



Beyond eosinophilia: inflammatory patterns in patients with asthma

Martín Bedolla-Barajas , Jaime Morales-Romero , Dante Daniel Hernández-Colín , Désirée Larenas-Linnemann , Juan Mariscal-Castro , Miriam Montserrat Flores-Razo & Angie Bedolla-Pulido

To cite this article: Martín Bedolla-Barajas , Jaime Morales-Romero , Dante Daniel Hernández-Colín , Désirée Larenas-Linnemann , Juan Mariscal-Castro , Miriam Montserrat Flores-Razo & Angie Bedolla-Pulido (2020): Beyond eosinophilia: inflammatory patterns in patients with asthma, Journal of Asthma, DOI: [10.1080/02770903.2020.1852413](https://doi.org/10.1080/02770903.2020.1852413)

To link to this article: <https://doi.org/10.1080/02770903.2020.1852413>



Accepted author version posted online: 17 Nov 2020.



Submit your article to this journal [↗](#)



Article views: 11



View related articles [↗](#)



View Crossmark data [↗](#)

Running title: Inflammation in asthma

Beyond eosinophilia: inflammatory patterns in patients with asthma

Martín Bedolla-Barajas, MD, MSc^{*1}, Jaime Morales-Romero, MD, PhD², Dante Daniel Hernández-Colín, MD, MSc¹, Désirée Larenas-Linnemann, MD³, Juan Mariscal-Castro, MD¹, Miriam Montzerrat Flores-Razo, MD¹, Angie Bedolla-Pulido, MD⁴

¹Department of Allergy and Clinical Immunology, Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”; Salvador Quevedo y Zubieta No. 750, Colonia la Perla, Guadalajara, Jalisco, Mexico. P. C. 44340.

²Instituto de Salud Pública, Universidad Veracruzana; Av. Luis Castelaz Ayala s/n., Colonia Industrial Ánimas, Xalapa, Veracruz, Mexico. P. C. 91190.

³Department of Investigation, Hospital Médica Sur; Puente de Piedra No. 150, Colonia Toriello Guerra, Mexico City, Mexico. P. C. 14050.

⁴Centro Universitario en Ciencias de la Salud, Universidad de Guadalajara; Sierra Mojada No. 950, Colonia Independencia Oriente, Guadalajara, Jalisco, Mexico. P. C. 44340.

***Corresponding Author:** Martín Bedolla-Barajas, MD, MSc Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”; Salvador Quevedo y Zubieta No. 750, Colonia la Perla, Guadalajara, Jalisco, México. P. C.: 44340. Phone number: 52+ 33+ 36625644. E-mail: drmbdobar@gmail.com

Abstract

Background: Recently, inflammatory cell ratios have gained importance as useful indicators in the categorization of asthma. **Objective:** We compared the concentration of white blood cells in peripheral blood, as well as their respective inflammatory cell ratios, between patients with asthma and a healthy control group. **Methods:** We performed cross-sectional analyses of the data obtained from 53 adult patients with asthma and 109 adult controls. In our study, we estimated and compared the following inflammatory cell ratios: Neutrophil-Lymphocyte Ratio (NLR), Eosinophil-Lymphocyte Ratio (ELR), Eosinophil-Neutrophil Ratio (ENR), Eosinophil-Monocyte Ratio (EMR), and Platelet-Lymphocyte Ratio (PLR). The magnitude of association was quantified with the odds ratio. **Results:** In both groups, the average age was 33 years. In asthmatic patients, we obtained the following results: eosinophils ≥ 400 cells/ μ l, accounted for 37.7%; basophils ≥ 110 cells/ μ l, comprised 37.7%; and monocytes < 320 cells/ μ l, reached 11.3%. In the control group, the results were as follows: 4.6%, 9.2% and 0.9%, respectively. When compared to the control group, asthmatic patients had higher odds of eosinophils ≥ 400 cells/ μ l (OR = 12.61, $p < 0.0001$); higher odds of basophils ≥ 110 cells/ μ l (OR = 6.00, $p < 0.0001$); and increased odds of monocytes < 320 cells/ μ l (OR = 13.79, $p = 0.017$). NLR did not differ between our two groups; however, ELR, ENR, EMR and PLR were significantly higher in the asthma group. **Conclusions:** Overall, patients with asthma have a higher concentration of eosinophils and basophils, fewer monocytes in their blood, and higher ratios of increased chronic inflammation.

Keywords: eosinophils; basophils; monocytes; asthma; inflammation.

Introduction

Chronic inflammation of the respiratory tract and variable airflow obstruction (along with other symptoms) are some of the primary characteristics that define asthma (1-3). However, previous findings have shown that asthmatics not only experience local inflammation, but systematic

inflammation as well; biomarkers, such as: fibrinogen, amyloid protein and C-reactive protein, tend to be more elevated in asthmatic patients (4, 5). A decade ago, Nadif et al. demonstrated that it was possible to categorize asthma based on the number and type of inflammatory cells in a patient's blood (6). Interestingly, different indicators of chronic inflammation obtained by a white blood cell count have been used as prognostic biomarkers for cardiovascular diseases and various types of cancer (7, 8), while others have been useful in identifying the eosinophilic asthma phenotype (9). They have also been used to predict mortality associated with cardiovascular diseases and recurrent nasal polyposis (10, 11). Thus, detecting systemic inflammation from a peripheral blood sample is a helpful tool that is readily available in daily clinical practice. Due to the use of biological medications that selectively regulate the inflammatory process, we must reevaluate how white blood cells are expressed in asthmatic patients. Little is known about whether the rates of chronic inflammation differ between asthmatics and healthy individuals. In our study, we compared the concentration of eosinophils, basophils, and monocytes found in peripheral blood, between a group of patients with asthma and a control group. We also evaluated the following indicators of chronic inflammation: Neutrophil-Lymphocyte Ratio (NLR), Eosinophil-Lymphocyte Ratio (ELR), Eosinophil-Neutrophil Ratio (ENR), Eosinophil-Monocyte Ratio (EMR), and Platelet-Lymphocyte Ratio (PLR). These ratios were evaluated to observe the differences between patients with asthma and the control group.

Methods

Ethics

Our study was approved by the Research Ethics Committee of *Nuevo Hospital Civil de Guadalajara "Dr. Juan I. Menchaca"*. All asthma patients and controls gave written consent prior to partaking in our investigation.

Setting

Our facility is located in Guadalajara's central metropolitan district. In terms of population, Guadalajara is Mexico's second largest city, and it is located in the western part of the country, at an elevation of 1566 meters above sea level.

Design and participants

In our cross-sectional study, we compared the data that corresponded to two groups: 53 patients with asthma, and 109 healthy individuals (control group). All participants were recruited consecutively from June of 2018 to December of 2018. Asthmatic participants were 18 years or older. Our asthmatic group was comprised of newly diagnosed patients from the Allergy Department of a teaching hospital. For our asthma group, we excluded all patients with a personal history of diabetes, hypertension, rheumatic diseases, urticaria, chronic renal diseases or cancer. We did not include patients that had taken systemic steroids during the previous month; those that had experienced a respiratory tract infection during the previous month; pregnant women or those that were breastfeeding. The control group consisted of individuals that had donated to our hospital's blood bank and met the criteria established by Mexico's blood donor eligibility standards, which are as follows: ages 18 to 65 years, body weight of 50 kg or more, no tattoos or piercings, no drug use, no precedent of high-risk sexual practice the absence of any infectious and contagious diseases, no chronic diseases such as asthma, diabetes, renal failure, history of a previous blood transfusion, and more (12).

Additionally, both groups were asked about their current personal intake of alcohol and tobacco.

Definition of asthma

Asthma is clinically defined by the following symptoms that can vary in intensity: wheezing, coughing, and chest tightness or dyspnea (13). Patients with a clinical asthma diagnosis underwent a forced spirometry test, which provided evidence of an obstructed airway and its reversibility after inhaling salbutamol.

Body mass index

In order to measure the body mass index (BMI), the patient's body weight was divided by his/her height in meters squared. We used the World Health Organization's standards of body mass index (BMI) defined as: underweight, BMI < 18.5 kg/m²; normal, 18.5 and < 25kg/m²; overweight BMI ≥ 25 and < 30 kg/m²; and obesity, BMI ≥ 30 kg/m².

Forced spirometry technique

All spirometry tests were performed in the morning by using a Master Screen-Body PFT (Jaeger®, Care Fusion, Baesweiler, Germany). Each spirometry measurement was taken while patients were seated with their nostrils closed. To meet the criteria for acceptable and reproducible tests, every patient provided three measurements. In order to determine the reversibility of airflow obstruction, each patient inhaled 400 mcg of salbutamol with a metered dose inhaler. After 15 minutes, the test was retaken. If the forced expiratory volume per second (FEV₁) exceeded 12% and more than 200 ml, the test was deemed positive (14). The FEV₁ was used to determine the severity of airway obstruction (mild intermittent, moderate intermittent and severe intermittent).

White blood cell count

The white blood cell count (neutrophils, lymphocytes, monocytes, basophils and eosinophils) was done in our hospital's clinical laboratory by using a flow cytometer, CELL-DYN Ruby™ (Abbott Diagnostics Division, Abbott Laboratories, IL, USA). CELL-DYN Ruby uses optical precision methods, consisting of four different scattering angles of laser beams, located at 0° (size), 10° (complexity), 90° (globularity), and 90°D (granularity). This technology is known as *Multi-Angle-Polarized-Scatter-Separation* (MAPSS™). The light source consists of a 10 miliwatt helium-neon laser, which releases a 632.8 nm laser beam.

Statistical analysis

We compared continuous variables with the Student's t-test or the Mann-Whitney U test. In regard to categorical variables, these were compared by using the Chi-squared test or Fisher's exact test. The measure of association between variables was estimated with Spearman's rho test or the Odds Ratio (OR), we also used 95% confidence intervals (95% CI). The statistical significance was set with a value of $p \leq 0.05$. Lastly, IBM® SPSS® Statistics 20 (IBM Corp., Armonk, NY, USA) was used to analyze the data.

Results

In both groups, the average age was 33 years. Notably, no significant differences were found regarding sex (Table 1). Though the mean of BMI did not differ between the groups, obesity was

more prevalent in the asthma group, and being overweight was more recurrent in the control group ($p = 0.039$). There was no difference with regard to tobacco intake between the groups; however, the control group had a considerably higher alcohol intake ($p = 0.014$). One third of the patients underwent hospitalization due to a previous asthma exacerbation in the last year. Less than 30% of the patients had controlled asthma. Overall, the predicted basal FEV1 was of 72.7 ± 18.0 on average, and after the use of bronchodilator, the FEV1 was of 87.0 ± 16.7 . Median total serum IgE accounted for 233 UI/mL. Additionally, patients with asthma had greater sensitization to mites and house dust, followed by tree pollens.

Table 2 shows a comparison of the white blood cell count derived from both groups. Overall, the amount of leukocytes, neutrophils and lymphocytes did not vary between the two groups. The absolute and relative concentration of eosinophils and basophils were considerably higher in patients with asthma ($p < 0.0001$, respectively) (Table 2). Interestingly, the asthma group also proved to have fewer monocytes ($p < 0.005$). In this study, 37.7% (95% CI: 25.9% to 51.2%) of asthmatic patients had an eosinophil count of ≥ 400 cells/ μ L; additionally, 37.7% (95% CI: 25.9% to 51.2%) had a basophil count of ≥ 110 cells/ μ L. With regard to the total number of monocytes, patients with < 320 cells/ μ L accounted for 11.3% (95% CI: 4.9% to 22.9%) (Table 3). Through univariate analysis, we observed that patients with asthma had significantly higher odds of having an eosinophil count that was ≥ 400 cells/ μ L and $\geq 5\%$ (OR = 12.61, $p < 0.0001$ and OR = 6.49, $p = 0.0001$, respectively); increased odds of having a basophil count that was ≥ 110 cells/ μ L and $\geq 1.5\%$ (OR = 6.00, $p < 0.0001$ and OR = 6.50, $p < 0.0001$, respectively), plus higher odds of monocytopenia < 320 cells/ μ L and $< 5\%$ (OR = 13.79, $p = 0.017$ and OR = 4.51, $p = 0.039$, respectively).

A comparison between the NLR of both groups is displayed (Table 4). As presented, there is no difference between our two groups. In regard to the ELR, ENR, EMR and PLR, these were significantly higher in patients with asthma.

We compared inflammatory blood cell ratios between both groups (Table 5). With the exception of NLR, all other rates proved to be significantly associated with asthma. When EMR was ≥ 0.43 , it had the greatest measure of association (OR = 7.1, 95% CI 3.4 to 14.8), followed by ELR ≥ 0.11 (OR = 6.7, 95% CI 3.2 to 13.8), ENR ≥ 0.06 (OR = 5.6, 95% CI 2.7 to 11.4), and PLR ≥ 150 (OR = 2.9, 95% CI 1.2 to 7.0).

The following blood inflammatory cell ratios were negatively associated with ACT: ELR ($\rho = -0.379$, $p = 0.005$), ENR ($\rho = -0.336$, $p = 0.014$) and EMR ($\rho = -0.412$, $p = 0.002$) (Table 6).

Discussion

As a result of our findings, we would like to highlight three main points: first, when white blood cell screening was performed in the control group, our results provide a relative and absolute parameter to determine normal amounts of white blood cell counts; secondly, when we used these parameters to compare both groups, we were able to note that patients with asthma not only had a higher concentration of eosinophils, but that they also had a higher count of basophils, and a reduced count of monocytes; thirdly, based on the diverse estimates of inflammatory blood ratios observed in our study, it is evident that patients with asthma have a higher probability of experiencing chronic inflammation. A blood biometry allowed us to observe that patients with asthma have a considerably higher basophil count than those in the control group. Since the first debates, on which cells are involved in the pathogenesis of asthma, mentioned basophils as possible culprits, recently this vision has again gained new interest although still their role is not fully understood. A previous study compared a small sample of asthmatic patients to a healthy group of individuals, and it was discovered that asthmatic patients had a considerably higher basophil count in their sputum sample;

moreover, it is even more common among patients with eosinophilic asthma (15). This study also found that basophils are correlated to the number of eosinophils in blood, though the opposite is true when it comes to neutrophils. In regard to the respiratory function tests; in the same study, there appeared to be no association. The authors mentioned that determining the number of basophils and eosinophils can help identify the TH2-high asthma phenotype, furthermore, this might aid in understanding the role that different cells play in the pathogenesis of asthma (15). In another study, a group of patients with asthma produced similar results: the percentage of basophils found in sputum samples was correlated to sputum eosinophils, as well as mast cells, the level of exhaled nitric oxide and the amount of eosinophils in blood. Notably, there was no direct correlation with the asthma control test point system, FEV1, IgE serum level concentration, or the basophil count in blood (16). In our study, no association was observed between the concentration of eosinophils and basophils (results not shown). Thus, it is evident that basophils play an important role in asthma, but its effects are still unclear. When comparing the mean and median of absolute and relative monocyte concentration between patients with asthma and the control group, we observed that asthmatics had a significantly lower amount of these cells. To our knowledge, this is the first time this observation is being made. As of this moment, we are unclear as to the origin or the consequences of this finding, and additional studies are required to further elucidate on the matter.

When evaluating the systemic inflammatory state between both groups, we calculated the different inflammatory cell ratios by using a peripheral blood sample. First, we evaluated NLR, as it has been proven to be useful when predicting various cardiovascular diseases and different types of cancer (7, 8), yet, we found no difference in the mean and median between our two groups. This occurrence could be due to the fact that patients had not experienced asthma exacerbation, at least not during the month prior to our study. Another study included 54 children with asthma exacerbation and 94 healthy children to assess NLR as an inflammatory marker; though NLR was estimated for all participants, when it came to children with asthma, their NLR was calculated during a period of exacerbation and, then again, three months later. In their results, NLR levels among children during an episode of asthma exacerbation were considerably higher than during an asymptomatic period, and when they were compared to the control group (17). Another explanation may be due to the absence of an asthma categorization based on inflammatory patterns, as it is more likely that NLR increases when asthma is expressed as a neutrophilic phenotype (9). Therefore, when using NLR as an inflammatory marker, we must consider the possibility that it may only be altered during an episode of exacerbated asthma or when it comes to neutrophilic asthma. In our investigation, we found that normal NLR values derived from healthy patients are similar to those documented in previous studies (8, 18). Secondly, we observed that eosinophil ratios (ELR, ENR, and EMR) were significantly higher in patients with asthma than those in the control group. Zhang et al, included 164 patients with controlled asthma in a study, and they found that ELR, ENR and EMR can be used as predictable factors when identifying the eosinophilic asthma phenotype (9). Additionally, there is evidence that some of these ratios have been used to accurately predict mortality among patients that have experienced acute myocardial infarction (10); they have also been used for the prognosis of recurrent nasal polyposis (11). In the context of previous findings and our own, we deduce that eosinophils play an important role in the systemic inflammatory process. Thus, it is likely that patients with asthma and higher ELR, ENR and EMR, should not only be treated with inhaled steroids, but also with short-term systemic steroid therapy, and perhaps anti-IL-5 monoclonal antibodies, naturally, further studies are required on the matter. Thirdly, we proved that patients with asthma express higher PLR than healthy controls. To our knowledge, PLR has been previously used as a predictive biomarker for acute asthma exacerbations (19). Thus far, PLR has served as a predictive biomarker of pneumonia (20), and a prognostic marker for cancer (21), however, it is also a predictor of poor prognosis in patients with sudden hearing loss (22), sepsis mortality (23), or pulmonary thromboembolism (24), among other things. In brief, the estimation of the aforementioned ratios highlights the important role that chronic inflammation plays in patients with

asthma. It also denotes a predominant systemic inflammatory pattern dominated by eosinophils, which is found in the majority of asthmatic patients. Other groups focusing on biochemical markers of inflammation in asthma were also able to show a rise in IL-6 in patients with mild-moderate allergic asthma, which was directly related to a decline in lung-function (25). Also, elevated C-reactive protein levels have been demonstrated, although it is questionable if these inflammatory markers are directly related to asthma or more to the comorbidities (26). This leads us to two important questions that urgently await a response: a) Should we use systemic anti-inflammatory therapy to treat these patients? b) And if so, what is the best treatment option?

Due to the technical complexity of induced sputum cytology, we were unable to compare this to our peripheral blood findings. Nor were we able to compare other inflammatory markers to the diverse ratios of chronic inflammation that were obtained in our study (ELR, ENR, EMR and PLR). An additional limitation to the study was the inability to record the asthma control medications that patients used at the time of their admission. However, in a general view, we know that most patients used short-acting β_2 -agonists as rescue therapy in a regular manner and just a few of them regularly used inhaled steroids due to their recent asthma diagnosis. On the other hand, the majority of patients with asthma have allergic rhinitis, but it turned out that determining its role over the blood eosinophil concentration hindered the study. Furthermore, analyzing the role of air pollution over blood eosinophil expression became another obstacle. Ultimately, defining blood eosinophilia continues to be a challenge, since there is no consistency regarding the cut-off point that should be used to do so. While these are important limitations to our investigation, we do not believe they detract from the validity of our findings.

Conclusions

When compared to healthy individuals, patients with asthma have higher levels of eosinophils and basophils in their blood; they also have a reduced amount of monocytes. Interestingly, they have higher levels of ELR, ENR, EMR and PLR, all of which suggest that these measurements can be used as markers to help identify chronic inflammation in patients with asthma.

Declaration of interests and acknowledgements

Author contributions

Martín Bedolla-Barajas conceived the study design, oversaw its conduct, did the statistical analysis, and wrote the first draft of the article; Jaime Morales-Romero did the statistical analysis, and wrote the first draft of the article; Dante Daniel Hernández-Colín, Désirée Larenas-Linnemann, Juan Carlos Fonseca-López, Miriam Montzerrat Flores-Razo and Angie Bedolla-Pulido, contributed elements of the study design, collected patient information and interpreted the data, helped write and revise the article and conduct the study.

Conflict of interests

The authors declare no conflicts of interest.

References

1. GEMA 4.3. Guía española para el manejo del asma. Spain; 2018. 179p. Available from:

https://www.semg.es/images/documentos/docs_varios/GEMA_43.pdf

2. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. 2020. 162p. Available from: <https://ginasthma.org/wp-content/uploads/2020/04/GINA-2020-full-report-final-wms.pdf>
3. British Thoracic Society/ Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. 2019. 214p. Available from: <https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma/>
4. Jousilahti P, Salomaa V, Hakala K, Rasi V, Vahtera E, Palosuo T. The association of sensitive systemic inflammation markers with bronchial asthma. *Ann Allergy Asthma Immunol.* 2002;89(4):381-5.
5. Monadi M, Firouzbaji A, Hosseini A, Javadian Y, Sharbatdaran M, Heidari B. Serum C-reactive protein in asthma and its ability in predicting asthma control, a case-control study. *Caspian J Intern Med.* 2016;7(1):37-42.
6. Nadif R, Siroux V, Oryszczyn MP, Ravault C, Pison C, Pin I, Kauffmann F; Epidemiological study on the Genetics and Environment of Asthma (EGEA). Heterogeneity of asthma according to blood inflammatory patterns. *Thorax.* 2009;64(5):374-80.
7. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. *Int Arch Med.* 2012;5(1):2.
8. Forget P, Khalifa C, Defour JP, Latinne D, Van Pel MC, De Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio? *BMC Res Notes.* 2017;10(1):12.
9. Zhang XY, Simpson JL, Powell H, Yang IA, Upham JW, Reynolds PN. Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy.* 2014;44(9):1137-45.
10. Deng X, Wang X, Shen L, Yao K, Ge L, Ma J, et al. Association of eosinophil-to-monocyte ratio with 1-month and long-term all-cause mortality in patients with ST-elevation

- myocardial infarction undergoing primary percutaneous coronary intervention. *J Thorac Dis.* 2018;10(9):5449-5458.
11. Yenigün A. Assessment of patients with nasal polyposis by the neutrophil-to-lymphocyte ratio and eosinophil-to-lymphocyte ratio. *Kulak Burun Bogaz Ihtis Derg.* 2015;25(4):193-9.
 12. Norma Oficial Mexicana NOM-253-SSA1-2012, para la disposición de sangre humana y sus componentes con fines terapéuticos. Mexico; 2012. Available from:
http://www.dof.gob.mx/nota_detalle.php?codigo=5275587&fecha=26/10/2012
 13. Larenas-Linnemann D, Salas-Hernández J, Vázquez-García JC, Ortiz-Aldana I, Fernández-Vega M, Del Río-Navarro BE, et al. Guía Mexicana del Asma: GUIMA 2017. *Rev Alerg Mex.* 2017; 64 Supl 1:s11-s128.
 14. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force. Standardization of spirometry. *Eur Respir J* 2005;26(2):319-38.
 15. Brooks CR, van Dalen CJ, Hermans IF, Gibson PG, Simpson JL, Douwes J. Sputum basophils are increased in eosinophilic asthma compared with non-eosinophilic asthma phenotypes. *Allergy.* 2017;72(10):1583-86.
 16. Suzuki Y, Wakahara K, Nishio T, Ito S, Hasegawa Y. Airway basophils are increased and activated in eosinophilic asthma. *Allergy.* 2017;72(10):1532-39.
 17. Nacaroglu HT, İsgüder R, Bent S, Bahceci SE, Ceylan G, Korkmaz HA, et al. Can neutrophil/lymphocyte ratio be a novel biomarker of inflammation in children with asthma? *Eur J Inflamm.* 2016;14(2):109-112.
 18. Lou H, He L, Zhang G, Yu J, Chen Y, Yin H, et al. Normal Reference Intervals of Neutrophil-To-Lymphocyte Ratio, Platelet-To-Lymphocyte Ratio, Lymphocyte-To-Monocyte Ratio, and Systemic Immune Inflammation Index in Healthy Adults: a Large Multi-Center Study from Western China. *Clin Lab.* 2019;65(3).
 19. Shi G, Zhao JW, Ming L. *Nan Fang Yi Ke Da Xue Xue Bao.* 2017;37(1):84-88.

20. Kartal O, Kartal AT. Value of neutrophil to lymphocyte and platelet to lymphocyte ratios in pneumonia. *Bratisl Lek Listy*. 2017;118(9):513-16.
21. Valga F, Monzón T, Henriquez F, Antón-Pérez G. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as biological markers of interest in kidney disease. *Nefrología*. 2019;39(3):243-49.
22. Sifuentes-Cock DM, Romero-Moroni F, Romero-Orellano F. Índice neutrófilo/linfocito y plaquetas/linfocito como marcador en la hipoacusia súbita. *Revista Faso*. 2018;25(1): 26-31.
23. Shen Y, Huang X, Zhang W. Platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: interaction effect with disease severity-a retrospective study. *BMJ Open*. 2019;9(1):e022896.
24. Wang Q, Ma J, Jiang Z, Ming L. Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in acute pulmonary embolism: a systematic review and meta-analysis. *Int Angiol*. 2018;37(1):4-11.
25. Neveu WA, Allard JL, Raymond DM, et al. Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. *Respir Res*. 2010;11(1):28.
26. Ilmarinen P, Tuomisto LE, Niemelä O, et al. Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. *Eur Respir J*. 2016;48(4):1052-62.

Table 1. Clinical characteristics by groups.

	Asthma n = 53	Control n = 109	p value
Age, years, mean $\pm$ SD	33.8 $\pm$ 12.0	32.4 $\pm$ 10.4	0.432
Gender, n (%)			0.083
Male	21 (39.6)	59 (54.1)	
Female	32 (60.4)	50 (45.9)	
Somatometry			
Weight (kg), mean $\pm$ SD	76.4 $\pm$ 19.8	75.5 $\pm$ 12.0	0.737
Height (m), mean $\pm$ SD	1.64 $\pm$ 8.3	1.67 $\pm$ 8.2	0.011
BMI (kg/m ²), mean $\pm$ SD	28.5 $\pm$ 6.5	27.1 $\pm$ 3.9	0.154
BMI categories, n (%)			0.039
Underweight	2 (3.8)	0 (0)	
Normal	14 (26.4)	32 (29.4)	
Overweight	19 (35.8)	54 (49.5)	
Obesity	18 (34.0)	23 (21.1)	
Current tobacco use, n (%)	7 (13.2)	17 (15.6)	0.624
Current alcohol intake, n (%)	15 (28.3)	53 (48.6)	0.014
Hospitalization due to asthma in previous year, n (%)	17 (32.1)	---	
ACT, points			
Mean $\pm$ SD	14.6 $\pm$ 5.9	---	
≥ 20 , n (%)	15 (28.3)	---	
Respiratory function test, mean $\pm$ SD			
% Predicted baseline			
FVC	87.4 $\pm$ 18.0	---	
FEV ₁	72.7 $\pm$ 18.0	---	
FEV ₁ /FVC	69.4 $\pm$ 10.1	---	
% Predicted post- β 2 agonist			
FVC	95.5 $\pm$ 15.5	---	
FEV ₁	87.0 $\pm$ 16.7	---	
FEV ₁ /FVC	76.2 $\pm$ 10.3	---	
Asthma severity, n (%)			
Mild persistent	18 (34.0)	---	
Moderate persistent	24 (45.3)	---	

Severe persistent	11 (20.8)		---		
Serum IgE , UI/mL					
Median	233		---		
IgE $\geq$ 100, n (%)	39 (73.6)		---		
Allergic sensitization, n (%)					
House dust mite	39 (73.6)		---		
Epithelia	21 (39.6)		---		
Cockroach mix	25 (47.2)		---		
Trees	32 (60.4)		---		
Grass	20 (37.7)		---		
Weeds	29 (54.7)		---		
Fungi	8 (15.1)		---		

SD: Standard Deviation.

SD: Standard Deviation.

ACT: Asthma Control Test.

BMI: Body Mass Index.

FVC: Forced Vital Capacity.

FEV₁: Forced Expiratory Volume at 1 second.

Comparison of means was made for independent groups with Student's t-Test.

Comparison of proportions was made with Chi square or Fisher tests accordingly.

Table 2. Leukocyte formula according to study population.

	Asthma n = 53	Control n = 109	p value
Leukocytes, X 10 ⁹ /L			
Mean ± SD	7.47±2.05	7.29±1.16	0.473
Median	7.23	7.16	0.739
P ₂₅ -P ₇₅	6.25 – 8.91	6.37 – 8.15	
Min-Max	3.63 – 13.32	5.11 – 9.74	
Neutrophils, X 10 ⁹ /L			
Mean ± SD	4.13±1.47	4.02±0.96	0.585
Median	4.01	3.96	0.918
P ₂₅ -P ₇₅	3.13 – 5.11	3.34 – 4.61	
Min-Max	1.55 – 8.66	2.19 – 6.90	
Neutrophils, %			
Mean ± SD	54.52±7.80	54.56±7.17	0.973
Median	54.10	54.10	0.877
P ₂₅ -P ₇₅	48.17 – 59.70	49.85 – 60.40	
Min-Max	36.50 – 70.11	38.70 – 70.80	
Lymphocytes, X 10 ⁹ /L			
Mean ± SD	2.37±0.70	2.45±0.49	0.409
Median	2.39	2.39	0.317
P ₂₅ -P ₇₅	1.94 – 2.70	2.15 – 2.47	
Min-Max	1.02 – 4.65	1.41 – 4.03	
Lymphocytes, %			
Mean ± SD	32.18±6.26	33.90±6.69	0.120
Median	31.80	33.20	0.165
P ₂₅ -P ₇₅	27.78 – 37.72	28.55 – 39.10	
Min-Max	20.10 – 46.00	20.10 – 49.20	
Monocytes, X 10 ⁹ /L			
Mean ± SD	0.51±0.18	0.58±0.13	0.005
Median	0.48	0.58	0.002
P ₂₅ -P ₇₅	0.42 – 0.59	0.49 – 0.65	
Min-Max	0.00 – 1.08	0.30 – 1.01	
Monocytes, %			
Mean ± SD	6.72±1.77	7.96±1.46	< 0.0001

Median	6.60		7.90		< 0.0001
P ₂₅ -P ₇₅	5.91 – 7.90		7.25 – 8.80		
Min-Max	0.00 – 10.50		4.22 – 11.90		
Eosinophils, X 10 ⁹ /L					
Mean ± SD	0.38±0.26		0.18±0.09		< 0.0001
Median	0.29		0.16		< 0.0001
P ₂₅ -P ₇₅	0.21 – 0.57		0.11 – 0.21		
Min-Max	0.05 – 1.22		0.05 – 0.42		
Eosinophils, %					
Mean ± SD	4.87±3.73		2.45±1.26		< 0.0001
Median	4.10		2.17		< 0.0001
P ₂₅ -P ₇₅	2.70 – 5.86		1.50 – 3.02		
Min-Max	0.76 – 18.70		0.63 – 5.72		
Basophils, X 10 ⁹ /L					
Mean ± SD	0.10±0.04		0.07±0.02		< 0.0001
Median	0.09		0.07		< 0.0001
P ₂₅ -P ₇₅	0.07 – 0.12		0.06 – 0.09		
Min-Max	0.00 – 0.22		0.03 – 0.16		
Basophils, %					
Mean ± SD	1.36±0.67		1.03±0.31		< 0.0001
Median	1.30		1.01		< 0.001
P ₂₅ -P ₇₅	0.90 – 1.92		0.82 – 1.23		
Min-Max	0.00 – 2.60		0.51 – 1.81		

SD: Standard Deviation.

P₂₅, P₇₅: Percentile 25th, Percentile 75th.

Min, Max: Minimum, Maximum.

Comparison of means was made for independent groups with Student's t-Test.

Comparison of proportions was made with Chi square or Fisher tests accordingly.

Table 3. Univariate analysis of the comparison of the eosinophil, basophil and monocyte count between subjects with asthma and the control group.

	Asthma		Control					
	n = 53		n = 109					
	n	%	n	%	OR	95% CI	<i>p</i>	
Eosinophils, X 10 ⁹ /L								
≥ 0.400	20	37.7	5	4.6	12.61	4.39 to 36.22	< 0.0001	
< 0.400	33	62.3	104	93.4	1			
Eosinophils, %								
≥ 5	18	34.0	8	7.3	6.49	2.59 to 16.25	0.0001	
< 5	35	66.0	101	92.7	1			
Basophils, X 10 ⁹ /L								
≥ 0.110	20	37.7	10	9.2	6.00	2.55 to 14.11	< 0.0001	
< 0.110	33	62.3	99	90.8	1			
Basophils, %								
≥ 1.5	21	39.6	10	9.2	6.50	2.77 to 15.23	< 0.0001	
< 1.5	32	60.4	99	90.8	1	---		
Monocytes, X 10 ⁹ /L								
< 0.320	6	11.3	1	0.9	13.79	1.61 to 117.72	0.017	
≥ 0.320	47	88.7	108	99.1	1			
Monocytes, %								
< 5	6	11.3	3	2.8	4.51	1.08 to 18.81	0.039	
≥ 5	47	88.7	106	92.2	1			

Comparison of proportions was made with Chi square or Fisher tests accordingly.

OR: Odds Ratio.

CI: Confidence Interval.

Table 4. Comparison of the inflammatory cell ratios between patients with asthma and the control group.

	Asthma n = 53	Control n = 109	p value
NLR			
Mean $\pm$ SD	1.80 $\pm$ 0.61	1.71 $\pm$ 0.54	0.335
Median	1.71	1.63	0.448
P ₂₅ -P ₇₅	1.34 – 2.13	1.29 – 2.11	
Min-Max	0.90 – 3.48	0.79 – 3.21	
ELR			
Mean $\pm$ SD	0.17 $\pm$ 0.12	0.07 $\pm$ 0.04	< 0.0001
Median	0.13	0.06	< 0.0001
P ₂₅ -P ₇₅	0.08 – 0.22	0.04 – 0.09	
Min-Max	0.02 – 0.63	0.02 – 0.24	
ENR			
Mean $\pm$ SD	0.10 $\pm$ 0.09	0.05 $\pm$ 0.03	< 0.0001
Median	0.08	0.04	< 0.0001
P ₂₅ -P ₇₅	0.05 – 0.11	0.03 – 0.06	
Min-Max	0.01 – 0.46	0.01 – 0.12	
EMR			
Mean $\pm$ SD	0.77 $\pm$ 0.62	0.32 $\pm$ 0.20	< 0.0001
Median	0.52	0.27	< 0.0001
P ₂₅ -P ₇₅	0.37 – 0.98	0.20 – 0.40	
Min-Max	0.13 – 3.16	0.08 – 1.30	
PLR			
Mean $\pm$ SD	128.1 $\pm$ 49.81	111.77 $\pm$ 27.72	0.008
Median	115.22	110.38	0.084
P ₂₅ -P ₇₅	94.73 – 149.83	89.54 – 128.66	
Min-Max	48.24 – 300.98	58.88 – 181.25	

NLR: Neutrophil/Lymphocyte Ratio.

ELR: Eosinophil/Lymphocyte Ratio.

ENR: Eosinophil/Neutrophil Ratio.

EMR: Eosinophil/Monocyte Ratio.

PLR: Platelet/Lymphocyte Ratio.

P₂₅, P₇₅: Percentile 25th, Percentile 75th.

Min, Max: Minimum, Maximum.

Comparison of means was made for independent groups with Student's t-Test.

Comparison of medians was made with the Mann-Whitney U test.

Accepted Manuscript

Table 5. Univariate analysis of blood inflammatory cell ratios.

	Asthma		Control						
	n = 53		n = 109						
	n	%	n	%		OR	95% CI	<i>p</i>	
NLR									
≥ 1.65	29	54.7	53	48.6		1.28	0.66 – 2.46	0.467	
< 1.65	24	45.3	56	51.4		1	---		
ELR									
≥ 0.11	34	64.2	23	21.1		6.69	3.24 – 13.83	< 0.0001	
< 0.11	19	35.8	86	78.9		1	---		
ENR									
≥ 0.06	36	67.9	30	27.5		5.58	2.73 – 11.38	< 0.0001	
< 0.06	17	32.1	79	72.5		1	---		
EMR									
≥ 0.43	36	67.9	25	22.9		7.12	3.43 – 14.76	< 0.0001	
< 0.43	17	32.1	84	77.1		1	---		
PLR									
≥ 150	13	24.5	11	10.1		2.90	1.20 – 7.00	0.0183	
< 150	40	75.5	98	89.9		1	---		

NLR: Neutrophil/Lymphocyte Ratio.

ELR: Eosinophil/ Lymphocyte Ratio.

ENR: Eosinophil/Neutrophil Ratio.

EMR: Eosinophil/Monocyte Ratio.

PLR: Platelet/Lymphocyte Ratio.

OR: Odds Ratio.

CI: Confidence Interval.

Comparison of proportions was made with Chi square or Fisher tests accordingly.

Table 6. Correlation between blood inflammatory cell ratios and asthma severity.

	ACT	FEV ₁ Predicted baseline	Asthma severity	NLR	ELR	ENR	EMR	PRL
ACT	1							
FEV ₁ Predicted baseline	0.511 p < 0.0001	1						
Asthma severity*	- 0.429 p = 0.001	- 0.927 p < 0.0001	1					
NLR	- 0.023 p = 0.868	- 0.258 p = 0.062	0.219 p = 0.115	1				
ELR	- 0.379 p = 0.005	- 0.214 p = 0.123	0.190 p = 0.172	- 0.028 p = 0.844	1			
ENR	- 0.336 p = 0.014	- 0.152 p = 0.278	- 0.131 p = 0.349	- 0.409 p = 0.002	0.898 p < 0.0001	1		
EMR	- 0.412 p = 0.002	- 0.245 p = 0.077	0.226 p = 0.104	- 0.180 p = 0.198	0.844 p < 0.0001	0.842 p < 0.0001	1	
PRL	0.017 p = 0.905	- 0.173 p = 0.215	0.045 p = 0.750	0.389 p = 0.004	- 0.021 p = 0.884	- 0.154 p = 0.270	- 0.053 p = 0.704	1

FEV₁: Forced Expiratory Volume at 1 second.

ACT: Asthma Control Test.

NLR: Neutrophil/Lymphocyte Ratio.

ELR: Eosinophil/ Lymphocyte Ratio.

ENR: Eosinophil/Neutrophil Ratio.

EMR: Eosinophil/Monocyte Ratio.

PLR: Platelet/Lymphocyte Ratio.

* Asthma severity was determined as mild intermittent, moderate intermittent and severe intermittent.

Correlation estimated through Spearman's rho.

Accepted Manuscript