

RELATIONSHIP OF INFLAMMATORY ENDOTHELIAL MARKERS WITH THE SEVERITY OF ASTHMA IN CHILDREN

Nataliia Makieieva, Maryna Biriukova, Natalia Alekseeva, Iryna Piddubna, Yuliia Vasylchenko, Mariia Yavorovych, Victoria Koval, Dmytro Butov
Kharkiv National Medical University, Kharkiv, Ukraine

ODNOS INFLAMATORNIH ENDOTELIJALNIH MARKERA SA TEŽINOM ASTME KOD DECE

Natalija Makijejeva, Marina Birjukova, Natalija Aleksejeva, Irina Pidubima, Julija Vasilčenko, Marija Javorovič, Viktorija Koval, Dmitro Butov
Nacionalni medicinski univerzitet Harkov, Harkov, Ukrajina

ABSTRACT

Objective. Monocyte chemoattractant protein-1 (MCP)-1 and soluble vascular cell adhesion molecule-1 (sVCAM-1) are key regulators of the inflammation sites infiltrated with monocytes. The aim of the study was to investigate the role of these molecules in children with asthma.

Methods. The levels of MCP-1 and sVCAM-1 during asthma exacerbation on the background of therapy in children with various degrees of asthma severity as well as correlation relationships between MCP-1, sVCAM-1 and parameters of respiration function were determined.

Results. The level of MCP-1 and sVCAM-1 in all groups of the examined patients at the period of exacerbation of the disease was significantly higher than in children of the control group. On the background of therapy the levels of MCP-1 and sVCAM-1 were decreased in patients of all groups, regardless of asthma severity. Negative correlation relationships between MCP-1 and FEV₁, PEF, VC, FVC, FEV₁/FVC were revealed before and after the therapy.

Conclusions. The increased levels of the above-mentioned biomarkers were preserved on the seventh day of therapy. This testifies to the involvement of these chemokines into the formation and prolongation of the inflammatory process. The revealed negative correlation between MCP-1 and the main parameters of pulmonary function testified to participation of chemoattractant MCP-1 in chronic inflammation of the airways.

Key words: asthma; inflammation; biomarkers; monocyte chemoattractant proteins; cell adhesion molecules.

INTRODUCTION

Asthma is defined as a chronic genetically determined respiratory disease (1-3). A long-term aberrant immune response to non-pathogenic stimuli, which determines the pathogenesis of asthma, leads to such structural changes of airways and vascular remodeling (4, 5). At the first stages, the inflammation has general mechanisms based on the action of the cytokines complex (5, 6). Endothelial cells (EC) of blood vessels become the targets of all these factors. Surface molecules expressed by EC control the intensive migration of leukocytes to the centers of

SAŽETAK

Cilj. Monocitni hemoatraktantni protein-1 (MCP) -1 i rastvorljivi molekularni adhezivni vaskularni ćelijski molekul-1 (sVCAM-1) ključni su regulatori mesta zapaljenja koje infiltriraju monociti. Cilj studije bio je da istraži ulogu ovih molekula kod dece sa astmom.

Metode. Određene su koncentracije MCP-1 i sVCAM-1 tokom terapije održavanja astme kod dece s različitim stepenom težine astme, kao i međusobna povezanost MCP-1, sVCAM-1 i parametara funkcije disanja.

Rezultati. Koncentracije MCP-1 i sVCAM-1 u svim grupama pregledanih pacijenata u periodu pogoršanja bolesti bile su znatno više nego kod dece iz kontrolne grupe. Tokom terapije održavanja koncentracije MCP-1 i sVCAM-1 smanjile su se kod pacijenata svih grupa bez obzira na težinu astme. Negativne korelacije između MCP-1 i FEV₁, PEF, VC, FVC, FEV₁/FVC ustanovljene su pre i posle terapije.

Zaključak. Povećane koncentracije pomenutih biomarkera održavaju se do sedmog dana terapije. Ovo ukazuje na to da postoji veza između ovih hemokina i stvaranja i održavanja zapaljenjskog procesa. Otkrivena negativna korelacija između MCP-1 i glavnih parametara plućne funkcije sugeriše učešće hemoatraktanata MCP-1 u hroničnom zapaljenju disajnih puteva.

Ključne reči: astma; zapaljenje; biomarkeri; monocitni hemoatraktantni proteini; ćelijski adhezivni molekuli.

inflammation (7, 8). The expression of soluble vascular cell adhesion molecule-1 (s-VCAM-1) on the endothelium is increased under the influence of cytokines (IL-1, IL-6, IL-8, etc.). In the site of inflammation, the activated endothelium synthesizes chemokines and cytokines which attract and activate neutrophils and monocytes (4, 9). Monocytic chemoattractant protein-1 (MCP-1) is one of the main chemokines (10). The purpose of our study was to identify possible dependence of the levels of MCP-1 and sVCAM-1 on the severity of asthma and the effect of anti-inflammatory therapy with corticosteroids on the levels of these inflammatory markers and parameters of pulmonary function in children with varying severity of asthma.

PATIENTS AND METHODS

Study population

All the examined patients with asthma underwent treatment in Kharkiv City Children's Clinical Hospital No.16 from September 2016 to December 2017. They were divided into groups according to asthma severity. Group I included children with mild asthma. Group II included children with moderate asthma. Group III included children with severe asthma. Diagnostic criteria of asthma and therapy comply with the recommendations of GINA 2017. The study involved 68 patients (42 males and 26 females) aged from 5 to 18 with persistent asthma. There was no significant difference in sex and age in all the groups (Table 1). Patients were excluded from the asthma groups if other acute or chronic respiratory diseases, congenital anomalies of the lungs, or hereditary diseases of the pulmonary system were detected.

Treatment regimen

The examination of children with asthma was carried out on the first day of hospitalization before the beginning, and on the seventh day of asthma exacerbation treatment. Treatment of asthma exacerbation included the use of short-acting beta agonists through a spacer with a facial mask or through a nebulizer. Also, the therapy included the use of systemic and inhaled corticosteroids.

Ethics approval and consent to participate

The project was approved by the Medical Ethics Committee of Kharkiv National Medical University, Ukraine. It was conducted in accordance with the Declaration of Helsinki standards. All of the patients and their parents gave written informed consent and explicitly provided permission for treatment and blood tests, as well as for the collection of relevant clinical data.

Measurement of serum biomarkers

Venous blood (2 ml) was taken in the morning in the fasting state, and centrifuged at 2,300 x g for 10 min. Serum at the lower part of the test tube was stored at -20°C. Serum levels of sVCAM-1 were determined by enzyme-linked immunosorbent assay (ELISA, catalog #BMS232, Austria). The level of MCP-1 in blood serum

was measured by enzyme-linked immunosorbent assay (Human MCP-1 Platinum ELISA, immundiagnostik, catalog #BMS281, Bender MedSystems GmbH, Austria). The pulmonary function test was performed using computerized spirometer SpiroCom "KhAIMedica".

Statistical analysis

Statistica Version 8 (Tulsa, OK) was used for statistical evaluation and graphical presentation. The Kruskal-Wallis One Way Analysis of Variance (ANOVA) on Ranks was used for testing statistically significant differences in the median values among all groups. Mean MCP-1 and sVCAM-1 of two independent groups were compared by the Mann-Whitney U-test. The non-parametric Wilcoxon test (T) was used for comparison of two dependent samples. The correlation between MCP-1, sVCAM-1 and parameters of lung function was assessed by Spearman's linear correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

RESULTS

Levels of MCP-1 before the therapy and on the 7th day of treatment

During asthma exacerbation the levels of MCP-1 in all groups were higher than those in children of the control group. MCP-1 rates declined with the increase in the disease severity, but did not reach the healthy children values. There was a significant difference between groups I and II, III (p_{I-II}=0.005, p_{I-III}=0.001). The levels of MCP-1 in group I significantly differed only from the ones in the control group (p<0.0001). There was no significant difference between groups II, III (p=0.573) and between groups II, III and control group (p_{II-control}=0.113, p_{III-control}=0.620) (Fig. 1).

Follow-up levels of MCP-1 on the background of therapy were decreased in patients of all groups. The decrease in MCP-1 depended on the severity of asthma. MCP-1 serum levels were significantly decreased in all the groups (p_I<0.0001, p_{II}=0.043, p_{III}=0.027) on the background of treatment. MCP-1 levels significantly differed between group I and III (p=0.014). There were no significant differences between the groups I and II (p=0.230), II and III (p=0.423). The highest levels

Table 1. General information of patients with asthma

Groups	Number of cases	Males*	Females**	Age (years)***
Group I	30	18	12	10.78±3.62
Group II	11	7	4	10.75±2084
Group III	11	7	4	11.00±4.00
Control group	16	10	6	10.45±2065

*p_{I-II} 0.4312, p_{II-III} 0.5000, p_{I-III} 0.4312, p_{I-control} 0.4477, p_{II-control} 0.4792, p_{III-control} 0.4792

**p_{I-II} 0.4167, p_{II-III} 0.3777, p_{I-III} 0.3284, p_{I-control} 0.4347, p_{II-control} 0.4687, p_{III-control} 0.3415

***the mean ± standard deviation

remained in group I. Only group I ($p=0.001$) was found to have a significant difference from the control group. There were no significant differences between the groups II, III and control group ($p_{II-control}=0.238$, $p_{III-control}=0.268$) (Fig. 2).

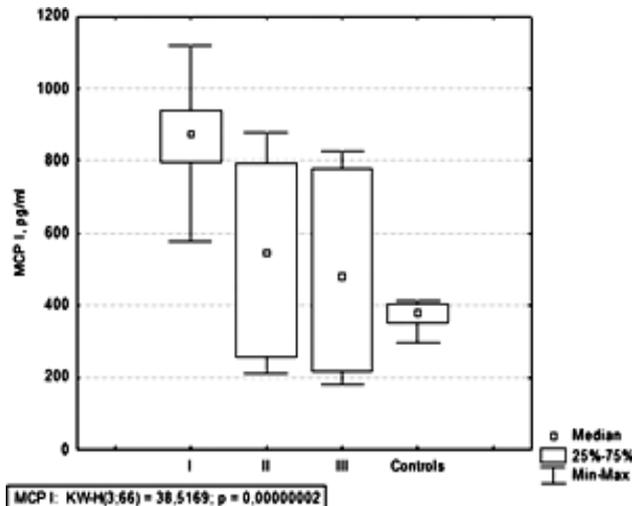


Figure 1. MCP-1 levels in the three groups of patients with asthma before starting of therapy and in the control group. The levels of MCP-1 in all groups were higher than those in children in the control group. There was significant difference between groups I and II, III ($p_{I-II}=0.005$, $p_{I-III}=0.001$). The levels of MCP-1 in the group I significantly differed only from ones in the control group ($p<0.0001$). There was no significant difference between groups II, III ($p=0.573$) and between groups II, III and control group ($p_{II-control}=0.113$, $p_{III-control}=0.620$).

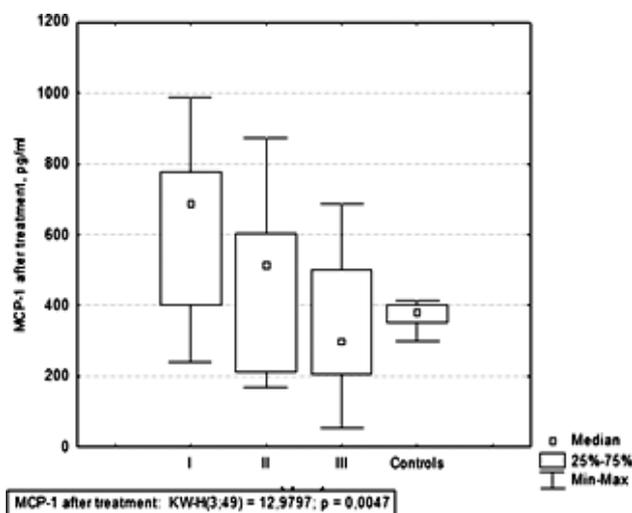


Figure 2. MCP-1 levels in patients with asthma on the 7th day of treatment. Levels of MCP-1 were decreased in patients of all groups. On the background of treatment MCP-1 levels in the serum was significantly decreased in all groups ($p<0.0001$, $p_{II}=0.043$, $p_{III}=0.027$). The highest levels remained in group I. Significant differences from the control group occurred only in group I ($p<0.0001$). There was no significant difference between the groups II, III and the control group ($p_{II-control}=0.238$, $p_{III-control}=0.268$).

Levels of sVCAM-1 before the therapy and on the 7th day of treatment

The levels of sVCAM-1 in patients of all groups were significantly increased in comparison with the values in children of the control group ($p<0.0001$). An increase in the severity of asthma was associated with an increase in sVCAM-1. The highest sVCAM-1 values were observed in patients with severe asthma. The levels of sVCAM-1 significantly differed between group I and III ($p=0.012$) and between group II and III ($p=0.017$). There were no significant differences between groups I and II ($p=0.345$) (Fig. 3).

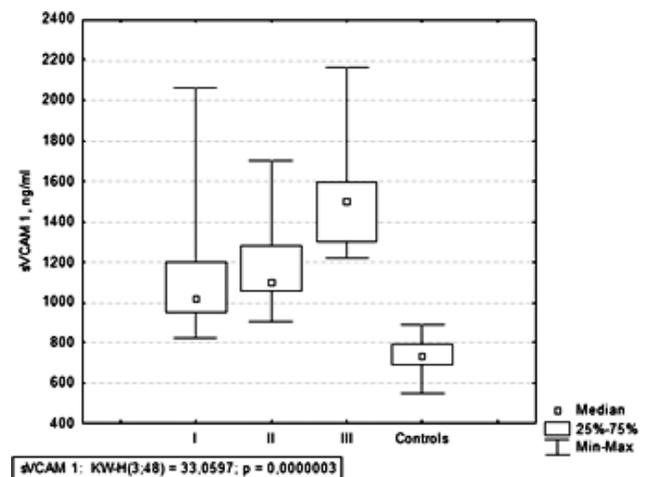


Figure 3. sVCAM-1 levels in the three groups of patients with asthma before start of therapy and in the control group. The levels of sVCAM-1 in patients of all groups were significantly increased in comparison with the values of children from the control group ($p<0.0001$). The highest sVCAM-1 values were in patients with severe asthma. Levels sVCAM-1 were significant different between group I and III ($p=0.012$) and between group II and III ($p=0.017$). There was no significant difference between the groups I and II ($p=0.345$).

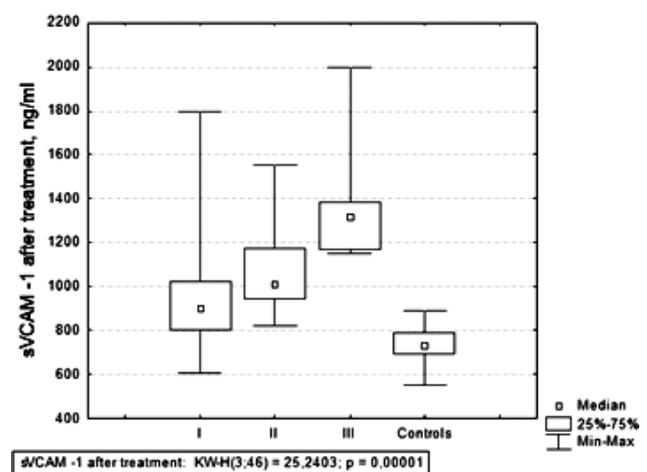


Figure 4. sVCAM-1 levels in patients with asthma on 7th day of treatment. sVCAM-1 levels in the serum were significantly decreased in all groups ($p<0.0001$, $p_{II}=0.011$, $p_{III}=0.027$). Despite the decrease in sVCAM-1 values during the treatment, they remained significantly higher than those in the control group ($p<0.0001$).

Serum sVCAM-1 level was significantly decreased in all the groups ($p_{I}<0.0001$, $p_{II}=0.011$, $p_{III}=0.027$) on the background of treatment. Despite the decrease in sVCAM-1 during treatment, its values remained significantly higher than those in the control group ($p <0.0001$). The levels of sVCAM-1 were significantly different between group I and III, II and III ($p_{I-III}=0.011$, $p_{II-III}=0.038$). There were no significant differences between groups I and II ($p=0.140$) (Fig. 4).

Correlation between MCP-1 and the main parameters of pulmonary function

The study showed a significant correlation between the levels of MCP-1 and FEV₁, PEF, VC, FVC, FEF₁/FVC in group I patients before the treatment. The r values of MCP-1 and FEV₁, PEF, FVC, VC, FEF₁/FVC were - 0.65, - 0.47, - 0.45, - 0.52, - 0.59, respectively ($p<0.05$). There was also a significant correlation between MCP-1 and FEV₁, VC, FVC, FEF₁/FVC in group I patients after the treatment ($r = - 0.52$, $r = - 0.46$, $r = - 0.69$, $r = - 0.56$ ($p<0.05$)). MCP-1 correlated negatively with VC ($r = - 0.90$, $p<0.05$) in group III after treatment. There was no significant correlation between MCP-1 and group II indices. There were no significant correlations between soluble adhesion molecules sVCAM-1 and the above parameters.

DISCUSSION

The study involved determination of the levels of MCP-1 and sVCAM-1 during exacerbation of asthma, and after cessation of clinical signs on the 7th day of therapy. The ability of these cells to release pro-inflammatory molecules is one of the pathogenic factors of the inflammatory response development in asthma (11, 12). MCP-1 also triggers proliferation of smooth muscle cells in vessels with secretion of pro-inflammatory cytokines leading to the disease progression due to vascular damage (3, 13). It should be noted that individuals with asthma have higher levels of MCP-1 (14, 15). However, these studies were conducted among adults. Such trials in pediatric population are limited, which prompted us to perform our study. The study showed that the level of this chemoattractant was increased in all pediatric patients with asthma as compared to the control group. The highest MCP-1 levels were detected in patients with mild persistent asthma course. An increase in asthma severity was associated with a decrease in MCP-1 levels. But these levels did not reach values of healthy children. We hypothesized that this was probably due to intensification of therapy. Anti-inflammatory basic therapy, including inhaled corticosteroids, becomes more intensive with an increase in asthma severity. Corticosteroids block many pathways of inflammation in asthma (16). Blockage of several genes encoding the synthesis of cytokines, chemokines, adhesion molecules,

inflammatory enzymes, receptors and proteins (active in chronic inflammation) is one of the main effects of corticosteroids. In higher concentrations they additionally affect synthesis of anti-inflammatory proteins and exert a post-genomic effect (16, 17).

The study also showed an increase in the level of sVCAM-1 in all groups of patients. The more severe asthma course was, the higher sVCAM-1 levels were observed in patients during exacerbation. The increase in the level of sVCAM-1 in patients with asthma can be regarded as a marker of the severity of chronic inflammatory process in view of the fact that this molecule has relatively selective inhibition of leukocyte adhesion. It provides the accumulation of mononuclear cells in the transition of acute phase of inflammation to the chronic one. Increased sVCAM-1 levels in patients with asthma in the absence of clinical manifestations after therapy testifies to the persistence of endothelial dysfunction. The severity of endothelial dysfunction is stipulated by the level of sVCAM-1 expression (4, 18, 19). The role of adhesion molecules in asthma has been confirmed by several studies which demonstrated an increase in the expression of adhesion molecules in bronchial epithelial and endothelial cells in patients with asthma (20, 21). Increased adhesion of eosinophils to sVCAM-1 was also studied in patients with labile asthma (22, 23), during seasonal birch blossom in individuals with sensitization to birch pollen (24). After a series of studies, serum adhesion molecules were proposed as markers of inflammation in asthma and allergies (25).

Long-term persistence of the antigen prolongs the course of alteration and exudation secondary to the developed proliferation, triggering chronic inflammation (4, 22). Macrophage cells are the main cells in the regulation of proliferation. Activated macrophages are accumulated in the focus of inflammation. In addition to the production of chemokines and cytokines, they synthesize the growth factor of fibroblasts and attract fibroblasts to the focus of inflammation, stimulate the proliferation of endothelial and smooth muscle cells of the vascular wall and basal membrane. Chronic exposure to cytokines has a special effect on endothelial cells. Adhesion molecules, such as sVCAM, previously diffusely expressed on the luminal surface of endothelial cells, are redistributed into intercellular clefts. A vicious circle is formed in the focus of chronic inflammation: lymphocytes activate macrophages through lymphokine secretions, and macrophages secrete monokines, thereby activating lymphocytes (9, 11, 14). In the late stages of chronic immune inflammation of EC, mast cells, eosinophils, basophils and mediators of inflammation produced by them, cause remodeling of the bronchi with the development of irreversible sclerotic changes due to angiogenesis, thickening of the basal membrane and proliferation of smooth muscles.

After the 1st week of the treatment MCP-1 levels in patients with severe asthma decreased to the control group levels, while in patients with mild and moderate course of the disease, these parameters, although decreased, did not reach the normal values. Perhaps this is due to administration of not only bronchodilators, but also of systemic corticosteroids, used to treat exacerbation of severe asthma in doses significantly exceeding the doses of inhaled steroids, which are usually used in the treatment of the attack period of mild and moderate asthma. As mentioned above, corticosteroids have a powerful anti-inflammatory effect. Inhibition of chemokine formation as an inflammation factor is one of the mechanisms of anti-inflammatory action of corticosteroids. The levels of sVCAM-1 during therapy were decreasing in all groups of patients, but they did not reach the normal values. Previous studies have also shown a decrease in sVCAM-1 levels in patients receiving higher doses of inhaled steroids. This might probably be caused by steroid-induced inhibition of IL production (26).

Assessing the level of inflammation biomarkers MCP-1 and sVCAM-1 we suggested that the inflammatory process in patients with asthma of different severity persisted even after reversal of clinical signs of exacerbation. Prolonged inflammation requires anti-inflammatory therapy during a longer period of time. Levels of MCP-1 and sVCAM-1 can be considered to be one of the laboratory criteria for the duration and effectiveness of anti-inflammatory therapy of asthma.

The revealed correlations between endothelial markers of inflammation and the main parameters reflecting the functional state of the respiratory tract allows us to confirm that the chemoattractant MCP-1 plays a direct role in inflammation of the airways, promotes the growth of hypersensitivity and bronchial hyperactivity (27). The remaining elevated levels of endothelial markers of inflammation after the therapy create conditions for the development of chronic inflammation which stipulates progressive tracheobronchial tree remodeling (3, 28, 29). Ultimately irreversible changes in the bronchial ways lead to a decrease in FVC, VC. This is confirmed by the detected negative correlations between MCP-1 (after the therapy) and such parameters as FEV₁, VC, FVC, FEV₁/FVC in group I patients and between MCP-1 (after the therapy) and VC in group III patients.

In conclusion, the levels of MCP-1 and sVCAM-1 were increased in patients of all groups at the time of hospitalization and remained high on the 7th day of therapy. This indicates the involvement of these chemokines into the formation and prolongation of the inflammatory process. A decrease in MCP-1 level along with an increase in severity of the disease may reflect the progression of inflammation with the formation of bronchial tree remodeling with elements of irreversible sclerotic changes. The revealed negative correlation between MCP-1 and the main parameters of pulmonary function testified

to participation of MCP-1 in chronic inflammation leading to remodeling of airways.

AUTHORS' CONTRIBUTIONS

All authors took part in planning and designing of the study, data analysis, drafting and critical revising of the article. Each author contributed as follows: N.Makieieva, D.Butov, N. Alekseeva developed the clinical study design; Y.Vasylchenko, V.Koval carried out patients recruitment and clinical data analysis; M.Biriukova, M.Yavorovych performed statistical analysis; M.Biriukova, N.Makieieva, I.Poddubnaya wrote the article. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors are grateful to all research volunteers who participated in this study. We acknowledge the wholehearted support of the clinical physicians, nurses, and lab personnel who contributed their efforts and made this study possible. This work was supported by the Kharkiv National Medical University (No 0111U001173). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the paper.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Vogelberg C. Preschool children with persistent asthmatic symptoms. *Ther Clin Risk Manag* 2019; 15: 451-60.
2. Gruffydd-Jones K. Unmet needs in asthma. *Ther Clin Risk Manag* 2019; 15: 409-421.
3. Singh SR, Sutcliffe A, Kaur D, et al. CCL2 release by airway smooth muscle is increased in asthma and promotes fibrocyte migration. *Allergy* 2014; 69: 1189-97.
4. Elliot JG, Jones RL, Abramson MJ, et al. Distribution of airway smooth muscle remodelling in asthma: relation to airway inflammation. *Respirology* 2015; 20: 66-72.
5. King GG, James A, Harkness L, Wark PAB. Pathophysiology of severe asthma: we've only just started. *Respirology* 2018; 23: 262-71.
6. Castro-Rodriguez JA, Saglani S, Rodriguez-Martinez CE, Oyarzun MA, Fleming L, Bush A. The relationship between inflammation and remodeling in childhood asthma: A systematic review. *Pediatr Pulmonol* 2018; 53: 824-35.

7. Shoda T, Futamura K, Orihara K, Emi-Sugie M, Saito H, Matsumoto K, Matsuda A. Recent advances in understanding the roles of vascular endothelial cells in allergic inflammation. *Allergol Int* 2016; 65: 21-9.
8. Soveg F, Abdala-Valencia H, Campbell J, Morales-Nebreda L, Mutlu GM, Cook-Mills JM. Regulation of allergic lung inflammation by endothelial cell transglutaminase 2. *Am J Physiol Lung Cell Mol Physiol* 2015; 309: 573-83.
9. Foster PS, Maltby S, Rosenberg HF, et al. Modeling TH2 responses and airway inflammation to understand fundamental mechanisms regulating the pathogenesis of asthma. *Immunol Rev* 2017; 278: 20-40.
10. Yao Y, Tsirka SE. Mouse monocyte chemoattractant protein 1 (MCP1) functions as a monomer. *Int J Biochem Cell Biol* 2014; 55: 51-9.
11. Velikova TV, Krasimirova E, Lazova SM, et al. MCP-1/CCL2 in a Bulgarian cohort of children with bronchial asthma and cystic fibrosis. *Archives of Immunology and Allergy* 2018; 1: 1-5.
12. Zonneveld R, Martinelli R, Shapiro NI, Kuijpers TW, Plotz FB, Carman CV. Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. *Crit Care* 2014; 18: 204. (doi: 10.1186/cc13733).
13. Yeryomenko G. The role of fractalkine and monocyte chemoattractant protein-1 in the progression of asthma. *Georgian Med News* 2018; 274: 125-30.
14. Roy RM, Wuthrich M, Klein BS. Chitin elicits CCL2 from airway epithelial cells and induces CCR2-dependent innate allergic inflammation in the lung. *J Immunol* 2012; 189: 2545-52.
15. Lee YG, Jeong JJ, Nyenhuis S, et al. Recruited alveolar macrophages, in response to airway epithelial-derived monocyte chemoattractant protein 1/CCL2, regulate airway inflammation and remodeling in allergic asthma. *Am J Respir Cell Mol Biol* 2015; 52:772-84.
16. Ramamoorthy S, Cidlowski JA. Corticosteroids-mechanisms of action in health and disease. *Rheum Dis Clin North Am* 2016; 42: 15-31.
17. Marandi Y, Farahi N, Hashjin GS. Asthma: beyond corticosteroid treatment. *Arch Med Sci* 2013; 9: 521-6.
18. Bi J, Hu Y, Peng Z, Liu H, Fu Y. Changes and correlations of serum interleukins, adhesion molecules and soluble E-selectin in children with allergic rhinitis and asthma. *Pak J Med Sci* 2018; 34: 1288-92.
19. Mishra A1, Guo Y2, Zhang L1. A critical role for P2X7 receptor-induced VCAM-1 shedding and neutrophil infiltration during acute lung injury. *J Immunol* 2016; 197: 2828-37.
20. Rahbarghazi R, Keyhanmanesh R, Aslani MR, Hassanpour M, Ahmadi M. Bone marrow mesenchymal stem cells and condition media diminish inflammatory adhesionmolecules of pulmonary endothelial cells in an ovalbumin-induced asthmatic rat model. *Microvasc Res* 2019; 121: 63-70.
21. Keyhanmanesh R, Rahbarghazi R, Ahmadi M. Systemic transplantation of mesenchymal stem cells modulates endothelial cell adhesionmolecules induced by ovalbumin in rat model of asthma. *Inflammation* 2018; 41: 2236-45.
22. Incorvaia C, Masieri S, Cavaliere C, Makri E, Sposato B, Frati F. Asthma associated to rhinitis. *J Biol Regul Homeost Agents* 2018; 32: 67-71.
23. Pope CA 3rd, Bhatnagar A, McCracken JP, Abplanalp W, Conklin DJ, O'Toole T. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. *Circ Res* 2016; 119: 1204-14.
24. Hakansson L, Heinrich C, Rak S, Venge P. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. *J Allergy Clin Immunol* 1997; 99: 551-62.
25. Paone G, Leone V, Conti V, et al. Blood and sputum biomarkers in COPD and asthma: a review. *Eur Rev Med Pharmacol Sci* 2016; 20: 698-708.
26. Umland SP, Nahrebne DK, Razac S, et al. The inhibitory effects of topically active glucocorticoids on IL-4, IL- 5, and interferon-gamma production by cultured primary CD4 T cells. *J Allergy Clin Immunol* 1997; 100: 511-19.
27. Di Stefano A, Coccini T, Roda E, et al. Blood MCP-1 levels are increased in chronic obstructive pulmonary disease patients with prevalent emphysema. *Int J Chron Obstruct Pulmon Dis* 2018; 13: 1691-700.
28. Grigoras A, Grigoras CC, Giusca SE, Caruntu ID, Amalinei C. Remodeling of basement membrane in patients with asthma. *Rom J Morphol Embryol* 2016; 57: 115-9.
29. Bush A. Cytokines and chemokines as biomarkers of future asthma. *Front Pediatr* 2019; 7: 72. (doi: 10.3389/fped.2019.00072).