a nonspecific marker of inflammation and is often elevated in various other inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus (Gabay and Kushner 1999). Monteukast's potential to mitigate CRP-related inflammation suggest its utility beyond asthma management and warrant further exploration in these disease contexts.

NEUTROPHIL TO LYMPHOCTYE RATIO (NLR) is a marker of chronic inflammation, is known as a simple, widely available and inexpensive index measured from complete blood counts (Huang et al., 2020). NLR may reflect ethnicity, neurohumoral activation, renal dysfunction, thyroid disease, hepatic function, inflammatory diseases, chronic or acute systemic inflammation (Balta *et al.,* 2015). Cytokines in the pathogenesis of asthma cause an increase in neutrophils

Montelukast, a highly selective antagonist of cysteinyl leukotriene (CysLT) receptors, is widely used in the treatment of bronchial asthma, primarily as an adjustment to corticosteroids (Anonymous, 2004; Currie *et al.,* 2005; Diamant and van der Molen, 2005; Riccioni *et al.,* 2007).

The therapeutic activity of Montelukast is achieved through antagonism of CysLT-mediated bronchoconstriction, increased vascular permeability and mucus secretion, following release of these mediators, mainly from monocyte/macrophages, eosinophils,

mast cells and basophils as well as by anti-inflammatory actions targeting type 2 helper CD4 T-lymphocyte (Peters-Golden and Henderson, 2007). Montelukast has been reported to reduce NLR and modulate airway remodeling in patients with chronic asthma, compatible with an extended spectrum of anti-inflammatory activity (Henderson *et al.,* 2006; Muz *et al.,* 2006). Montelukast has also be reported to possess therapeutic activity in other diseases such as chronic obstructive pulmonary disease, a disorder that is believed to be of neutrophilic aetiology (Rubinstein *et al.,* 2004; Celik *et al.,* 2005). Although they do not produce CysLTs, neutrophils do possess receptor for Cysteinyl Leukotriene (LTC_4 and LTD_4),activation of which triggers relatively modest pro-inflammatory responses in these cells (Larfars *et al.,* 1999; Zhu *et al.,* 2005). Interference with neutrophil activation by CysLTs released from other cell types, such as monocytes, macrophages, mast cells or eosinophils, may therefore underlie the neutrophil-lymphocyte therapeutic efficacy of montelukast. Montelukast is a leukotriene D_4 (LTD_4) and a leukotriene E_4 (LTE_4) receptor antagonist. Inhibition of LTD_4 and LTE_4 reduces the bronchoconstriction and increased pulmonary vascular edema associated with an acute asthma attack. Montelukast is used chronically as a prophylactic agent for the treatment of asthma. Montelukast is not used to treat an acute asthma attack. It is administered orally, its oral bioavailability is 64% with 90% plasma protein binding (Bennett, 2012).

2.10 EFFECT OF HYDROCORTISONE ON INFLAMMATORY MARKERS

The effect of Hydrocortisone on some inflammatory, which are Interleukin-6, C-reactive protein and Neutrophil To Lymphocyte ratio.

INTERLEUKIN-6 (IL-6) is a pleotropic cytokine that has effect on inflammation, immune response and cardiovascular disease. IL-6 is elevated in bronchoalveloar lavage fluid and has a strong biological plausibility, pivotal cellular signaling modality in pathways. (Hirano, 1996). Hydrocortisone, is a glucocorticoid secreted by adrenal cortex (Jung *et al.,* 2014). Hydrocortisone is used to treat immune, inflammatory and neoplastic conditions (Sarkar *et al.,* 2015)

Hydrocortisone primarily acts by inhibiting the action of Glucocorticoid as a potent mediators of inflammation, glucocorticoid has a major role in the treatment of autoimmune, allergic disease. It is becoming increasily clear that lymphocyte and macrophages, as primary effectors of cellular and humoral immune responses, are the focal points for many of the clinically useful properties of glucocorticoids (Baxer and Harris 1995).

Several studies have investigated the impact of Hydrocortisone on IL-6. For instance, a study of conduct by Smith *et al.* In 1997 (Smith *et al.,* 1997) observed that hydrocortisone alone did not raise IL-6 production at any of the concentration tested. Peripheral blood mononuclear cells (PBMCs) were stimulated with Lipopolysaccharides stimulated PBMC was found to be dramatically sensitive to the presence of hydrocortisone. A dose-dependent hydrocortisone induced inhibition of IL-6 production was observed Lipopolysaccharide (LPS) induced IL-6 production by Mo in a dependent fashion. IL-6 was detected in Mo supernatants as early as 2 hours after stimulation, with peak IL-6 production observed by 16 hr. An attenuation of the inhibitory effect of Hydrocortisone occurred with greater concentrations of LPS and with the delay of Hydrocortisone addition until after LPS. However, there was no correlation between the quantity of IL-6 produced by Mo and the level of Hydrocortisone inhibition. The inhibitory effect of Hydrocortisone was greater if LPS, rather than IL-1β, were used as a stimulus to induce IL-6 production. The EC50 of LPS-induced IL-6 production by Hydrocortisone was 2.0 × 10-7*M.* The inhibitory effect of Hydrocortisone on LPS-stimulated IL-6 production was Glucocorticoids specific and receptor mediated because:

(i)equivalent inhibition was not observed with other endogenous steroids and

(ii) equimolar amounts of the GC antagonist RU 486 blocked the GC-mediated effect (Lisa *et al.,* 2002).

Hydrocortisone inhibited LPS-stimulated IL-6 production in a dose-dependent manner. However there was a wide variation in the response to hydrocortisone, namely from steroid-sensitive to steroid-resistant. The dramatic anti-inflammatory and immunosuppressive effects on glucocorticosteroids can be life-saving in autoimmune

diseases. The present findings suggested that there existed the difference in susceptibility to glucocorticosteroids even among normal subjects, providing some implications for the drug treatment, and also gave further evidence that there may exist an immunoregulatory feedback circuit between the immune and neuroendocrine system (Hirooka *et al.,* 1992).

Elevated levels of pro-inflammatory cytokines, especially interleukin-6 can mediates the greater risk for cardiovascular disease in individual with post-traumatic stress disorder (PSTD), particularly in those with comorbid major depressive disorder (MDD).

Sustained elevations of overnight IL-6 levels and relatively decreased sensitivity to hydrocortisone. IL-6 levels are not consistently elevated in either PSTD or MDD, although PSTD is associated with super sensitivity to glucocorticoids (Jessica *et al.,* 2010).

In a study aimed at determining the short term effect following montelukast treatment on female Wister rats, a total of twenty female rats were assigned into two equal groups. The control group received vehicle 0.2ml of saline solution (0.9%), while treated group received 10mg/kg/day of Montelukast diluted in saline 0.9% for five consecutive days. Results showed that there was significant reduction in the biomarkers of Interleukin-6, C-reactive protein and Neutrophil to lymphocyte ratio when compared to the control group (Al-Hayder *et al.,* 2022).

C-REACTIVE PROTEIN (CRP) is a well known inflammatory marker synthesized by hepatocytes. This marker increases in systemic inflammation such as diabetes, cardiovascular diseases, collagen vascular disease and obesity.

C-reactive protein is a marker of inflammation that predicts incidence of myocardial infarction, stroke and peripheral arterial disease.

In present study, the serum levels of hs-CRP of asthmatic patients with and without inhaled corticosteroids (ICS) treatment were compared to those of healthy controls, and the correlation between serum CRP levels (systemic inflammatory marker) and sputum eosinophils (airway inflammatory marker) was evaluated.

C-reactive protein is a non-specific systemic inflammatory marker protein (Alexandrov *et al.*, 2015), which is a typical acute-phase reaction protein produced and secreted by

hepatocyte (Mayer *et al.,* 2005). The level of CRP is associated with inflammatory response and repair level of the body, when inflammation occurs, CRP level rises rapidly in short time, with the recovery and remission of the disease.

Glucocorticoids (hydrocortisone) are an important class of regulatory molecules in the body (Conway *et al.,* 2011). They have important regulatory roles in development, growth, metabolism and immune function (Oppert et al., 2005; Xiang et al., 2018; Annane *et al.,* 2009). In clinical practice, they are widely used as effective anti-inflammatory and immunosuppressive agents (Laviolle at al., 2012). However, possible adverse reactions include secondary infections, gastrointestinal bleeding, and hyperglycemia (Shapiro *et al.,* 2010).

In the present study, hydrocortisone was administered to high inflammation and low inflammation rats to assess its treatment effect on immune body.

A female Wister rats was established by lipopolysaccharide (LPS) injection. The rats were divided into control (Control), high-inflammation treated with hydrocortisone (HT), high-inflammation non treatment (HNT), low inflammation treated with hydrocortisone (LT) and low-inflammation non-treatment groups according to the levels of serum C-reactive protein (CRP), Serum CRP levels decreases in high inflammation female wistar rats following glucocorticoid therapy.

A previous study demonstrated that high dose of glucocorticoids increases the possibility of double infection and mortality, therefore the use of high doses of glucocorticoids was prohibited (Bone *et al.,* 1987). Hydrocortisone treatment significantly attenuates pro-inflammatory cytokines in patients (Zhao *et al.,* 2018). The early application of low-dose hydrocortisone can improve patients prognosis (Greenberg and Coursin, 2014).

The present study grouped rats according to the level of blood inflammatory factors to evaluate the effectiveness of hydrocortisone in the treatment of high-inflammation and low-inflammation. CRP rat serum level decreased significantly following 7 days of glucocorticoids treatment, with the arterial pressure, heart rate and liver function of the female Wister rats.

The present study analyzed the effect of the glucocorticoids on inflammatory markers in female wistar rats and provided a theoretical basis for the use of glucocorticoids in the treatment of asthma induced female Wister rats according to the level of inflammation.

NEUTROPHIL TO LYMPHOCTYE (NLR) is an index calculated by dividing the neutrophil count by lymphocyte count, which maybe associated with cortisol levels. Studies show that the NLR is associated with disease activity and prognosis in patients with cardiovascular disease and autoimmune disease (He *et al.,* 2017; Passardi *et al.,* 2016; Qin *et al.,* 2016; Xu *et al.,* 2019; Condado *et al.,* 2016).

The neutrophil to lymphocyte is also calculated as a simple ratio between the neutrophil and lymphocyte count measured in peripheral blood, is a biomarker which conjugates two faces of immune system: the innate immune response due to neutrophils and adaptive immunity supported by lymphocyte (Song et al., 2021).

The leukocyte count is frequently performed to evaluate inflammatory conditions while the lymphocyte count will decrease.

Neutrophil to lymphocyte ratio is a prognostic factors in many diseases such as inflammatory disease, cardiovascular disease and cancer.

Glucocorticoids were recognized to modulate the immune system in 1924, when it was observed that adrenalectomy caused theymic hyperplasia in female Wister rat (Jaffe, 1924). cortisone acetate and adrenocorticotropic hormones were used to treat rheumatoid arthritis (Hench et al., 1950). Numerous studies have evaluated the effects of corticosteroids on inflammatory biomarkers in animals (mice) and in human cells cultured in vitro (Ashwell and Vacchio, 2000; Leung and Bloom, 2003; Gills *et al.,* 1979). Dale and colleague demonstrated that hydrocortisone administered intravenously to healthy patients at doses as high as 400mg for two consecutive days was well tolerated and cause rapid mobilization of neutrophils (Dale *et al.,* 1975), 400mg hydrocortisone transiently decreased recirculating concanavalin- A responsive peripheral (T) cells, while 100mg of hydrocortisone did not induce lymphopenia in humans (Fauci and Dale, 1975).

The proliferative response of Wister rats peripheral blood lymphocyte and spleen cells to phytohaemagglutinin (PHA) was studied following treatment with multiple doses of

hydrocortisone. Hydrocortisone in vitro caused a dose-dependent depression of blood lymphocyte response to PHA but enhanced the response of spleen cells when the steroid was added after the mitogen. Blood lymphocyte from multiple dose-treated rats has an enhanced response to PHA (Mansour *et al.,* 1978)

CHAPTER THREE

3.0 RESEARCH DESIGN AND METHOLOGY

3.1 MATERIALS

Sprague Dawley rats

Feed (top grower mash)

Cages

Chloroform

Nebulizer

Dissection materials

Stop watch

Electronic scale

Picric acid

Universial bottle

Drinking bowls

Syringes

Cotton wool

Gloves

Orogastric tubes

Aluminium hydroxide

Ovalbumin

Hydrocortisone

Montelukast

Saline solution.

3.2 EXPERIMENTAL ANIMALS

This study will involve the use of female Sprague-Dawley rats. They would all receive proper animal care in line with international guidelines for experimental animal handling. Ethical approval obtained from the College of Medical Sciences ethics board. The Sprague-Dawley rats will be housed in a clean, cool and sterile environment at 22^0C room temperature, they will be kept in cages, where they would have access to food and water *ad libitum* throughout the period of the experimental process.

3.3 STUDY DESIGN

Sprague-Dawley rats weighing between 180-250 g will be divided into two (2) main groups; the Control group and Test group. The test group will be further divided into four (4) subgroups treated with anti-asthmatic drugs. All the groups will consist of twenty (20) rats each (n=5). The control group will receive normal rat chow and water throughout the experimental period while the test groups will be exposed to concentrations of Ovalbumin (OVA, egg albumin grade II) and aluminum hydroxide to induce asthma after which they would be treated with Montelukast and Hydrocortisone.

Experimental protocol/design

Experiment will be carried out in phases

Phase 1

Rats will be acclimatized into their new environment for two (2) weeks after which they will be divided into four (4) groups of twenty (20) rats per group.

Test groups

GROUP 1: Control

GROUP 2: Asthmatic not treated

GROUP 3: Asthmatic and treated with montelukast

GROUP 4: Asthmatic and treated with hydrocortisone

All test groups will be induced with asthma following the modified guideline outlined by (Bai *et al*., 2019; Wu *et al*., 2019). All experimental groups (2, 3 and 4) will be sensitized 1 mg OVA and 200 mg aluminum hydroxide dissolved in 0.9 saline on day 0 and 7, challenged with OVA (1 % w/v, adsorbed in 0.9 saline) twice weekly from day 7 of treatment until the last day.

For the challenge rats will be placed in a plastic chamber measuring 70 cm in diameter and 40 cm in length connected to a Medel family nebulizer (REF 90543 MEDEL FAMILY SILVER AEROSOL) with aerosol delivery of 0.28 ml/min.

Normal control group will be sensitized and challenged with intraperitoneal injection and aerosolized saline respectively. Asthma induction will be verified first week after challenge with evidence of neutrophilia and eosinophilia in all test groups compared to control (Bai *et al.,* 2019; Wu *et al*., 2019).

Phase 2

After confirmation of asthma in all test groups, treatment would begin with 5 mg/kg hydrocortisone (Ekpo and Pretorius, 2008), 10 mg/kg Montelukast.

During this period of treatment, the blood pressure of all groups will be monitored via non-invasive tail-cuff method.

Phase 3

At the end of drug administration, all animals will be euthanized. Blood and tissue samples collected for biomarker assay and histology.

Phase 4

Inflammatory markers such as Interleukin-6 (IL-6), C-Reactive Protein (HS-CRP) and Neutrophil to Lymphoctye ratio (NLR) were assayed in blood plasma

Blood Pressure Measurement

i. Day 7 of treatment
ii. Day 28 (last day) of treatment

3.4 DETERMINATION OF INFLAMMATORY MARKERS

The inflammatory cytokine Interleukin(6) (IL-6), C-reactive Protein (HS-CRP) and Neutrophil to lymphoctye ratio (NLR) were measured by enzyme-linked immunosorbent assay (ELISA) in the serum using spectrophotometry-based kits. All the plates were analysed on an automated plate reader.

Histological analysis

Dissected heart, aorta, portal vessels and kidney tissues will be washed with normal saline, immersed in 10% (v/v) formaldehyde solution, and embedded in paraffin. Tissue specimens will be sectioned and stained with haematoxylin and eosin (H & E) dye. Images of selected sections captured at 10X magnifications using a zoom digital camera (Thakur *et al*., 2019).

3.5 BLOOD SAMPLING AND SERUM ISOLATION

Blood will be collected from retro-orbital plexus of rats under light diethyl ether anaesthesia in a non-heparinized tube. They will be kept at room temperature for 30 min, followed by centrifugation at 5000 rpm (rounds per minute) for 15 min, and serum

isolated by aspiration. The separated serum will be stored at frozen for the later quantitative determination of some biomarkers (Thakur *et al*., 2019).

Determination of total and differential cell counts

Total and differential leukocytes (Neutrophils, Eosinophils, Lymphocytes, and Monocytes) will be measured in the blood using a fully automated cell counter.

3.6 STATISTICAL ANALYSIS

All the data obtained from the experiments will be expressed as mean ± Standard Error of Mean (SEM). Statistical analysis performed by one way analysis of variance (ANOVA) for assessing differences amongst multiple groups, followed by Tukey's test using Graphpad Prism 8.1 software (Graphpad, San Diego, CA). $P < 0.05$ will be considered statistically significant.

CHAPTER FOUR

RESULTS

Figure 4.1 shows the effect of montelukast and hydrocortisone on IL-6 in asthma induced Sprague Dawley rats. Interleukin 6 of asthma induced Sprague Dawley rats was

significantly ($p<0.05$) higher after treatment with Hydrocortisone (2.6293pg/ml) compared to montelukast (2.4983pg/ml) treatment, control (2.0588 pg/ml) and negative control (2.2173 pg/ml).

The effect of montelukast and hydrocortisone on high sensitivity-creatinine reactive protein (Hs-CRP) in asthma induced Sprague Dawley rats is presented in figure 4.2. Hydrocortisone was 0.0053 mg/L), montelukast was 0.0033 mg/L compared to control (0.0050 mg/L) and negative control (0.0055 mg/L) on Hs-CRP in asthma induced Sprague Dawley rats. A significant ($P<0.05$) difference in Hs-CRP was observed between hydrocortisone, control, negative control and montelukast

Figure 4.3 show effect of montelukast and hydrocortisone on Neutrophil/Lymphocyte ratio in asthma induced Sprague Dawley rats. Neutrophil/Lymphocyte ratio of asthma induced Sprague Dawley rats were lower after montelukast (0.3002) and hydrocortisone (0.3340) treatment treated when compared to control (0.5892) while negative control had lower Neutrophil/lymphocytes ratio (0.1673) compared to montelukast and hydrocortisone treatment and negative control. Neutrophil/lymphocytes ratio of negative control was significantly different from control, montelukast and hydrocortisone treatments.

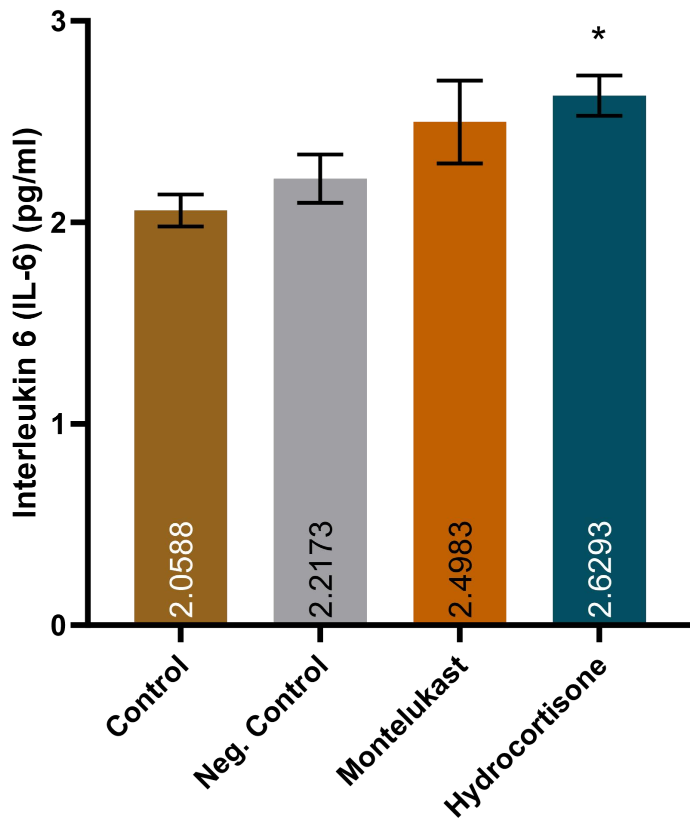

Fig. 4.1: chart show effect of montelukast and hydrocortisone on IL-6 in asthma induced Sprague Dawley rats

Result shows a statistically significant difference in IL-6 $p<0.05$

$^{*}p<0.05$ compared to control

$^{\alpha}p<0.05$ compared to negative control

$^{\phi}p<0.05$ compared to montelukast

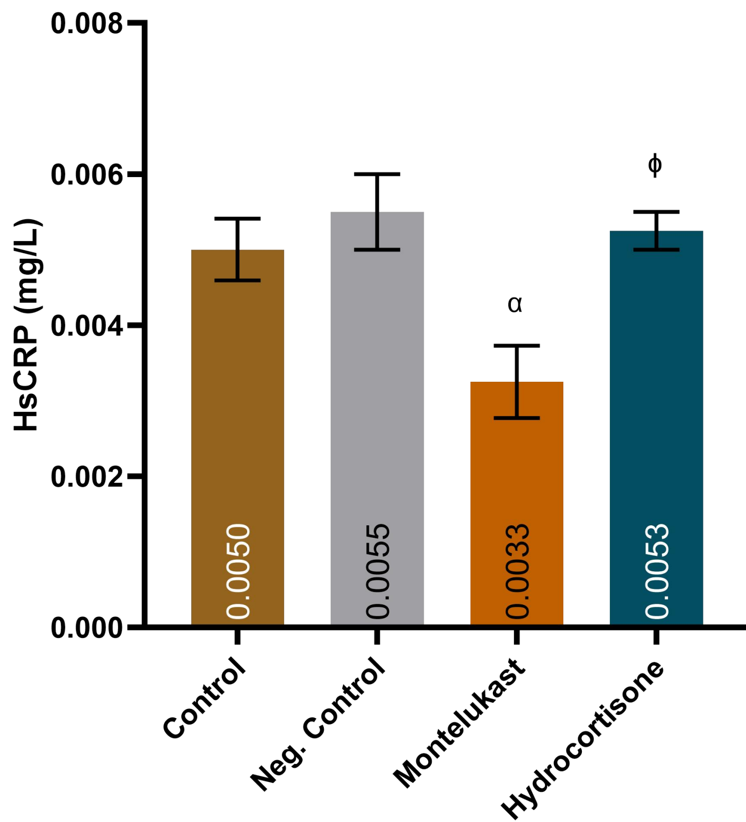

Fig. 4.2: chart show effect of montelukast and hydrocortisone on Hs-CRP in asthma induced Sprague Dawley rats

Result shows a statistically significant difference in Hs-CRP $p<0.05$

$^{*}p<0.05$ compared to control

$^{\alpha}p<0.05$ compared to negative control

$^{\phi}p<0.05$ compared to montelukast

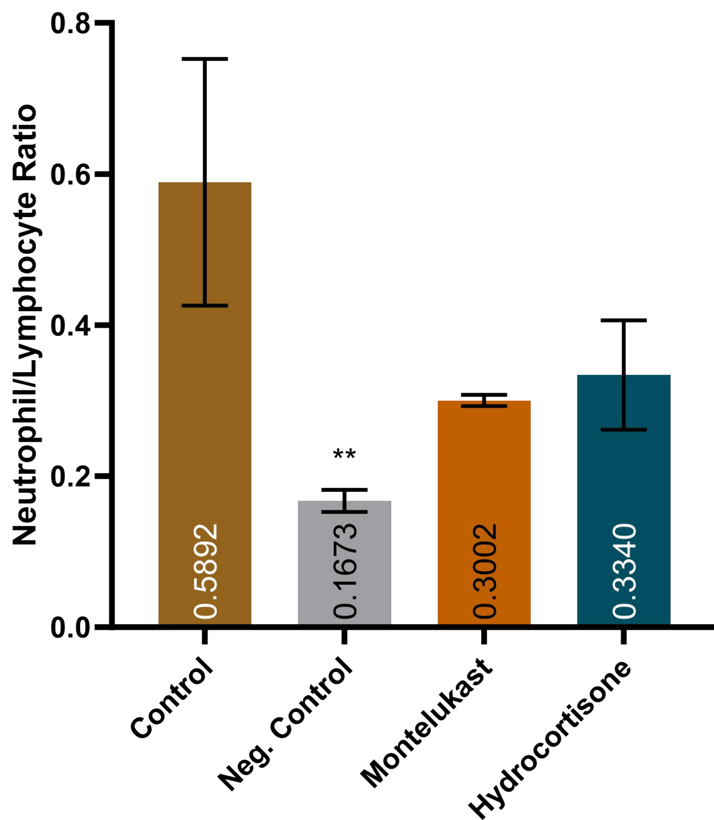

Fig. 4.3: chart show effect of montelukast and hydrocortisone on NEU/LYM ratio in asthma induced Sprague Dawley rats

Result shows a statistically significant difference in NEU/LYM ratio $p<0.05$

$^{*}p<0.05$ compared to control

$^{\alpha}p<0.05$ compared to negative control

$^{\phi}p<0.05$ compared to montelukast

CHAPTER FIVE

DISCUSSION

The effects of montelukast and hydrocortisone on some inflammatory markers in asthma-induced sprague-dawley rats was investigated. The findings of this study shows that hydrocortisone was more effective in the treatment of asthma as a high increase in Interleukin 6 was observed in the asthma induced Sprague Dawley rats. This finding agrees with the study of Li *et al.* (2020) who reported that survival with hydrocortisone was increased significantly in rats challenged with *B. anthracis*. The effectiveness of hydrocortisone in this study has be reported by Horvath and Wanner (2006) to be likely relates to the direct effect endogenous or exogenous glucocorticoids have on inhibiting nuclear factor-κβ (NF-κβ), a transcription factor central to host cell pro-inflammatory responses. Hirooka *et al*. (1992) reported that a wide variation in the response to hydrocortisone treatment of patients with asthma which agrees with the findings of this study. Montelukast treatment used in this study was also found to have high effects on IL-6 in asthma induced Sprague Dawley rats. Anti-inflammatory effects of montelukast of asthma-induced rat was reported by Rostevanov *et al*. (2022) which agrees with this study. This finding is also supported by the report of Basyigit *et al*. (2010) who reported a high increase in interleukin 6 of asthma induced rats treated with 2mg/ml concentration of montelukast.

Montelukast and hydrocortisone were observed to have a significant effects of high sensitivity-creatinine reactive protein in asthma induced Sprague Dawley rats. This finding is supported by previous report of Nagai et al. (1983) who reported a high sensitivity-creatinine reactive protein in asthma induced-rats. Hydrocortisone has been reported by Azari et al. (2015) to be effective in the treatment of asthma which is confirmed in this study. According to the reports of Tsutomu (2014) combination of hydrocortisone therapy and oral antihistamine help improves high sensitivity-creatinine reactive protein in asthma patients

Neutrophil/lymphocytes ratio of asthma induced Sprague Dawley rats were lower after montelukast and hydrocortisone treatment. This finding agrees with the study of Olnes *et*

al. (2016) who reported that hydrocortisone exerts differential effects on B and T lymphocytes and natural killer cells in humans. Mansour, A and Nelson (1978) reported that after multiple doses of hydrocortisone serum enhanced blood lymphocyte responses to phytohaemagglutinin. Anderson et al. (2009) reported that montelukast inhibits neutrophil pro-inflammatory activity which was observed in this study. Beneficial therapeutic effects of montelukast have been reported for diverse diseases in which neutrophils play a pathogenetic role, including chronic obstructive pulmonary disease, respiratory bronchiolitis, cystic fibrosis and atherosclerosis (Celik et al., 2005; Fitzgerald and Mellis, 2006; Riccioni et al., 2007).

5.1 Conclusion

Montelukast and hydrocortisone used in this study has proven to have significant inflammatory effects against asthma-induced sprague-dawley rats with hydrocortisone proven to be most effective. Furthermore, a synergic effect of anti-inflammatory activity of Montelukast and hydrocortisone on asthma might exist.

REFERENCES

Adamstein, N.H., MacFadyen, J.G., Rose, L.M., Glynn, R.J., Dey, A.K., Libby, P., Tabas, I.A., Mehta, N.N. and Ridker, P.M. (2021). The neutrophil–lympho cyte ratio and incident atherosclerotic events: Analyses from five contemporary randomized trials. *European Heart Journal* **42**:896–903.

Alangari, A. A. (2014). Corticosteroids in the treatment of acute asthma. *Annals of Thoracic Medicine* **9**(4):187–192.

Al-Athari, A.H. Aldhalmi, A.K. and Al-Hindy, H. (2022). Association of High-Sensitivity C-Reactive Protein and Vitamin D with Bronchial Asthma. *Open Access Macedonian Journal of Medical Sciences* **10**(2):931-936

Alexandrov, P.N., Kruck, T.P. and Lukiw, W.J. (2015). Nanomolar aluminum induces expression of the inflammatory systemic biomarker C-reactive protein (CRP) in human brain microvessel endothelial cells (hBMECs). *Journal of Inorganic Biochemistry* **152**:210–213.

Al-Hayder, M., Almiah, Z., Al-Mayyahi, R. and Hussein, Q. (2022). Short-term effects of the anti-asthmatic drug, montelukast, in female rats. *Bulletin of Pharmaceutical Sciences* **14**:70-77

Anderson, R., Theron, A., Gravett, M.C., Steel, H., Tintinger, G.C. and Feldman, C. (2009). Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism. *British Journal of Pharmacology* **156**: 105–115

Annane, D., Bellissant, E., Bollaert, P.E., Briegel, J., Confalonieri, M. and Meduri, G.U. (2009). Corticosteroids in the treatment of severe sepsis and septic shock in adults: A systematic review. *Journal of America Medical Association* **301**:2362–2375.

Azari, O., Kheirandish, R., Azizi, S., Abbasi, M., Chaman, S. and Bidi, M. (2015). Protective effects of hydrocortisone, vitamin c and e alone or in combination against renal ischemia-reperfusion injury in rat. *Iran Journal of Pathology* **10**(4): 272 – 280

Bai, F., Fang, L., Hu, H., Yang, Y., Feng, X. and Sun, D. (2019). Vanillic acid mitigates the ovalbumin (OVA)-induced asthma in rat model through prevention of airway inflammation. *Bioscience, Biotechnology and Biochemistry*, **83**(3): 531–537.

Balta, I., Ozlem, E., Pınar, O., Ihsan, U., Semsettin, K., Seval, D. K. and Meral, E. (2015). Epub **54**(6):662-666.

Bara, I., Ozier, A., Tunon, de Lara J.M., Marthan, R., Berger, P. (2010). Pathophysiology of bronchial smooth muscle remodelling in asthma. *European Respiration Journal* **36**(5):1174-1184.

Barnes, P.J. (2010), Inhaled Corticosteroids. *Pharmaceutical* **3**(3):514-540

Barnes, P.J. (2010). Inhaled Corticosteroids. Pharmaceuticals. Basel, Switzerland. pp.514–540.

Barnes, T.C. Marina, E., Anderson, Robert, J. and Moots, D. (2011). The Many Faces of Interleukin-6: The Role of IL-6 in Inflammation, Vasculopathy, and Fibrosis in Systemic Sclerosis. *International Journal of Rheumatology* **72**: 160-168

Basyigit, I., Sahin, M., Sahin, M., Yildiz, F., Boyaci, H., Sirvanci, S. and Ercan, F. (2010). Anti-inflammatory effects of montelukast on smoke-induced lung injury in rats. *Multidisciplinary Respiratory Medicine* **5**(2): 92-98

Beasley, R., Semprini, A. and Mitchell, E. (2015). Risk factors for asthma: is prevention possible? *Lancet*. **386**:1075–1085.

Benson, M., Berrill, W., Cruickshank, J., & Sterling, G. (1978). A comparison of four beta-adrenoceptor antagonists in patients with asthma. *British Journal of Clinical Pharmacology* **5**(5): 415–419.

Bilal, A., Farooq, J. H., Kiani, I., Assad, S., Ghazanfar, H. and Ahmed, I. (2016). Importance of Mean Red Cell Distribution Width in Hypertensive Patients. *Cureus* **8**(11): 45-49.

Black, S. (2004). *Journal of Biological Chemistry* 279(47) 48487-48490

Blair, D. R., Lyttle, C. S., Mortensen, J. M., Bearden, C. F., Jensen, A. B., Khiabanian, H., Melamed, R., Rabadan, R., Bernstam, E. V., Brunak, S., Jensen, L. J., Nicolae, D., Shah, N. H., Grossman, R. L., Cox, N. J., White, K. P. and Rzhetsky, A. (2013). A nondegenerate code of deleterious variants in mendelian loci contributes to complex disease risk. *Cell* **155**(1): 70–80.

Bone, R., Fisher, C.J., Clemmer, T.P., Slotman, G.J., Metz, C.A. and Balk, R.A. (1987). A controlled trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. *New England Journal of Medicine* **317**:653–658.

Bouchard, J. (2021). Asthma, C-reactive protein (CRP), hypersensitivity inflammation, steroids. *England Medical Journal of Allergy Immunology* **6**(1) 53-60.

Brinkman, P., Wagener, A.H., Hekking, P.P., Bansal, A.T., Maitland-van der Zee, A.H., Wang, Y., Weda, H., Knobel, H.H., Vink, T.J., Rattray, N.J., D'Amico, A., Pennazza, G., Santonico, M., Lefaudeux, D., De Meulder, B., Auffray, C., Bakke, P.S., Caruso, M., Chanez, P., Chung, K.F., Corfield, J. and Dahlén, S.E. (2019). Identification and prospective stability of electronic nose (eNose)-derived inflammatory phenotypes in patients with severe asthma. *Journal of Allergy and Clinical Immunology* **143**(5):1811-1820.

Büyüköztürk, S., Gelincik, A.A. and Genç, S. (2004). Acute phase reactants in allergic airway disease. *Tohoku Journal Experimental Medicine* **204**:209–213.

Calapai, G., Casciaro, M., Miroddi, M., Calapai, F., Navarra, M. and Gangemi, S. (2014). Montelukast-induced adverse drug reactions: a review of case reports in the literature. *Pharmacology* **94**(1-2):60–70.

Celik, P., Sakar, A., Havlucu, Y., Yuksel, H., Turkdogan, P. and Yorganioglu, A. (2005). Short-term effects of montelukast in stable patients with moderate to severe COPD. *Respiratory Medicine* **99**: 444–450.

Celik, P., Sakar, A., Havlucu, Y., Yuksel, H., Turkdogan, P. and Yorganioglu, A. (2005). Short-term effects of montelukast in stable patients with moderate to severe COPD. *Respiratory Medicine* **99**:444–450.

Center for Health Statistics, N. (2011). *Health, United States, 2011*.

Centers for Disease Control and Prevention (CDC). (2011). Vital signs: asthma prevalence, disease characteristics, and self-management education: United States, 2001--2009. *MMWR. Morbidity and Mortality Weekly Report*, ***60***(17), 547–552.

Centres for Disease Control and Prevention (2011). Vital signs: asthma prevalence, disease characteristics, and self-management education: United States, 2001–2009. *Morbidity and Mortality Weekly Report* **60**:547–552.

Christiansen, S. C., Schatz, M., Yang, S. J., Ngor, E., Chen, W. and Zuraw, B. L. (2016). Hypertension and Asthma: A Comorbid Relationship. *Journal of Allergy and Clinical Immunology: In Practice* **4**(1): 76–81.

Condado, J. F., Junpaparp, P. and Binongo, J. N. (2016). Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can risk stratify patients in transcatheter aortic-valve replacement (TAVR). *International Journal of Cardiology* **223**: 444–449.

Conway-Campbell, B.L., George, C.L., Pooley, J.R., Knight, D.M., Norman, M.R., Hager, G.L. and Lightman, S.L. (2011). The HSP90 molecular chaperone cycle regulates cyclical transcriptional dynamics of the glucocorticoid receptor and its coregulatory molecules CBP/p300 during ultradian ligand treatment. *Molecular Endocrinology* **25**:944–954.

Crompton, G. (2006). A brief history of inhaled asthma therapy over the last fifty years. *Primary Car. Respiratory Journal* **15**(6):326–331.

Currie, G.P., Srivastava, P., Dempsey, O.J. and Lee, D.K. (2005). Therapeutic modulation of allergic airways disease with leukotriene receptor antagonists. *Qatar Journal of Medicine* **98**:171–182.

Dale, D., Fauci, A. S., Guerry, D. and Wolff, S. (1975). Comparison of agents producing a neutrophilic leukocytosis in man: hydrocortisone, prednisone, endotoxin and etiocholanolone. Journal of Clinical Investigation **56**: 808–813.

Daley-Yates, P.T. (2015). Inhaled corticosteroids: potency, dose equivalence and therapeutic index. *British journal of Clinical Pharmacology* **80**(3):372–380.

Daudt, L.D., Cavagni, J., Souza, A., Torres, I.L.S., Ferreira, B.C. and Rosing, C.K. (2011). Effect of inhaled corticosteroids on TNF-α production and alveolar bone loss in Wistar rats. *Archives of Oral Biology,* **56**(**11**):109-112

Davis, A. P., Joshua, S., Lauren, T., Sharon, S., Holte, S., Dunaway, S. and Marra, C. (2018). Montelukast reduces Interleukin-6 levels in individuals with allergic rhinitis. *Annals of Allergy, Asthma & Immunology* **121**(2): 248-253.

Delves, P., Martin, S., Burton, D. and Roitt, I. (2006) Essentials immunology. Chapter 1, eleventh edn. Blackwell Publishing, Malden

DeNicola, L.K., Gayle, M.O. and Blake, K.V. (2001). Drug Therapy Approaches in the Treatment of Acute Severe Asthma in Hospitalised Children. *Paediatrics Drugs* **3:** 509–537.

Diamant, Z. and van der Molen, T. (2005). Treating asthma: is there a place for leukotriene receptor antagonists? *Respiratory Medicine* **99**:655–662.

Dogra, S., Ardern, C. I. and Baker, J. (2007). The relationship between age of asthma onset and cardiovascular disease in Canadians. *Journal of Asthma* **44**(10): 849–854.

Dogru, M. and Yesiltepe, M.G. (2016). The evaluation of neutrophil-lymphocyte ratio in children with asthma. *Allergology and Immunopathology* **44**:292–296.

Doyle, L.W., Ehrenkranz, R.A. and Halliday, H.L. (2010). Postnatal hydrocortisone for preventing or treating bronchopulmonary dysplasia in preterm infants: a systematic review. *Neonatology* **98**(2):111–117.

Ekpo, O. E., & Pretorius, E. (2008). Using the BALB/c asthmatic mouse model to investigate the effects of hydrocortisone and a herbal asthma medicine on animal weight. *Scandinavian Journal of Laboratory Animal Science* **35**(4): 265–280.

Fajt, M.L. and Wenzel, S.E. (2015). Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. *Journal Allergy Clinical Immunology* **135**:299–310.

Fauci, A. S. and Dale, D. (1994). The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. *Journal of Clinical Investigation* **53**: 240-246.

Fauci, A. S. and Dale, D. C. (1975). The effect of Hydrocortisone on the kinetics of normal human lymphocytes. *Blood* **46**: 235–243

Fest, J., Ruiter, T.R., Koerkamp, B.G., Rizopoulos, D., Ikram M.A., Van Eijck, C.H.J. and Stricker, B.H. (2019). The neutrophil-to-lymphocyte ratio is associated with mortality in the general population: The Rotterdam Study. *European Journal of Epidemiology* **34**:463–470.

Fitzgerald, D.A. and Mellis, C.M. (2006). Leukotriene receptor antagonists in virus-induced wheezing: evidence to date. *Treatment Respiratory Medicine* **5**: 407– 417

Freeman, A. F., Avila, E. M., Shaw, P. A., Davis, J., Hsu, A. P., Welch, P., Matta, J. R., Hadigan, C., Pettigrew, R. I., Holland, S. M. and Gharib, A. M. (2011). Coronary artery abnormalities in hyper-IgE syndrome. *Journal of Clinical Immunology*, *31*(3), 338–345. https://doi.org/10.1007/s10875-011-9515-9

Fu, J.J., McDonald, V.M., Gibson, P.G. and Simpson, J.L. (2014). Systemic inflammation in older adults with asthma- COPD overlap syndrome. *Allergy, Asthma and Immunology Research* **6**: 316-324.

Fu, JJ., McDonald, V.M., Gibson, P.G. and Simpson, J.L. (2014). Systemic inflammation in older adults with asthma- COPD overlap syndrome. *Allergy Asthma and Immunology Research* **6**: 316-324.

Fujita, M.B.S., Shigeharu, U., Wataru, I., Takahito, C., Masahide, T., Norihiro, S. and Hiroyuki, K. (2007). Annals of Allergy. *Asthma & Immunology* **99**(1): 48-53.

Gabay, C. (2006). *Arthritis Research Therapy* **8**(2): 3.

Gabay, C. and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* **340**(6): 448-454.

Gabay, C. and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* **340**(6): 448-454.

Gabay, C., Lamacchia, C and Palmer, G. (2010). IL-6 and chronic inflammation. *Arthritis Research and Therapy* **12**(6): 105-111.

Goldsby, R.A., Kindt, T.J., Osborne, B.A. and Kuby, J. (2007) Immunology. Chapter 1, Fifth edn. W.H Freeman

Grossman, E. and Messerli, F. H. (2012). Drug-induced hypertension: An unappreciated cause of secondary hypertension. In *American Journal of Medicine* **125**(1): 14–22

Guyton, A.C. and Hall, J.E. (2001) Resistance of the body to infection: chapter 1.Leukocytes, granulocytes, the monocyte-macrophage system and inflammation. Textbook of medical physiology. Tenth edn. Gopsons Papers Ltd., Noida, pp 397–399

H. (2007). Drugs for the Geriatric Patient. pp. 581–607.

Ha, E.K., Park, J.H., Lee, S.J., Yon, D.K., Kim, J.H., Jee, H.M., Lee, K.S., Sung, M., Kim, M.A. and Shin, Y.H. (2020). Shared and unique individual risk factors and clinical biomarkers in children with allergic rhinitis and obstructive sleep apnea syndrome. *Clinical Respiratory Journal* **14**:250–259.

Han, R. T., Back, S. K., Lee, J., Kim, H. Y., Kim, H. J., & Na, H. S. (2017). Effects of exposure of formaldehyde to a rat model of atopic dermatitis induced by Neonatal Capsaicin treatment. *Journal of Visualized Experiments* **127**: 181–189.

He, Y. F. Luo, H. Q. and Wang, W. (2017). Preoperative NLR and PLR in the middle or lower ESCC patients with radical operation. *European Journal of Cancer Care* **26**(2): 205-215

Health, United States, 2015: With Special Feature on Racial and Ethnic Health Disparities - PubMed. (n.d.).

Hidalgo, C. A., Blumm, N., Barabási, A. L. and Christakis, N. A. (2009). A Dynamic Network Approach for the Study of Human Phenotypes. *PLoS Computational Biology* **5**(4): 101-113.

Hirano, T. (1998). Interleukin 6 and its receptor: ten years later. *International Review Immunology* **16**:249–284.

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K. and Iwamatsu, A. (1986). Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* 324: 73–76.

Hirooka, Y., Mitsuma, T., Nogimori, T. and Ishizukis, Y. (1992). Effect of hydrocortisone on interleukin-6 production in human ,peripheral blood rnononuclear ceils. *Mediators of Inflammation* **1**: 9-13.

Hirooka, Y., Mitsuma, T., Nogimori, T. and Ishizukis, Y. (1992). Effect of hydrocortisone on interleukin-6 production in human, peripheral blood rnononuclear ceils. *Mediators of Inflammation* 1: 5-9.

Holgate, S. T. (2012). Innate and adaptive immune responses in asthma. In *Nature Medicine* (Vol. 18, Issue 5, pp. 673–683). https://doi.org/10.1038/nm.2731

Holgate, S.T. (2012). Innate and adaptive immune responses in asthma. *Nature Medicine* **18**: 673-673.

Holgate, S.T., Wenzel, S., Postma, D.S., Weiss, S.T., Renz, H. and Sly, P.D. (2015). Asthma. *Nature Review and Disease* **1**(1): 15-25.

Horvath, G. and Wanner, A. (2006). Inhaled corticosteroids: effects on the airway vasculature in bronchial asthma. *European Respiratory Journal* **27**: 172–187

Hoshino, M., Akitsu, K. and Ohtawa, J. (2019). Comparison between montelukast and tiotropium as add-on therapy to inhaled corticosteroids plus a long-acting β2-agonist in for patients with asthma. *Official Journal of the Association for the Care of Asthma* **56**(9): 995–1003.

Huang, W.J., Huang, G.T., Zhan, Q.M., Chen, J.L., Luo, W.T. and Wu, L.H. (2020). The neutrophil to lymphocyte ratio as a novel predictor of asthma and its exacerbation: A systematic review and meta-analysis. *European Review of Medical Pharmacological Science* **24**(22): 11719–11728.

Huang, Z., Chen, C., Li, S., Kong, F., Shan, P. and Huang, W. (2016). Serum Markers of Endothelial Dysfunction and Inflammation Increase in Hypertension with Prediabetes Mellitus. *Genetic Testing and Molecular Biomarkers* **20**(6): 322–327.

Ibáñez, K., Boullosa, C., Tabarés-Seisdedos, R., Baudot, A. and Valencia, A. (2014). Molecular Evidence for the Inverse Comorbidity between Central Nervous System Disorders and Cancers Detected by Transcriptomic Meta-analyses. *PLoS Genetics* **10**(2): 104-117.

Ilmarinen, P., Tuomisto, L.E. and Niemelä, O. (2016). Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. *European Respiratory Journal* **48**:1052–1062.

Iwalewa, E.O., McGaw, L.J., Naidoo, V. and Eloff, J.N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal Biotechnology* **6**:2868–2885.

Jiang, J., Liu, R., Yu, X., Yang, R., Xu, H., Mao, Z. and Wang, Y. (2019). The neutrophil-lymphocyte count ratio as a diagnostic marker for bacteraemia: A systematic review and meta-analysis. *America Journal of Emergence Medicine* **37**:1482–1489.

Jiang, T., Dai, L., Li, P., Zhao, J., Wang, X., An, L., Liu, M., Wu, S., Wang, Y., Peng, Y., Sun, D., Zheng, C., Wang, T., Wen, X., & Cheng, Z. (2021). Lipid metabolism and identification of biomarkers in asthma by lipidomic analysis. Biochimica et biophysica acta. *Molecular and Cell Biology of Lipids* **18**(2):158-158.

Jousilahti, P., Salomaa, V., Hakala, K., Rasi, V., Vahtera, E. and Palosuo, T. (2002). The association of sensitive systemic inflammation markers with bronchial asthma. *Annual Allergy Asthma Immunology* **89**: 381-385

Jung, C., Greco, S., Nguyen, H.H., Ho, J.T., Lewis, J.G., Torpy, D.J. and Inder, W.J. (2014). Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone in normal volunteers. *British Medical Council Endocrine Disorders* **26:** 91-97.

Kishimoto, T. (2005). Interleukin-6: From basic science to medicine—40 years in immunology. *Annual Review of Immunology* **23**: 1-21.

Kony, S., Zureik, M., Driss, F., Neukirch, C., Leynaert, B. and Neukirch, F. (2004). Association of bronchial hyperresponsiveness and lung function with C-reactive protein (CRP): a population based study. *Thorax* **59**(10):892–896.

Kossaify, A., Garcia, A., Succar, S., Ibrahim, A., Moussallem, N., Kossaify, M. and Grollier, G. (2013). Perspectives on the value of biomarkers in acute cardiac care and implications for strategic management. *Biomarker Insights* **8**: 115–126.

Kudo, M., Ishigatsubo, Y. and Aoki I (2013). Pathology of Asthma. *Frontiers in Microbiology,* **4**(263):95-99.

Kudo, M., Ishigatsubo, Y. and Aoki, I. (2013). Pathology of asthma. *Frontiers in Microbiology* 4:103-113.

Kumar, M. and Sarin, S.K. (2009). Biomarkers of diseases in medicine. Current trends in science. *Platinum Jubilee Special* **13**:112-120.

Landstra, A.M., Boezen, H.M., Postma, D.S., van Aalderen, W.M.C. (2003). Effect of intravenous hydrocortisone on nocturnal airflow limitation in childhood asthma. *European Respiratory Journal* **21**:627-632

Landstra, A.M., Kauffman, H.F., Marike Boezen, H., van Aalderen, W.M., Zonderland, J. and Postma, D.S. (2005). The influence of intravenous hydrocortisone on cytokine levels in children with asthma. *Pediatric Allergy and Immunology: Official Publication of the European Society of Pediatric Allergy and Immunology* **16**(4):299–305.

Laviolle, B., Annane, D., Fougerou, C. and Bellissant, E. (2012). Gluco- and mineralocorticoid biological effects of a 7-day treatment with low doses of hydrocortisone and fludrocortisone in septic shock. *Intensive Care Medicine* **38**:1306–1314.

Lee, M.J., Park, S.D., Kwon, S.W., Woo, S.I., Lee, M.D., Shin, S.H., Kim, D.H., Kwan, J. and Park, K.S. (2016). Relation between Neutrophil-to-Lymphocyte Ratio and Index of Microcirculatory Resistance in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention. *America Journal of Cardiology* **118**:1323–1328.

Lee, P., Oen, K., Lim, G., Hartono, J., Muthiah, M., Huang, D., Teo, F., Li, A., Mak, A. and Chandran, N. (2021). Neutrophil-to-Lymphocyte Ratio Predicts Development of Immune-Related Adverse Events and Outcomes from Immune Checkpoint Blockade: A Case-Control Study. *Cancers* **13**:130-138.

Li, J., Chen, J. and Kirsner, R. (2007). Pathophysiology of acute wound healing. *Clinical Dermatology* **25**:9–18.

Li, W., Hou, M., Ding, Z., Liu, X., Shao, Y. and Li, X. (2021). Prognostic Value of Neutrophil-to-Lymphocyte Ratio in Stroke: A Systematic Review and Meta-Analysis. *Frontiers in Neurology* **12**:686–983.

Li, Y., Cui, X., Shiloach, J., Wang, J., Sufredini, D., Xu, W., Liu, W., Fitz, Y., Sun, J. and Eichacker, P. (2020). Hydrocortisone decreases lethality and inflammatory cytokine and nitric oxide production in rats challenged with *B. anthracis* cell wall peptidoglycan. *Intensive Care Medicine Experimental* **8**:61-67.

Li, Y., Wang, W., Yang, F., Xu, Y., Feng, C. and Zhao, Y. (2016). The regulatory roles of neutrophils in adaptive immunity. *Cell Community Signal* **17**:14-17.

Libby P (2007) Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutrition Review* **65**:140–146.

Litonjua, A. A., Tantisira, K. G., Lake, S., Lazarus, R., Richter, B. G., Gabriel, S., Silverman, E. S. and Weiss, S. T. (2005). Polymorphisms in signal transducer and activator of transcription 3 and lung function in asthma. *Respiratory Research* 6(1): 52-59.

Liu, X., Zhang, Q., Wu, H., Du, H., Liu, L., Shi, H., Wang, C., Xia, Y., Guo, X., Li, C., Bao, X., Su, Q., Sun, S., Wang, X., Zhou, M., Jia, Q., Zhao, H., Song, K., and Niu, K. (2015). Blood neutrophil to lymphocyte ratio as a predictor of hypertension. *American Journal of Hypertension*, *28*(11), 1339–1346. https://doi.org/10.1093/ajh/hpv034

Lowsby, R., Gomes, C., Jarman, I., Lisboa, P., Nee, P.A., Vardhan, M., Eckersley, T., Saleh R. and Mills, H. (2015). Neutrophil to lymphocyte count ratio as an early

indicator of blood stream infection in the emergency department. *Emergence Medicine Journal* **32**:531–534.

Luan, Y. and Yao, Y. (2018) Search in PubMedSearch in NLM Catalog **7**:9:13-22

Lucia Cazzoletti, A., Corsico, A., Janson, C., Jarvis, D., Pin, I., Accordini, S., Bugiani, M., Cerveri, I., Gislason, D., Gulsvik, A. and de Marco, A. (2010). Therapy and Health Economics Group of the European Community Respiratory Health Survey. *International Archives of Allergy and Immunology* **151**(1):70-79.

Lucian D.K., Gayle, M.O. and Blake, K.V. (2001). Drug Therapy Approaches in the Treatment of Acute Severe Asthma in Hospitalised Children. *Paediatrics Drugs* **3**: 509–537.

Ma, C.S., Deenick, E.K., Batten, M. and Tangye, S.G. (2012). The origins, function, and regulation of T follicular helper cells. *Journal Experimental Medicine* **209**: 1241–1253.

Magen, E., Yosefy, C., Viskoper, R. J. and Mishal, J. (2006). Treatment of allergic rhinitis can improve blood pressure control. *Journal of Human Hypertension* 20(11): 888–893.

Mansour, A. and Nelson, D.S. (1978). Effect of hydrocortisone on the response of rat lymphocytes to phytohaemagglutinin. *Australian Journal of Experimental Biology Medicine Science* **56**(3):301-311.

Mansour, A. and NelsonAust, D.S. (1978). *Journal of Experimental Biology and Medical Science* **10**:103-108

Marini, M., Vittori, E., Hollemborg, J. and Mattoli, S. (1992). Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *Journal Allergy Clinical Immunology* **89**:1001–1009.

Masoli, M., Fabian, D., Holt, S. and Beasley, R. (2004). The global burden of asthma: Executive summary of the GINA Dissemination Committee Report. In *Allergy: European Journal of Allergy and Clinical Immunology* **59**(5): 469–478.

Mayer, C.M., Gruben, H.J., Land, E.M., Scharnag, H. and Marz, W. (2005). Statin induced inhibition of CRP expression in primary human hepatocytes. *Atherosclerosis* **6**:70–71.

Mayeux R (2004) Biomarkers: potential uses and limitations. *NeuroRx* **1**:182–188.

McCarthy, M. W. (2023). Montelukast as a potential treatment for COVID-19. *Expert Opinion on Pharmacotherapy* **24**(5):551–555.

Melissa, D.G. and Tatyana, G. (2020). Understanding the immunology of asthma: Pathophysiology, biomarkers, and treatments for asthma endotypes. *Paediatric Respiratory Reviews* **36**:118-127.

Melissa, D.G. and Tatyana, G. (2020). Understanding the immunology of asthma: Pathophysiology, biomarkers, and treatments for asthma endotypes. *Paediatric Respiratory Reviews* **36**:118-127.

Mims, J.W. (2015). Asthma: definitions and pathophysiology. *International forum of Allergy and Rhinology* **5**(1):2-6.

Monadi, M., Firouzjahi, A. and Hosseini, A. (2016). Serum c-reactive protein in asthma and its ability in predicting asthma control, a case-control study. *Caspian Journal of Internal Medicine* **7**(1): 37–42.

Moore, W. C., Meyers, D. A., Wenzel, S. E., Teague, W. G., Li, H., Li, X., D'Agostino, R., Castro, M., Curran-Everett, D., Fitzpatrick, A. M., Gaston, B., Jarjour, N. N., Sorkness, R., Calhoun, W. J., Chung, K. F., Comhair, S. A. A., Dweik, R. A., Israel, E., Peters, S. P. and Bleecker, E. R. (2010). Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *American Journal of Respiratory and Critical Care Medicine* **181**(4):315–323.

Moosmann, J., Krusemark, A., Dittrich, S., Ammer, T., Rauh, M., Woelfle, J., Metzler, M. and Zierk J. (2022). Age-and sex-specific pediatric reference intervals for neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and platelet-to-lymphocyte ratio. *International Journal of Laboratory Hematology* **44**:296–301.

Morales, D. R., Lipworth, B. J., Donnan, P. T., Jackson, C. and Guthrie, B. (2017). Respiratory effect of beta-blockers in people with asthma and cardiovascular

disease: Population-based nested case control study. *British Medical Council* **15**(1). 111-118.

Morris, I.P., Goel, N., Chakraborty, M. (2019). Efficacy and safety of systemic hydrocortisone for the prevention of bronchopulmonary dysplasia in preterm infants: a systematic review and meta-analysis. *European Journal of Pediatrics* **178**(8):1171–1184.

Mortaz, E., Alipoor S.D., Adcock I.M., Mumby S. and Koenderman, L. (2018). Update on Neutrophil Function in Severe Inflammation. *Frontiers in Immunology* **9**:21-27.

Muz, M.H., Deveci, F., Bulut, Y., Illhan, N., Yekeler, H. and Turgut, T. (2006). The effects of low dose leukotriene receptor antagonist therapy on airway remodeling and cysteinyl leukotriene expression in a mouse asthma model. *Experimental and Molecular Medicine* **38**:109–118.

Nagai, H., Takizawa, T., Nakatomi, I., Matsuura, N. and Koda, A. (1983). Anti-allergic action of glucocorticoids in rats. *Japan Journal of Pharmacology* **33**: 349-355

Nair, P. and Prabhavalkar, K. (2021). Anti-Asthmatic Effects of Saffron Extract and Salbutamol in an Ovalbumin-Induced Airway Model of Allergic Asthma. *Sinusitis* **5**: 17-31.

Nakamura, H. and Yamashita, T. (2002). Predictive value of cardiovascular events by high sensitivity CRP. *Nihon Rinsho* **60**(5):916–921.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K. and Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *Journal of Clinical Investigation* **113**: 1271–1276.

Neveu, W. A., Allard, J. L., Raymond, D. M., Bourassa, L. M., Burns, S. M. and Bunn J. Y. (2010). Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. *Respiratory Resource* **11**:28.

Niimi A. (2013). Cough, asthma, and cysteinyl-leukotrienes. *Pulmonary Pharmacology and Therapeutics* **26**(5):514–519.

Niimi, A. (2013). Cough, asthma, and cysteinyl-leukotrienes. Pulmonary Pharmacology & Therapeutics **26**(5):514–519.

Niu, D., Huang, Q., Yang, F., Tian, W., Li, C., Ding, L., Fang, H.C. and Zhao, Y. (2021). Serum biomarkers to differentiate Gram-negative, Gram-positive and fungal infection in febrile patients. *Journal of Medical Microbiology* **70**:136-139.

Olafsdottir, I.S., Gislason, T. and Thjodleifsson, B. (2005). C reactive protein levels are increased in non-allergic but not allergic asthma: a multicentre epidemiological study. *Thorax* 6b0:451–454.

Olnes, M.J., Kotliarov, Y., Biancotto, A., JinguoChen, F. and Robert, N. (2016). Effects of systemically administered hydrocortisone on the human immunome**.** *Scientific Reports* **6**:23-32.

Oppert, M., Schindler, R., Husung, C., Offermann, K., Gräf, K.J., Boenisch, O., Barckow, D., Frei, U. and Eckardt, K.U. (2005). Low-dose hydrocortisone improves shock reversal and reduces cytokine levels in early hyperdy-namic septic shock. *Critical Care Medicine* **33**:2457–2464.

Oțelea, M.R., Andrei, N. and Moise, L.G. (2018). The significance of the neutrophil to lymphocyte ratio in silicosis. *Journal of Contemporary Clinical Practice* **4**: 53-59.

Paljarvi, T., Forton, J., Luciano, S., Herttua, K. and Fazel, S. (2022). Analysis of Neuropsychiatric Diagnoses after Montelukast Initiation. *Journal of American Medical Association Network Open* **5**(5):221-236.

Pan, R., Ren, Y., Li, Q., Zhu, X., Zhang, J., Cui, Y. and Yin, H. (2023). Neutrophil–lymphocyte ratios in blood to distinguish children with asthma exacerbation from healthy subjects. *International Journal of Immunopathology and Pharmacology* 37:394-404

Park, J., Lee, D. S., Christakis, N. A. and Barabási, A. L. (2009). The impact of cellular networks on disease comorbidity. *Molecular Systems Biology* 5: 103-118.

Park, J.M. (2017). Neutrophil-to-lymphocyte ratio in trauma patients. *Journal of Trauma Acute Care Surgery* **82**:225–226.

Passardi, A., Scarpi, E., and Cavanna, L. (2016). Inflammatory indexes as predictors of prognosis and bevacizumab efficacy in patients with metastatic colorectal cancer. *Oncotarget* 7(22): 332-340

Peters-Golden, M. and Henderson, W. (2007). Medicine Mechanisms of disease: Leukotrienes **10**:105-116

Peters-Golden, M., Swern, A., Bird, S.S. and Hustad, E. (2017). Leukotrienes. *New England Journal of Medicine* **357**(18): 1841-1854.

Pierson, W.E., Bierman, C.W. and Kelley, V. (1974). A double-blind trial of corticosteroid therapy in status asthmaticus. *Pediatrics* **54**(3):282–288.

Pilaczynska-Cemel, M., Golda, R., Dabrowska, A. and Przybylski, G. (2019). Analysis of the level of selected parameters of inflammation, circulating immune complexes, and related indicators (neutrophil/lymphocyte, platelet/lymphocyte, CRP/CIC) in patients with obstructive diseases. *Centre European Journal of Immunology* **43**:292–298.

Poynter, M.E. and Irvin, C.G. (2016). *European Respiratory Journal* **48**: 979-981.

Qin, A.B., Ma, N. and Tang , Q. (2016). Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. *Modern Rheumatology* **26**(3): 372–376

Rademaker, K.J., de Vries, L.S., Uiterwaal, C.S., Groenendaal, F., Grobbee, D.E. and van Bel, F. (2008). Postnatal hydrocortisone treatment for chronic lung disease in the preterm newborn and long-term neurodevelopmental follow-up. Archives of disease in childhood. *Fetal and Neonatal* **93**(1):58–63.

Raissy, H.H., Kelly, H.W., Harkins, M. and Szefler, S.J. (2013). Inhaled corticosteroids in lung diseases. *American Journal of Respiratory and Critical Care Medicine* **187**(8):798–803.

Reinke, S.N., Gallart-Ayala, H., Gómez, C., Checa, A., Fauland, A., Naz, S., Kamleh, M.A., Djukanović, R., Hinks, T.S.C., Wheelock, C.E. (2017). Metabolomics

analysis identifies different metabotypes of asthma severity. *European Respiration Journal* **49**(3):160-174.

Rhen, T. and Cidlowski, J.A. (2005). Antiinflammatory action of glucocorticoids–new mechanisms for old drugs. *New England Journal of Medicine* **353**(16):1711-1723.

Riccioni, G., Bucciarelli, T., Mancini, B., Di Ilio, C. and D'Orazio, N. (2007). Antileukotriene drugs: clinical application, effectiveness and safety. *Current Medicine Chemistry* **14**: 1966–1977.

Riccioni, G., Bucciarelli, T., Mancini, B., Di, I. and D'Orazio, N. (2007). Antileukotriene drugs: clinical application, effectiveness and safety. *Current Medicine Chemistry* **14**:1966–1977.

Rincon, M. and Irvin, C.G. (2012). Role of IL-6 in asthma and other inflammatory pulmonary diseases. *International Journal of Biological Science* b:1281–1290.

Rodríguez-Iturbe, B., Pons, H., Quiroz, Y., & Johnson, R. J. (2014). The immunological basis of hypertension. *American Journal of Hypertension* **27**(11): 1327–1337.

Roger, I., Milara, J., Montero, P. and Cortijo, J. (2021). The Role of JAK/STAT Molecular Pathway in Vascular Remodeling Associated with Pulmonary Hypertension. *International Journal of Molecular Sciences* **22**: 49-58.

Rostevanov, I.S., Betesh-Abay, B., Nassar, A., Rubin, E., Uzzan, S., Kaplanski, J., Biton, L. and Azab, A.N. (2022) Montelukast induces beneficial behavioral outcomes and reduces inflammation in male and female rats. *Frontiers in Immunology* **13**:98-104

Rostevanov, I.S., Betesh-Abbay, B.N.A., Rubin, E., Uzzan, S., Kaplanski, J., Biton, L. and Azab, A.N. (2022). Montelukast induces beneficial behavioural outcomes and reduces inflammation in male and female rats. *Frontiers in Immunology* **13**:65-68

Rubinstein, I., Kumar, B. and Schriever, C. (2004). Long-term montelukast therapy in moderate to severe COPD- a preliminary observation. *Respiratory Medicine* **98**:134–138.

Rubio-Perez, C., Guney, E., Aguilar, D., Piñero, J., Garcia-Garcia, J., Iadarola, B., Sanz, F., Fernandez-Fuentes, N., Furlong, L. I. and Oliva, B. (2017). Genetic and

functional characterization of disease associations explains comorbidity. *Scientific Reports* 7(1): 111-117.

Sabin, B.R., Avila, P.C., Grammer, L.C. and Greenberger, P.A. (2012). Chapter 15: Lessons learned from clinical trials of asthma. *Asthma Proceedings* **33**(1):51–54.

Saik, O. V., Demenkov, P. S., Ivanisenko, T. V., Bragina, E. Y., Freidin, M. B., Goncharova, I. A., Dosenko, V. E., Zolotareva, O. I., Hofestaedt, R., Lavrik, I. N., Rogaev, E. I. and Ivanisenko, V. A. (2018). Novel candidate genes important for asthma and hypertension comorbidity revealed from associative gene networks. *British Medical Council Genomics* **11**(1): 118-126.

Salem, S., Jankowski, V., Asare, Y., Liehn, E., Welker, P., Raya-Bermudez, A., Pineda-Martos, C., Rodriguez, M., Muñoz-Castañeda, J. R., Bruck, H., Marx, N., Machado, F. B., Staudt, M., Heinze, G., Zidek, W. and Jankowski, J. (2015). Identification of the Vasoconstriction-Inhibiting Factor (VIF), a potent endogenous cofactor of angiotensin II acting on the angiotensin II type 2 receptor. *Circulation*, **131**(16): 1426–1434.

Samet, J. M. (2013). Tobacco smoking. The leading cause of preventable disease worldwide. *Thoracic Surgery Clinics* 23(2): 103–112.

Sarkar, U., Rivera-Burgos, D., Large, E.M., Hughes, D.J., Ravindra, K.C., Dyer, R.L., Ebrahimkhani, M.R., Wishnok, J.S., Griffith, L.G. and Tannenbaum, S.R. (2015). Metabolite profiling and pharmacokinetic evaluation of hydrocortisone in a perfused three-dimensional human liver bioreactor. *Drug Metabolism Disposition* **43**(7):1091-1099.

Sävykoski, T., Harju, M., Paldanius, H. Kuitunen, A., Bloigu, E., Wahlström, P., Rytilä, V., Kinnula, P. and Saikku, M. (2004). *Chlamydia pneumoniae* infection and inflammation in adults with asthma. *Respiration* **71**(2):120-125.

Scott, H. A., Gibson, P. G., Garg, M. L., Pretto, J. J., Morgan, P. J., Callister, R. and Wood, L. G. (2013). Dietary restriction and exercise improve airway inflammation and clinical outcomes in overweight and obese asthma: A randomized trial. *Clinical and Experimental Allergy* **43**(1): 36–49.

Seiko, K., Kenji, B., Ayako, M., Hiroyuki, S., Tadashi, O. and Etsuro, Y. (2008). The Efficacy of Montelukast and Airway Mast Cell Profiles in Patients with Cough Variant Asthma. *Journal of Asthma* **45**(3):243-250.

Sesso, H. D., Jiméenez, M. C., Wang, L., Ridker, P. M., Buring, J. E. and Michael Gaziano, J. (2015). Plasma inflammatory markers and the risk of developing hypertension in men. *Journal of the American Heart Association* **4**(9): 61-68

Shapiro, N.I., Trzeciak, S., Hollander, J.E., Birkhahn, R., Otero, R., Osborn, T.M., Moretti, E., Nguyen, H.B., Gunnerson, K. and Milzman, D. (2010). The diagnostic accuracy of plasma neutrophil gelatinase-associated lipocalin in the prediction of acute kidney injury in emergency department patients with suspected sepsis. *Annual Emergence Medicine* **56**:52–59.

Shere, A., Eletta, O. and Goyal, H. (2017). Circulating blood biomarkers in essential hypertension: a literature review. *Journal of Laboratory and Precision Medicine* **2**: 99–109.

Shi, H.Z., Xiao, C.Q. and Zhong, D. (1998). Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *American Journal of Respiratory and Critical Care Medicine* **157**: 204–209.

Silvestri, M., Serpero, L., Petecchia, L., Sabatini, F., Cerasoli, F. Jr. and Rossi, G.A. (2006). High serum levels of tumour necrosis factor-α and interleukin-8 in severe asthma: markers of systemic inflammation? *Clinical and Experimental Allergy* **36**: 1373–1381.

Simeone-Penney, M. C., Severgnini, M., Tu, P., Homer, R. J., Mariani, T. J., Cohn, L. and Simon, A. R. (2007). Airway Epithelial STAT3 Is Required for Allergic Inflammation in a Murine Model of Asthma. *The Journal of Immunology* **178**(10): 6191–6199.

Song, M., Graubard, B.I., Rabkin, C.S. and Engels, E.A. (2021). Neutrophil-to-lymphocyte ratio and mortality in the United States general population. *Science Reproduction* **11**:464- 480.

Stadnyk, A.W. (1994). Cytokine production by epithelial cells. *Faseb Journal* **8**:1041–1047.

Sule, D., Youssef, E.A., Densley, F., Rohit, P., Ramon, G., Miriam, S. and Clara, G. (2021). Methylprednisolone, dexamethasone or hydrocortisone for acute severe pediatric asthma: does it matter? *Journal of Asthma* **59**(3):590-596.

Sule, D., Youssef, E.A., Densley, F., Rohit, P., Ramon, G., Miriam, S. and Clara, G. (2021). Methylprednisolone, dexamethasone or hydrocortisone for acute severe pediatric asthma: does it matter? *Journal of Asthma* **59**(3):590-596.

Sule, D., Youssef, E.A., Densley, F., Rohit, P., Ramon, G., Miriam, S. and Clara, G. (2021). Methylprednisolone, dexamethasone or hydrocortisone for acute severe pediatric asthma: does it matter? *Journal of Asthma* **59**(3):590-596.

Sun, D., Li, X., Heianza, Y., Nisa, H., Shang, X., Rabito, F., Kelly, T., Harville, E., Li, S., He, J., Bazzano, L., Chen, W. and Qi, L. (2018). History of asthma from childhood and arterial stiffness in asymptomatic young adults: The Bogalusa heart study. *Hypertension* **71**(5): 928–936.

Szalai, A.J. (2004). C-reactive protein (CRP) and autoimmune disease: facts and conjectures. *Clinical Development Immunology* **11**(3-4):221–226.

Takemura, H. Ariel, R., Jonathan, W., Jason, D., Yeatman, B. A. Wandell, F. and Pestilli, A. (2006). Major human white matter pathway between dorsal and ventral visual cortex. *Cerebral Cortex* **26**(5): 2205–2214.

Takeshita, S., Kanai, T., Kawamura, Y., Yoshida, Y. and Nonoyama, S. A. (2017). Comparison of the predictive validity of the combination of the neutrophil- to-lymphocyte ratio and platelet-to-lymphocyte ratio and other risk scoring systems for intravenous immunoglobulin (ivig)- resistance in Kawasaki disease. *PLoS ONE* **12**:176-179.

Takeshita, S., Kanai, T., Kawamura, Y., Yoshida, Y. and Nonoyama, S. A. (2017). Comparison of the predictive validity of the combination of the neutrophil- to-lymphocyte ratio and platelet-to-lymphocyte ratio and other risk scoring systems

for intravenous immunoglobulin (ivig)- resistance in Kawasaki disease. *PLoS ONE* 12:176-1795.

Tashkin, D.P., Lipworth, B. and Brattsand, R. (2019). Benefit: Risk Profile of Budesonide in Obstructive Airways Disease. *Drugs* **79**(16):1757–1775.

Tenero, L., Piazza, M., Sandri, M., Azzali, A., Chinellato, I., Peroni, D., Boner, A. and Piacentini, G. (2016). Effect of montelukast on markers of airway remodeling in children with asthma. *Allergy and Asthma Proceedings* **37**(5):77–83.

Thakur, V. R., Khuman, V., Beladiya, J. V., Chaudagar, K. K. and Mehta, A. A. (2019). An experimental model of asthma in rats using ovalbumin and lipopolysaccharide allergens. *Heliyon* **5**(11):201-218

Tillie-Leblond, I., Pugin, J., Marquette, C. H., Lamblin, C., Saulnier, F. and Brichet, A. (1999). Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *America Journal of Respiratory and Critical Care Medicine* **159**: 487–494.

Tillie-Leblond, I., Pugin, J., Marquette, C.H., Lamblin, C., Saulnier, F. and Brichet, A. (1999). Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *America Journal of Respiratory and Critical Care Medicine* **159**:487–494.

Tsutomu, O. (2014). Different effects of oral cyclosporine therapy and oral antihistamine therapy to serum high-sensitivity CRP level, TARC level, and peripheral blood basophils in adult patients with AD were shown. A combination of these two therapies may be more effective for the treatment of AD in adults. *International Journal of Dermatology* **54**(6): 648-655

Tuleta, I., Skowasch, D., Aurich, F., Eckstein, N., Schueler, R., Pizarro, C., Schahab, N., Nickenig, G., Schaefer, C. and Pingel, S. (2017). Asthma is associated with atherosclerotic artery changes. *PLoS ONE* **12**(10): 71-79.

Ülger, Z., Gülen, F. and Özyürek, A. R. (2015). Abdominal aortic stiffness as a marker of atherosclerosis in childhood-onset asthma: A case-control study. *Cardiovascular Journal of Africa*, 26(1): 8–12.

Verma, S., Wang, C. H., Li, S. H., Dumont, A. S., Fedak, P. W. M., Badiwala, M. V., Dhillon, B., Weisel, R. D., Li, R. K., Mickle, D. A. G. and Stewart, D. J. (2002). A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation* **106**(8): 913–919.

Virchow, J.C., Kroegel, C. and Walker, C. (1996). Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. *Journal of Allergy and Clinical Immunology* **98**: 27–33.

Virchow, J.C., Kroegel, C., Walker, C. and Matthys, H. (1996). Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. *Journal of Allergy and Clinical Immunology* **98**:27–33.

Vos, T., Allen, C. and Arora, M. (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study. *Lancet* 388:1545–1602.

Wang, C. H., Li, S. H., Weisel, R. D., Fedak, P. W. M., Dumont, A. S., Szmitko, P., Li, R. K., Mickle, D. A. G. and Verma, S. (2003). C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* **107**(13): 1783–1790.

Wang, T. J., Gona, P., Larson, M. G., Levy, D., Benjamin, E. J., Tofler, G. H., Jacques, P. F., Meigs, J. B., Rifai, N., Selhub, J., Robins, S. J., Newton-Cheh, C. and Vasan, R. S. (2007). Multiple Biomarkers and the Risk of Incident Hypertension Epidemiology-Biomarkers and Hypertension.

Wenzel, S.E. (2006). Asthma: defining of the persistent adult phenotypes. *Lancet* **368**:804–813.

Wenzel, S.E. (2012). Asthma phenotypes: the evolution from clinical to molecular approaches. Nat. Med. 18(5):716-725.

Wilar, R. (2019). Diagnostic value of eosinopenia and neutrophil to lymphocyte ratio on early onset neonatal sepsis. *Korean Journal of Pediatrician* **62**: 217-223.

Williams, D.M. (2018). Clinical Pharmacology of Corticosteroids. *Respiratory Care* **63**(6):655-670.

Wood, C. D., Matthewman, R., Badve, V. C. and Conroy, C. (2001). A review of the nutritive value of dry season feeds for ruminants in Southern Rajasthan. Department for International Development

Wu, W., Li, Y., Jiao, Z., Zhang, L., Wang, X., & Qin, R. (2019). Phyllanthin and hypophyllanthin from Phyllanthus amarus ameliorates immune-inflammatory response in ovalbumin-induced asthma: role of IgE, Nrf2, iNOs, TNF-α, and IL's. *Immunopharmacology and Immunotoxicology* **41**(1): 55–67.

Xiang, G., Fan, M., Ma, Y., Wang, M., Gao, J., Chen, J., Li, X. and Gao, H. (2018). Anti-inflammatory actions of Caesalpinin M2 in experimental colitis as a selective glucocoricoid receptor modulator. *Biochemistry and Pharmacology* **150**:150–159.

Xu, M., Xu, J. and Yang, X. (2017). Asthma and risk of cardiovascular disease or all-cause mortality: A meta-analysis. *Annals of Saudi Medicine* **37**(2): 99–105.

Xu, N., Tang, X. F. and Xu, J. J. (2019). Predictive value of neutrophil to lymphocyte ratio on long-term outcomes of acute myocardial infarction patients with multivessel disease. *Chinese Journal of Cardiology* **47**(1): 42–48

Yi, F., Zhan, C., Liu, B., Li, H., Zhou, J., Tang, J., Peng, W., Luo, W., Chen, Q. and Lai, K. (2022). Effects of treatment with montelukast alone, budesonide/formoterol alone and a combination of both in cough variant asthma. *Respiratory Research* **23**(1):279.

Yokoyama, A., Kohno, N., Fujino, S., Hamada, H., Inoue, Y. and Fujioka, S. (1995). Circulating interleukin-6 levels in patients with bronchial asthma. *America Journal of Respiratory Critical Care Medicine* **151**: 1354–1358.

Zhan, J., Chen, W. and Cheng, L. (2020). Diagnosis of asthma based on routine blood biomarkers using machine learning. *Computational Intelligence and Neuroscience* **88**:410-412.

Zhang, H.P., Jia, C.E., Lv, Y., Gibson, P.G. and Wang, G. (2014). Montelukast for prevention and treatment of asthma exacerbations in adults: Systematic review and meta- analysis. *Allergy and Asthma Proceedings* **35**(4):278–287.

Zhang, X.Y., Simpson, J.L., Powell, H., Yang, I.A., Upham, J.W., Reynolds, P.N., Hodge, S., James, A.L., Jenkins, C. and Peters, M.J. (2014). Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clinical Experimental Allergy* 4b4:1137–1145.

Zhang, X.Y., Simpson, J.L., Powell, H., Yang, I.A., Upham, J.W., Reynolds, P.N., Hodge, S., James, A.L., Jenkins, C. and Peters, M.J. (2014). Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clinical and Experimental Allergy* 44:1137–1145.

Ziyab, A. H., Karmaus, W., Zhang, H., Holloway, J. W., Steck, S. E., Ewart, S. and Arshad, S. H. (2014). Allergic sensitization and filaggrin variants predispose to the comorbidity of eczema, asthma, and rhinitis: Results from the Isle of Wight birth cohort. *Clinical and Experimental Allergy* **44**(9): 1170–1178.

Zolotareva, O., Saik, O. V., Könings, C., Bragina, E. Y., Goncharova, I. A., Freidin, M. B., Dosenko, V. E., Ivanisenko, V. A. and Hofestädt, R. (2019). Comorbidity of asthma and hypertension may be mediated by shared genetic dysregulation and drug side effects. *Scientific Reports* **9**(1): 88-92