СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО
SERBIAN ARCHIVES
OF MEDICINE

Address: 1 Kraljice Natalije Street, Belgrade 11000, Serbia
☏ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

Borko Milanović[1,2], Gordana Vijatov-Đurić[1,2], Jelena Stojčević-Maletić[1,3], Vesna Stojanović[1,2,†]

Comparation between serum levels of interleukin-33 in children with allergic asthma before and after inhalatory corticosteroid treatment

Поређење серумских нивоа интерлеукина-33 код деце са алергијском астмом пре и после шестомесечне инхалаторне кортикостероидне терапије

[1]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia
[2]Institute for Child and Youth Health Care of Vojvodina, Pediatrics Clinic, Novi Sad, Serbia
[3]Clinical Center of Vojvodina, Center for laboratory medicine, Novi Sad, Serbia

Received: June 5, 2019
Revised: November 3, 2019
Accepted: November 22, 2019
Online First: November 28, 2019
DOI: https://doi.org/10.2298/SARH190605126M

*__Accepted papers__ are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.
Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.
When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

†**Correspondence to:**
Vesna STOJANOVIĆ
Institute for Child and Youth Health Care of Vojvodina
Hajduk Veljkova 10
21000 Novi Sad, Serbia
Email: **vesna.stojanovic@mf.uns.ac.rs**

Comparation between serum levels of interleukin-33 in children with allergic asthma before and after inhalatory corticosteroid treatment

Поређење серумских нивоа интерлеукина-33 код деце са алергијском астмом пре и после шестомесечне инхалаторне кортикостероидне терапије

SUMMARY

Introduction/Objective Interleukin 33 (IL-33) has a very significant function in inflammatory and autoimmune mechanisms, but it is significance in immunopathogenis mechanisms of different allergic diseases, including allergic asthma (AA) is more and more emphasized. Investigating serum levels of IL-33 in children with AA before and after six months from applying inhalation corticosteroid therapy (ICS Th) and correlating the gathered values of IL-33 with some clinical traits of the patient.

Methods The serum value of IL-33 has been determined in 61 children with AA before starting treatment and six months after treatment with ICS Th, and this was repeated in 30 healthy children.

Results Values of IL-33 in serum are significantly higher in children with AA that have not been treated with ICS Th during six months ($p = 0,00$; $p < 0,05$), as well as compared with healthy children ($p = 0,00$; $p < 0,05$). Serum values of IL-33in children with AA after six months of ICS Th and in healthy children do not show significant difference ($p = 0,88$; $p > 0,05$). The correlation between serum values of IL-33 before applying ICS Th and the severity, degree of AA control and the applied dose of ICS Th is statistically significant and positive.

Conclusion IL-33 values in the serum are significantly higher in children with AA that are not treated and in those that as badly controlled. Treatment with ICS Th during six months leads to significant reduction of IL-33 serum levels whose values are in positive correlation with the severity and control of AA.

Key words: interleukin-33, asthma, anti-inflammatory medication

САЖЕТАК

Увод/циљ Интерлеукин 33 (ИЛ-33) има веома битну функцију у инфламаторним и аутоимунским механизмима, али се све више истиче и значај у имунопатогенетским механизмима различитих алергијских обољења, укључујући и алергијску астму (АА). Испитивање серумских вредности ИЛ-33 код деце са АА пре и након шест месеци примене инхалаторне кортикостероидне терапије (ИЦС Тх) и корелације добијених вредности ИЛ-33 са појединим клиничким особинама ових пацијената.

Методе Одређена је серумска вредности ИЛ-33 код 61 детета са АА пре започињања лечења и шест месеци након третмана са ИЦС Тх као и код 30 здраве деце.

Резултати Вредности ИЛ-33 у серуму су значајно веће код деце са АА која нису лечена у односу на децу са АА код којих је спроведена ИЦС Тх током шест месеци ($p = 0,00$; $p < 0,05$), као и у односу на здраву децу ($p = 0,00$; $p < 0,05$). Серумске вредности ИЛ-33 код деце са АА након шест месеци ИЦС Тх и код здраве деце не показују значајне разлике ($p = 0,88$; $p > 0,05$). Корелација између серумских вредности ИЛ-33 пре примене ИЦС Тх и тежине, степена контроле АА као и примењене дозе ИЦС Тх је статистички значајна и позитивна.

Закључак Вредности ИЛ-33 у серуму су значајно веће код деце са АА која нису лечена и код којих је АА лоше контролисана. Третман са ИЦС Тх током шест месеци доводи до значајне редукције серумских нивоа ИЛ-33 чије вредности су у позитивној корелацији са тежином и контролом АА.

Кључне речи: интерлеукин-33; астма; дете; анти-инфламаторни лекови;

INTRODUCTION

Allergic asthma (AA) is a leading chronic disease in children; its incidence in last decades is on the constant increase on a global level, as well as in Serbia. Newest prevalence estimates across the world indicate

that 334 million people suffer from AA. It is estimated that the number of people with asthma will increase to over 400 million until 2025 [1].

Asthma is a chronic inflammatory disease of the airways that is characterized by episodes of reversible airway obstruction, bronchial hyperactivity, and chronic lung inflammation [2].

Interleukin-33 (IL-33) was initially identified in small veins with high endothelia when it was determined that it has similar molecular properties with certain members of IL-1 superfamily (IL-1α, IL-1β, IL-1Ra i IL-18) [3, 4, 5].

IL-33 can have pro, anti-inflammatory, and protective roles, so IL-33 represents a subject of numerous research in order to clarify the precise role of this cytokine in inflammatory diseases [6–9].

A large number of studies had as its aim the investigation of the exact role of IL-33 and ST2 receptors in Th2 mediated disorders. In most cases results pointed out that IL-33/ST2 axes stimulates the Th2 inflammatory response [10, 11, 12].

In the AA etiopathogenesis inflammatory cells and mediators that belong to the Th2 immune response, eosinophil and basophil granulocytes and mast cells have a key role. Environmental antigens like infections (virus, bacterial), allergens, and air pollution induce Th2 immune response that as an effect has the release of appropriate cytokines by epithelial cells. Function and significance of individual cytokines, among them IL-33, in patients suffering from AA, especially in children, are not precisely known [13, 14].

Up until now, research emphasizes the importance of IL-33 in initiating the differentiation of naïve CD4+ T cells and their maturation into Th2 cells that through their own specific cytokine profile lead to the activation of eosinophil granulocytes that support allergic inflammation, or create predispositions in an individual for the development of asthma and its exacerbation [15]. The direct influence of mast cells leads to the releasing of TNF that specifically emphasizes antigen sensibilization. Indirectly through mast cells and IL-13, IL-33 induces eosinophilia and hyper-reactivity of airways [16,17].

IL-33 is dominantly a product of tissue cells, although active leukocytes that are a classical source of other para-inflammatory cytokines present a significant source of this cytokine [5]. Tissue damage that occurs as a consequence of infection of exposure to hypersensitive individuals to an allergen can lead to the release of IL-33 [15, 18].

Results of other studies conducted on those suffering from AA shown an absence of correlation between serum IL-33 levels and other parameters of allergic inflammation (for instance Eo in blood, cumulative IgE in the blood) in persons with low atopic status while in those with high atopic status this correlation is present [19].

The explanation lies in the fact that IL-33 shows primarily the characteristics of a proinflammatory marker, while serum IgE and eosinophil granulocytes represent significant markers in the estimation of atopic status, but not the degree of inflammation [20, 21].

PATIENTS AND METHODS

We have performed a prospective study as the Institute for the Health of Youth and Children of Vojvodina in the period between September 2016 and March 2018. The study encompassed 61 children aged 6–18 with diagnoses AA. The control group was comprised from 30 healthy children of the same sex and age as the children in studied group.

Study protocol has been approved by the Ethical Commission (Faculty of Medicine Novi Sad, Institute for the Health of Youth and Children of Vojvodina, Clinical Center of Vojvodina). Signed informed consent has been attained from all parents, and from children older than 10 years. The study has been performed adhering to the principles of the Helsinki Declaration.

Inclusion criteria in the study group were age from 6 to 18 with newly diagnoses light to mild AA, or subjects with light to mild AA diagnosed earlier, but without ICS prophylaxis at least 6 months before inclusion into the study. Diagnosis and classification has been perfoemed by GINA guidelines [2].

Exclusion criteria were: existence of atopic dermatitis (AD), urticaria, food allergies, chronic respiratory infections, uncontrolled gastro-esophageal reflux, eosinophilic esophagitis, parasite infection, any other chronic infection, allergen specific immunotherapy (in e period before and during the study), acute infections and other acute illnesses, usage of systemic corticosteroids immediately before and during the planed study.

Children that comprised the study group underwent two additional examinations after the initial one, three, and six months after treatment. Anamnestic and hetero-anamnestic data for the first and the second control period were taken from all children in the study group. Type of hardship, need for the use of SABA (short lasting beta 2 agonist) and the frequency of it were especially noted. WE performed a clinical examination and specially noted the following: body mass and weight, nutrition levels (BMI, Z score, and percentiles), vital parameter values and transcutaneous oxygen saturation of hemoglobin as well as findings of a physical lung examination. All examines under wen an investigation of lung functioning via spirometer MasterScreenIOS, manufactured by Jaeger, Germany according to ATS guidelines (American Thoracic Society).

During the first examination and the follow up six months after all participants underwent laboratory examinations that amongst other thing encompassed determining levels of IL-33 in the serum. The measurement of IL-33 levels was performed at the laboratory of the Clinical Center of Vojvodina in Novi Sad. IL-33 levels were determined via direct sandwich enzyme linked immunosorbent assay test that contains recombined human IL-33 and polyclonal antibodies specific for IL-33 (commercial name for the test is Human IL-33 Quantikine®ELISA, produced by R&D systems, USA). |According to the specifications of the manufacturer, the minimal detectable level of IL-33 that can be determined by usage of this test is 0 pg/ml. All procedures have been performed by following the instructions of the manufacturer. The intensity of the colored reaction has been determined by an automatized immunochemical analyzing device ChemWell. Absorbance has been measure at 450 nm filter via standard curve of serially diluted standards. By using the standard curve we determined the levels of IL-33 in all 91 participants.

The therapeutic approach in asthma treatment with all participants was in accordance to GINA recommendations for the treatment of mild and medium asthma, in other words appropriately dosed ICS therapy was applied in all cases. During the study, on the follow up examinations at three and six months from the beginning of ICS therapy, allergic asthma difficulty based on GINA recommendations was determined based on the level of control (controlled, partially controlled, uncontrolled) and treatment intensity (low, medium, high ICS dosages).

All children in the control group underwent the following: anamnesis/hetero-anamnesis taken (except the information is the child being investigated for oversensitivity about any medication, the information pertaining to exclusion from the study were specially noted, like the absence of hardships and signs of acute infections two weeks before the examination, absence of chronic diseases). We performed a clinical examination and the following were especially noted: mass, height, nutrition level (BMI, Z score, and percentile), absence of clinical signs of infection. The level of IL-33 (pg/ml) in the serum was determined in the same way as in the investigated group.

Statistical analysis

Wilcoxon pair test, t-test for independent samples, Mann-Whitney U-test, test median i χ^2 test were used for the determination of statistical significance. Spearman's rank correlation coefficient was used to determine correlation. $p < 0.05$ is considered statistically significant. Data processing was performed by the statistical program package IBM SPSS Statistics 23.

RESULTS

Out of the total number of participants (n = 61) with an average age of nine years and six months, 32 (52.5%) were boys and 29 (47.5) were girls. The control group (n = 30) was made up of healthy children with an average age of nine years and eight months, 16 (53.3 %) boys and 14 (46.6 %) girls. (Table 1)

The severity and degree of AA control that the participants experienced after 3 and 6 months of ICS Th as well as the ICS dose that the participants received during the first 3 months and between the 3rd and 6th month of ICS Th are displayed in table 1. (Table 2)

IL-33 level values (pg/ml) in the investigated group before the application of ICS Th and 6 months after ICS Th are presented in table 2. Values of serum IL-33 (pg/ml) in the control group are also presented here. (Table 3)

Participant grouping with regards to serum IL-33 levels that the participants had before and after ICS Th are presented in table 3.

Children that suffered from AA before beginning ICS therapy had significantly higher values of IL-33 than healthy children of the same age ($U = 509$; $p = 0.00$; $p < 0.01$;).

In children where AA treatment has not commenced, IL-33 serum values were significantly higher compared to those that underwent 6 months of ICS Th ($Z = -4.394$; $p = 0.00$; $p < 0.01$). It has been determined that children that suffer from AA with higher values of IL-33 in the serum before ICS treatment have higher after six months of ICS therapy ($rs = 0.271$; $p = 0.04$; $p < 0.05$).

Results show that children with AA that have undergone six months of ICS Th do not show statistical differences in serum IL-33 values that healthy children of the same age ($U = 897$; $p = 0.88$; $p > 0.05$). (Table 4)

In table 4 we presented serum values of IL-33 (before and after six months of ICS T) referenced by AA severity and control level (that the children had 3 and 6 months from the introduction of ICS therapy) and applied medication dosages (during the first and second trimester of ICS Th)

DISCUSSION

In our study, we analyzed the values of IL-33 in the serum of children 6 to 18 yeas olf with allergic asthma and in healthy children. The average levels of IL-33 were higher in children with allergic asthma before ICS therapy and it was 2.550 ± 3.387 pg/ml. Lower values of IL-33 in the serum were detected in children after six months of ICS therapy with an average value of 0.838 ± 1.394 pg/ml. The lowest level of serum IL-33 has been measured in healthy children and is was 0.573 ± 0.632 pg/ml.

It has been determined that IL-33 serum levels are significantly higher in children that suffer from allergic asthma before the beginning of treatment concerning healthy children. Similar findings has been found in the research odBahrami and associates where IL-33 levels in 61 children with asthma were compared with IL-33 levels of children in the control group, those without asthma, and they were statistically significant. In the aforementioned study average values of IL-33 in the serum of children with asthma was 15.17 ± 32.3 pg/ml [22]. Higher IL-33 serum levels in children that suffer from asthma detected in the study by Bahrami and associates than those detected in our study can be explained by differences in inclusion criteria. In the research of Bahrami and associates participants with allergic asthma were included regardless of the length (intermittent, persistent) and asthma severity (light, intermediary, severe) in contrast to our study where participants with only the characteristics of persistent, light and intermediate asthma were included. Other studies that compared IL-33 levels in children with allergic asthma with those in healthy children also produced similar results. A meta-analysis that encompassed 8 previously conducted studies that cumulatively had 330 children with asthma and 248 healthy children shows that IL-33 serum levels were higher in children with asthma than in healthy children [23].

IL-33 serum values in healthy children that are in the control group of participants in our study are similar to those reported by other studies of pediatric populations. For instance, the average value of IL-33 in the serum of healthy children in the Iranian population was 0.61 ± 2.16 pg/ml [24]. But the results of research done on healthy adult population show higher IL-33 values that those detected in healthy children in our study. This fact suggests that patient age can be a significant factor in defining the normal span of IL-33 serum levels, and this is significant for interpreting laboratory findings in regular every day practice.

Our study shows that after six months of ICS therapy in children that suffer from allergic asthma, there is a significant decrease in serum IL-33 levels. We did not find similar research in literature available to us.

The relationship and influence of ICS on IL-33 in allergic asthma is not explained. Studies performed on cell cultures have pointed out the significance of IL-33 for the creation of corticosteroid resistance. Namely, the

research conducted by Kabata and associates point out that of the potential mechanisms of corticosteroid resistance that can emerge in Th2 mediated inflammation of the air ways can emerge is because of the influence of IL-33 on natural helper cells (NH) that represent a sort of ILCs2 (lymphoid cells of inborn immunity type 2) i.e. resistance can emerge as a consequence of IL-33 mediated proliferation and production of cytokines type 2 from the said cells [25]. In vitro research on cell cultures show that corticosteroids have a relatively efficient anti-inflammatory effect on IL-33 mediated inflammation [26].

In our study, children with allergic asthma that underwent six months of ICS treatment do not differ in IL-33 serum levels than healthy children of the same age. Studies that compared IL-33 serum levels before and after ICS therapy have not been found in the available literature. Similar values of IL-33 in the serum in children with allergic asthma after six months of ICS and in healthy children and the significant fall of IL-33 six months after ICS therapy can firstly be explained by the aforementioned anti-inflammatory effects of ICS. In addition, the results of our research can be explained by the characteristics of the participant group itself. Children included in our study exclusively had light and medium asthma severity while patients with severe forms of asthma and patients that required additional treatment in order to control their illness (LABA, combination of ICS+LABA, systemic corticosteroids) were excluded from our study. Maintaining high values of IL-33 despite the application of ICS monotherapy can perhaps be expected in patients with severe and/or steroid resistant form of allergic asthma, and this is not the subject of our study.

The incidence of participants regarding the severity and degree of AA control in our research is a direct consequence of inclusion and exclusion criteria of our study.

In our research the patients that had higher levels of IL-33 before ICS prophylaxis had a more severe form of allergic asthma during the follow up period, and worse control of asthma during the treatment period and they required higher dosages of ICS in the second trimester of treatment. Patients that after six months of ICS prophylaxis still had a severe form and worse control of allergic asthma had higher IL-33 levels in the serum. The connection of IL-33 and severity of allergic asthma is documented in other studies. Research performed by Bartami and associates on children with asthma has also shown a correlation between IL-33 in the serum and asthma severity. Lowest IL-33 serum levels were detected in children with mild asthma, somewhat higher values were identified in children with medium severity and highest in those with severe asthma [22]. In addition, studies conducted on adult populations have shown a significant difference in IL-33 values between patients with intermittent, light, medium severe and severe persistent asthma [27]. Guo and associates in a study conducted on 45 adult participants have shown a positive correlation between IL-33 levels in the serum and the

thickening of the basal membrane in bronchial biopsy samples and asthma severity [28]. Lower values of IL-33after six months of ICS monotherapy application confirm the anti-inflammatory effect of ICS and its suppressive potential on pro-inflammatory cytokines. Lower degrees of control and severe form of allergic asthma in participants that had higher values of IL-33 before and after six months of ICS therapy shows that IL-33 can be a useful marker when choosing the therapy type and dosage, i.e. to contribute to the optimal treatment of allergic asthma.

CONCLUSION

Results of our research and the cited results of other studies suggest that serum IL-33 can represent a potent biomarker for the severity of allergic asthma. The great importance of determining IL-33 serum levels during diagnostic evaluation of allergic asthma before starting the treatment shows a potential for better defining the asthma phenotype and with it an earlier optimization of therapy.

ACKNOWLEDGEMENT

This work is part of a doctoral dissertation: Milanović B. The effect of six-month inhaled corticosteroid treatment on IL-33 serum levels in children with allergic asthma (PhD thesis). Novi Sad: Univerzitet u Novom Sadu; 2019

Conflict of interests: None declared.

REFERENCES

1. Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017; 5:691–706.
2. Global Initiative for Asthma (GINA). 2019 GINA Report, global strategy for asthma management and prevention 2019. Revised 2018, April 12. Available from: http://www.ginasthma.org/
3. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin- 1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005; 23:479–90.
4. Gabryelska A, Kuna P, Antczak A, Białasiewicz P, Panek M. IL-33 mediated inflammation in chronic respiratory diseases—understanding the role of the member of IL-1 superfamily. Front Immunol. 2019; 10: 692.
5. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. J Clin Invest. 2019; 129(4):1441-51.
6. Nunez KG, Frank A, Gonzalez-Rosario J, Galliano G, Bridle K, Crawford D, et al.Interleukin-33 / Cyclin D1 imbalance in severe liver steatosis predicts susceptibility to ischemia reperfusion injury. PLoS One. 2019; 29;14(4):e0216242.
7. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. Nat Immunol. 2016; 17(2):122-31.
8. Huang P, Li X, Meng Y, Meng Y, Yuan B, Liu T et al. Interleukin-33 regulates hematopoietic stem cell regeneration after radiation injury. Stem Cell Res Ther. 2019; 10:123
9. Chan BCL, Lam CWK, Tam LS, Wong CK. IL33: Roles in allergic inflammation and therapeutic perspectives. Front Immunol. 2019;10:364.
10. Sjöberg LC, Gregory JA, Dahlén SE, Nilsson GP, Adner M. Interleukin-33 exacerbates allergic bronchoconstriction in the mice via activation of mast cells. Allergy. 2015; 70:514-21.
11. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell–derived cytokines: more than just signaling the alarm. J Clin Invest. 2019;129(4):1441–1451.
12. Zoltowska AM, Lei Y, Fuchs B, Rask C, Adner M, Nilsson GP. The interleukin-33 receptor ST2 is important for the development of peripheral airway hyperresponsiveness and inflammation in a house dust mite mouse model of asthma. Clin Exp Allergy. 2016; 46:479–90.
13. Khaitov MR, Gaisina AR, Shilovskiy IP, Smirnov VV, Ramenskaia GV, Nikonova AA, et al. The role of interleukin-33 in pathogenesis of bronchial asthma. Biochemistry (Mosc). 2018; 83(1):13–25.
14. Caminati M, Pham DL, Bagnasco D, Canonica GW. Type 2 immunity in asthma. World Allergy Organ J. 2018 Jun 26;11(1):13.
15. Fahy JV. Type 2 inflammation in asthma — present in most, absent in many. Nat Rev Immunol. 2015;15(1):57-65.
16. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family Nat Rev Immunol.2010; 10:103-10.
17. Johnston LK, Hsu CL, Krier-Burris RA, Chhiba KD, Chien KB, McKenzie A, et al. IL-33 precedes IL-5 in regulating eosinophil commitment and is required for eosinophil homeostasis. J Immunol. 2016; 197:3445–53.
18. Thio CL, Chi PY, Lai AC, Chang YJ. Regulation of type 2 innate lymphoid cell-dependent airway hyperreactivity by butyrate. J Allergy Clin Immunol. 2018: 142:1867–83.

19. Saglani, S, Lloyd CM. The Immunopathogenesis of Asthma. In: Wilmott RR, Bush A, editors. Kendig's Disorders of the Respiratory Tract in Children (9th edition). Saunders Eslevier, Philadelphia. 2019; 665-76.
20. Komai-Koma M, Brombacher F, Pushparaj PN, Arendse B, McSharry C, Alexander J, et al. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naïve mice. Allergy. 2012;67:1118–26.
21. Chan BCL, Lam CWK, Tam LS, Wong CK. IL33: Roles in Allergic Inflammation and Therapeutic Perspectives. Front Immunol. 2019;10:364.
22. Bahrami MS, Movahedi M, Aryan Z, Bahar MA, Rezaei A, Sadr M, et al. Serum IL-33 Is Elevated in Children with Asthma and Is Associated with Disease Severity. Int Arch Allergy Immunol. 2015;168:193-6.
23. Wang Y, Wang L, Hua S. Interleukin-33 in children with asthma: A systematic review and metaanalysis. AllergolImmunopathol. 2017;45:387-92.
24. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. Allergy. 2015;70:556-67
25. Kabata H, Moro K, Fukunaga K, Suzuki Y, Miyata J, Masaki K, et al. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation Nat Commun. 2013; 4: 2675.
26. Préfontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol. 2009; 183(8):5094-103.
27. Wang Y, Wang L, Hua S. Interleukin-33 in children with asthma: A systematic review and metaanalysis. AllergolImmunopathol. 2017;45:387-92.
28. Guo Z, Wu J, Zhao J, Liu F, Chen Y, Bi L, et al. IL-33 promotes airway remodeling and is a marker of asthma disease severity. J Asthma. 2014;51:863-9.

Table 1. Division of participants by asthma severity and control levels in the third and sixth month of ICS Th, as well as ICS dosages administered during the first three months and in the period from third to sixth month.

	After 3 months of ICS Th		**After 6 months of ICS Th**	
	Number of participants	Percentage	Number of participants	Percentage
Asthma severity				
Light	46	75.4%	39	63.9%
Medium difficulties	15	24.6%	18	29.5%
Difficult	0	0.0%	4	6.6%
Asthma control				
Controlled	59	96,7%	45	73.8%
Partially controlled	2	3.3%	15	24.6%
Uncontrolled	0	0.0%	1	1.6%
ICS medication dose	**During first 3 months**		**From trird to sixth month**	
Low	23	37.7%	47	77.0%
Medium	38	62.3%	14	23.0%

ICS Th – inhalation corticosteroid therapy

Table 2. Values of IL-33 serum levels (pg/ml) in the investigated group (before and six months after ICS Th) and in the control group.

	N	Min.	Max.	Mod	M	Mean	SD
Before ICS Th application	61	0.00	14.74	0.04	1.49	2.55	3.39
After 6 months of ICS Th application	61	0.00	7.80	0.00	0.26	0.83	1.39
Control group	30	0.00	2.68	0.00	0.26	0.57	0.63

N-number of participants; Mod-(most common value); M-media (medial value); SD-standard deviation (deviation from the mean) IL-33-interleukin 33; ICS Th- inhalation corticosteroid therapy

Table 3. Participants grouped by IL-33 serum levels before and after six months of ICS-Th

Participant groups based on IL-33 levels	Number of participants	Percentage
Higher values before ICS Th	45	73.8%
Higher values after ICS Th	13	21.3%
Same values before and after ICS Th	3	4.9%
Total/Ukupno	**61**	**100.0%**

IL-33-interleukin 33; ICS Th- Inhalation corticosteroid therapy

Table 4. Relationship between IL-33values in the serum with AA severity and control level, and applies ICS dose

Clinical indicators of the inflammation level	Before ICS Th-IL-33 (pg/ml)/		After six months ICS Th-IL-33 (pg/ml)/	
	r_s	p	r_s	p
AAseverity 3 months ICS Th	0.52**	0.00	0.23	0.08
AA severity 6 months ICS Th	0.42**	0.00	0.45**	0.00
AA control-3 months ICS Th	0.29*	0.02	0.14	0.28
AA control-6 months ICS Th	0.39**	0.00	0.39**	0.00
Medication dose: first 3 months of ICS Th	0.08	0.56	-0.02	0.87
Medication dose: second 3 months of ICS Th	0.48**	0.00	0.16	0.20

IL-33-interleukin 33; ICS Th- Inhalation corticosteroid therapy; AA-allergic asthma;
r_s – Spearman rank correlation coefficient $^{*}p<0.05$; $^{**}p<0.01$

