Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

Article

MCP-1/CCL2 in a Bulgarian cohort of children with bronchial asthma and cystic fibrosis

Tsvetelina Velikova[1,*], Ekaterina Krasimirova[2], Snezhina Lazova[3], Penka Perenovska[3], Anna Valerieva[4], Dimitrinka Miteva[3], Vasil Dimitrov[4], Maria Staevska[4], Dobroslav Kyurkchiev[2] and Guergana Petrova[3]

[1] Clinical Immunology, University Hospital Lozenetz, Sofia; tsvelikova@medfac.mu-sofia.bg
[2] Laboratory of Clinical Immunology, University Hospital St. Ivan Rilski, Medical University of Sofia, Sofia; katrinkrasimirova@gmail.com, dsk666@gmail.com
[3] Pediatric Clinic, University Hospital Alexandrovska, Medical University of Sofia, Sofia; snejina@lazova.com, perenovska@abv.bg, dimi_sm@abv.bg, gal_ps@yahoo.co.uk
[4] Clinical Centre of Allergology, University Hospital Alexandrovska, Medical University of Sofia; anna.valerieva@gmail.com, vddim2012@gmail.com, mari66stra@gmail.com

* Correspondence: tsvelikova@medfac.mu-sofia.bg; Tel.: +359883306049

Abstract: C-C motif chemokine ligand 2 (CCL2), also called monocyte chemoattractant protein-1 (MCP-1) is a key β-chemokine involved in the migration of monocytes and macrophages, playing a significant role in the inflammatory responses in the airways. We aimed to assess the serum levels of MCP-1/CCL2 in a pilot cross-sectional study of Bulgarian children with bronchial asthma (BA) and cystic fibrosis (CF). Forty-two children were recruited to the study as follows: twenty with BA, twelve with CF and ten healthy children. Serum MCP-1/CCL2 levels were measured using ELISA. We found higher serum level of MCP-1/CCL2 in children with BA (191.09±64.96 pg/ml) and CF (258.51±76.45 pg/ml) compared to healthy children (70.30±64.30 pg/ml, p=0.022, and p=0.068, respectively). Younger patients with BA had higher levels of MCP-1/CCL2, as well as children with CF, with levels decreasing gradually with age. We observed also higher levels of MCP-1/CCL2 in children with moderate to severe BA compared to mild BA. We documented the significantly higher level of MCP-1/CCL2 in children with these chronic pulmonary diseases than in healthy controls, which suggesting that investigation of serum MCP-1/CCL2 levels could turn out to be beneficial for the severity of the disease.

Keywords: monocyte chemoattractant protein-1; MCP-1; C-C motif chemokine ligand 2; CCL2; childhood asthma; bronchial asthma; severe asthma; cystic fibrosis; chronic obstructive pulmonary disease.

1. Introduction

Chemokines are immune chemoattractant mediators which are divided into four families depending on the number and spacing of the conserved cysteine residues in the N-terminal part of their protein molecule [1]. They exert their functions through the activation of G-protein-coupled receptors with one of their major roles to selectively direct immune cells, i.e., monocytes, neutrophils, and lymphocytes, from the blood stream across the vascular endothelium for routine immunological surveillance of tissues or in response to active inflammation – a process called chemotaxis [1].

C-C motif chemokine ligand 2 (CCL2), formally called monocyte chemoattractant protein-1 (MCP-1) is a key β-chemokine involved in the migration of monocytes and macrophages, especially in the lungs [2]. Human MCP-1 is encoded on chromosome 17 and it is a 13 kDa protein comprised

© 2018 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

of 76 amino acids [3]. The MCP family has at least four members: MCP-1, -2, -3, and -4, where the CCL2 is the first discovered human CC chemokine [1].

The major source of MCP-1/CCL2 is found to be monocyte and macrophages, but a variety of cell types could produce it, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, microglial cells, mesenchymal stem cells etc. [1]. The secretion of MCP-1/CCL2 could be constitutive or after induction by oxidative stress, cytokines, growth factors, etc. [1]. We have to mention also that several other chemokines are able to recruit monocytes in the inflamed tissues, such as RANTES (CCL5). Both MCP-1/CCL2 and its receptor CCR2 have been involved in various diseases [1,4]. One of them is bronchial asthma (BA) which is the most common chronic disease of childhood, as well as the most prevalent cause of hospital admission. Moreover, childhood BA incidence is on the increase throughout the last decades [5]. In general, chemokines have been demonstrated to participate in the induction of chronic inflammatory processes, as well as in the pathogenesis of acute asthma episodes. Furthermore, it has been suggested that chemokine production is related to the severity of asthmatic inflammation and reactive airway responses [6]. It is thought that MCP-1/CCL2 plays a significant role in allergic responses in the airways by inducing mast cell activation and the release of inflammatory mediators, such as leukotriene C4, which leads to airway hyper-responsiveness [6]. There is evidence also for the involvement of MCP-1/CCL2 in the conduction of normal/abnormal T-cell immune response [1]. It was previously demonstrated that different T cell subtypes actively participate in the BA pathogenesis [7].

In the context of cystic fibrosis (CF), the chronic host inflammatory response could be also associated with increased airway levels of chemoattractants leading to recruitment and activation of pro-inflammatory monocytes [2]. Nevertheless, CF patients often have a chronic Pseudomonas aeruginosa infection, leading to airway inflammation and remodeling, re-infection, chronic tissue damage and subsequent cicatrization [8].

However, there are few studies involving children with chronic obstructive pulmonary disease and chemokine levels. On this background, we aimed to assess the serum levels of MCP-1/CCL2 in a pilot cross-sectional study involving a Bulgarian cohort of children with BA and CF.

2. Materials and Methods

Forty-two children divided into the following groups: twenty with BA at mean age 11 ± 3 years, twelve with CF at mean age 9 ± 3 years and ten healthy children at mean age 9 ± 2 years were recruited to the study according to the following criteria: female and male patients from 1 to 18 years of age, diagnosed with the specific condition according to the international criteria for diagnosis of BA and CF. Disease clinical severity was evaluated by a specialist and blood samples collected outside disease exacerbation within the past 30 days.

All subjects included in the study were informed about the purpose of the project and volunteered to participate. A written informed consent was obtained from parents/legal guardians. The study design was approved by the Ethical Committee of the Medical University of Sofia. The protocols from the Ethical Committee were included in the relevant projects/grants for the study (Project no. 289/2015, grant no. 54/2015; Project No: 270/2015).

The serum samples (2 ml) from each subject were collected using serum separator tubes, centrifuged in room temperature, and frozen at -80°C before testing. MCP-1/CCL2 was measured using Human CCL2/MCP-1 DuoSet® ELISA, Development system, and DuoSet Ancillary Reagent Kit 2 (R&D Systems, Inc., USA & Canada). The analytical sensitivity of the detection assay was 0.57-10 pg/ml. Firstly, we coated the clear microplates with the Human MCP-1 Capture Antibody overnight, then we washed and blocked them, and afterward, the ELISA was performed according to the manufacturer`s protocol.

Statistical analysis was performed with the software package for statistical analysis (SPSS®, IBM 2009, version 19). We accepted the results for significant if $p<0.05$.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

3. Results

We found that the mean serum level of MCP-1/CCL2 in children with BA was 191.09±64.96 pg/ml and in children with CF – 258.51±76.45 pg/ml, whereas in healthy children the mean level was 70.30±64.30 pg/ml (Table 1).

Table 1. Serum levels of MCP-1 (pg/ml) in the different studied groups of children.

	Children with BA	Children with CF	Healthy children
Mean	191.09	258.51	70.30
Standard error of the mean	64.96	76.45	64.30
Standard deviation	283.15	253.54	90.93
Range (Min – Max)	5.60 - 1032.50	64.30 – 777.20	6.00 – 134.60
Median	77.40	107.35	70.30
Interquirtile range (25^{th} – 75^{th} percentile)	64.15 – 185.55	80.40 – 405.05	60.00 – 112.40

The difference found between patients with CF and healthy children was considered significant (p=0.022). Concerning the results in patients with BA versus healthy children, a clear trend was observed without reaching significance in this sample size (p=0.068) (Figure 1).

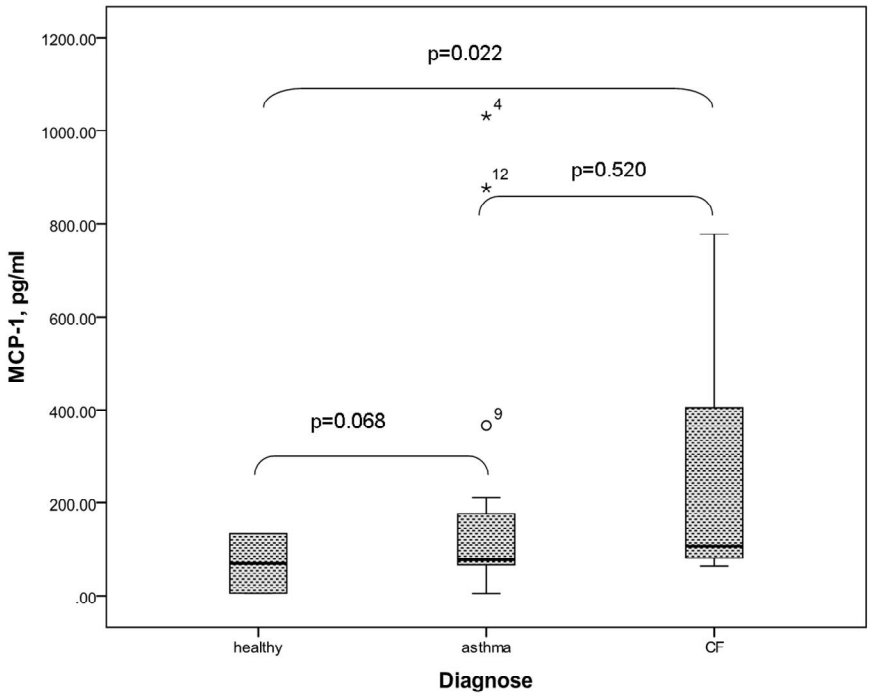

Figure 1. Serum levels of MCP-1/CCL2 in the different studied groups of children. The results are presented as Median [IQR].

We did not find differences in MCP-1/CCL2 level regarding sex affiliation in each studied group of children. According to age, we found higher levels of MCP-1/CCL2 in younger patients with BA (185.47±106.81 pg/ml in children below 10 years old vs. 77.40±45.01 pg/ml in children above 11 years of age). Similarly, the serum MCP-1/CCL2 levels decreased gradually with age in children with CF (405.05±117.34 pg/ml, 170.86±156.48 pg/ml, 98.12±39.11 pg/ml, in the age range below 5 years of age, 6-10 years, above 11 years, respectively). However, these differences did not reach significance.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

Dividing the children with BA into two groups regarding the severity of their asthma, we observed higher MCP-1/CCL2 levels in children with moderate to severe compared to those with mild asthma (203.64±81.56 pg /ml vs. 177.14±108.08 pg/ml). However, the difference could not reach significance due to the small sample size (Figure 2).

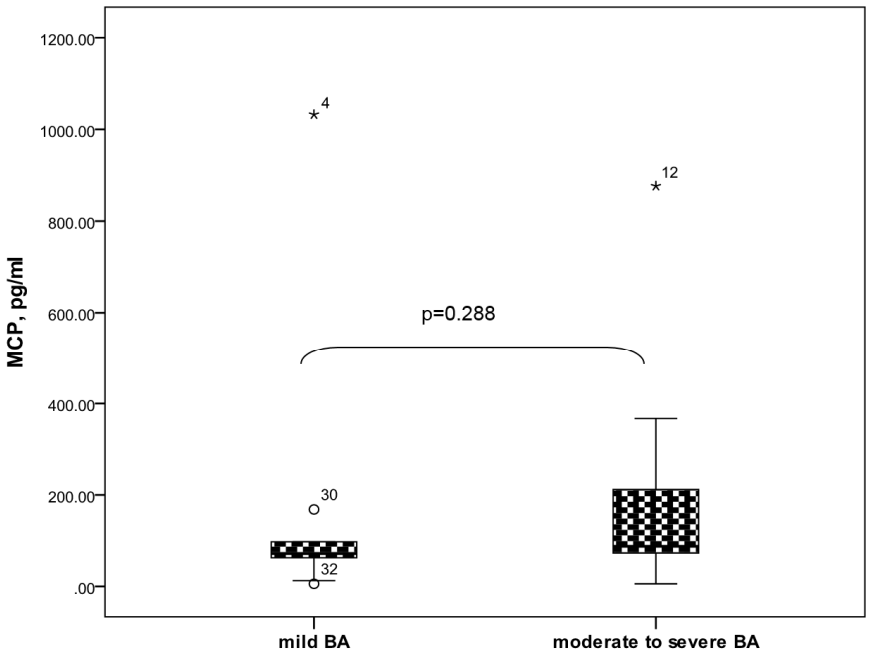

Figure 2. Mean levels of MCP-1/CCL in BA children with moderate to severe and mild asthma.

4. Discussion

In our pilot study, we assessed the serum levels of MCP-1/CCL2 in a Bulgarian cohort of children with BA and CF, as well as in healthy children. We found a significantly higher level of MCP-1/CCL2 in children with these two chronic pulmonary diseases than in healthy controls, with the chemoattractant levels being demonstrated higher in CF than BA patients.

Dividing the BA children into two groups regarding the severity of their asthma, higher levels were found in those children with moderate to severe disease compared to those having a milder course of the asthma. These results are in confirmation with other authors, who documented significantly higher serum levels of MCP-1 in children patients with an acute asthma attack than levels during asymptomatic phases (682.88±88.45 pg/ml vs. 329.57±99.20 pg/ml, $p<0.001$) [6]. Moreover, even in asymptomatic patients, the serum levels of MCP-1 were significantly higher than MCP-1 serum levels in the control group (329.57±99.20 pg/ml vs. 213.63±77.29 pg/ml, $p=0.007$) [6]. The observation that after treatment of the acute BA attacks, the serum levels of MCP-1 decreased rapidly, allowed the authors to suggest that MCP-1 might play a key role in the asthmatic inflammation and airway hyper responsiveness, and thus, in the acute asthma exacerbation pathogenesis [6]. Our results regarding MCP-1 levels and the severity of the asthma are in line with these considerations: children with moderate to severe asthma and poor disease control had higher levels of MCP-1/CCL2.

Chen et al. further extend their research in BA treatment and found that the concentration of MCP-1/CCL2 declined within 1 week after treatment but remained significantly higher than the levels in the control group for up to 2 months post exacerbation. They conclude that even after clinical manifestation had subsided, the ongoing airway inflammation is clearly demonstrated [6]. Cevit et al. and Hsieh et al. both reported increased chemokine production in BA patients which

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

decreased after successful immunotherapy [9,10]. All these studies, including ours, showed that investigation of MCP-1/CCL2 levels in BA children could be helpful for identifying those patients with systemic evidence of allergic inflammation, as well as for predicting the risk for exacerbations.

Other authors reported increased levels of MCP-1/CCL2 in the bronchial lavage of BA patients, which correlated with the serum levels [11]. However, since the bronchoscopy is an invasive and difficult method of examination, the serum testing of MCP-1/CCL2 should be preferable, especially in BA children.

However, Keszei et al. reported controversial results regarding MCP-1/CCL2 serum levels in BA children. They observed significantly lower concentrations in the BA than in the control children group (189 ± 85 pg/ml vs. 315 ± 106 pg/ml, respectively; $p<0.0001$) [5]. We should emphasize, however, that the levels in their group of children with BA were similar to our BA patients (191.09±64.96 pg/ml), which raises the doubt regarding the selection of the healthy children in their study.

Concerning the children with CF, we found the highest level of MCP-1/CCL2 (258.51±76.45 pg/ml). Rao et al. noticed increased CCL2, but not CX3CL1 in the airway and blood of CF patients [2]. The blood concentrations of CCL2 was increased in CF children compared with controls (1081 pg/ml vs. 295.5 pg/ml, respectively; $p=0.006$) [2]. They do not find an association of MCP-1/CCL2 serum levels with age, whereas we observed gradual decreasing of the serum MCP-1/CCL2 levels with age in children with CF. In their study, there was no association between CCL2 and lung function in CF patients, including FEV1% predicted, clinical exacerbations, etc. [2]. Lacking such associations, however, does not exclude the major role of CCL2 in CF-associated lung damage. Moreover, tissue-damaging airway inflammation precedes changes in lung function [2]. McAllister et al. reported no increase in CCL2 levels during an exacerbation in CF patients [12].

In our study, due to the cross-sectional design we could not establish associations between MCP-1/CCL2 levels and clinical course of the BA and CF. Thus, more longitudinal studies are needed to provide information regarding the role of serum MCP-1/CCL2 as predicting the risk of exacerbations, long-term tissue damage and cicatrization, and for the follow-up of the patients.

5. Conclusions

We documented a significantly higher level of MCP-1/CCL2 in children suffering from two chronic pulmonary diseases, such as BA and CF than in healthy controls. Moreover, children with moderate to severe asthma showed higher levels of MCP-1/CCL2 compared to those with mild asthma, although not reaching statistical significance. Our observations suggest a pathway involved in the chronic lung inflammation and pathogenesis of BA and CF, through a selectively recruited monocytes, neutrophils, and lymphocytes in the lung tissues. Thus, we could recommend investigation of serum MCP-1/CCL2 levels as possibly beneficial in children with BA and CF, and suggestive for the severity of the disease.

Acknowledgments: This work was supported by a grant from the Medical University of Sofia (Project no. 289/2015, grant no. 54/2015; Project No: 270/2015)..

Author Contributions: T. Velikova and A. Valerieva conceived and designed the experiments; S. Lazova, P. Perenovska, D. Miteva and G. Petrova selected, diagnosed and recruited the patients and healthy controls; T. Velikova and E. Krasimirova performed the testing; T. Velikova analysed and interpreted the data. T. Velikova and A. Valerieva have drafted the work; V. Dimitrov, M. Staevska, D. Kyurkchiev and G. Petrova substantively revised it. All authors has approved the submitted version..

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 April 2018 doi:10.20944/preprints201804.0278.v1

References

1. Deshmane, S.L.; Kremlev, S.; Amini, S. Sawaya, B.E. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. Journal of interferon & cytokine research, 2009, 29(6), 313-326, DOI: 10.1089/jir.2008.0027.
2. Rao, S.; Wright, A.K.A.; Montiero, W.; Ziegler-Heitbrock, L.; Grigg, J.; Monocyte chemoattractant chemokines in cystic fibrosis. Journal of Cystic Fibrosis, 2009, 8, 97–103, DOI 10.1016/j.jcf.2008.09.009.
3. Van Coillie, E.; Van Damme, J.; Opdenakker, G. The MCP/ eotaxin subfamily of CC chemokines. Cytokine Growth Factor Rev, 2009, 10, 61–86.
4. Valerieva, A.; Velikova, T.; Petkova, E.; Krasimirova, E.; Nedeva, D.; Krusheva, B.; Lazarova, T.; Dimitrov, V.; Staevska, M. Markers for systemic inflammation (CRP, ESR, IL-6, IL-10, IL-33, CCL2/MCP-1), and the disease severity in patients with chronic spontaneous urticaria. Allergy, 2017, 72, 153-154.
5. Keszei, M.; Nagy, A.; Kozma, G.T.; Radosits, K.; Tölgyesi, G.; Falus, A.; Szalai, C. Pediatric Asthmatic Patients Have Low Serum Levels of Monocyte Chemoattractant Protein-1. Journal of Asthma, 2006, 43, 399–404, DOI: 10.1080/02770900600710433.
6. Chan, C.K.; Kuo, M.L.; Yeh, K.W.; Ou, L.S.; Chen, L.C.; Yao, T.C.; Huang, J.L. Sequential Evaluation of Serum Monocyte Chemotactic Protein 1 Among Asymptomatic State and Acute Exacerbation and Remission of Asthma in Children Journal of Asthma, 2009, 46, 225–228, DOI 10.1080/02770900802553805.
7. Lazova, S.; Velikova, T.; Petrova, G.; Ivanova-Todorova, E.; Tumangelova-Yuzeir, K.; Kyurkchiev, D.; Perenovska, P. Immunological face of pediatric asthma. Detski i Infects. Bol, 2016, VIII(1), 8-15.
8. Murphy, B.S.; Bush, H.M.; Sundareshan, V.; Davis, C.; Hagadone, J.; Cory, T.J.; Hoy, H.; Hayes, D.; Anstead, M.I.; Feola, D.J. Characterization of macrophage activation states in patients with cystic fibrosis. Journal of Cystic Fibrosis, 2010, 9, 314–322, DOI 10.1016/j.jcf.2010.04.006.
9. Cevit, O.; Kendirli, S.G.; Yilmaz, M.; Altintas, D.U.; Karakoc, G.B. Specific allergen immunotherapy: effect on immunologic markers and clinical parameters in asthmatic children. J Investig Allergol Clin Immunol, 2007; 17, 286–291.
10. Hsieh, K.H.; Chou, C.C.; Chiang, B.L. Immunotherapy suppresses the production of monocyte chemotactic and activating factor and augments the production of IL-8 in children with asthma. J Allergy Clin Immunol, 1996; 98, 580–587.
11. Alam, R.; York, J.; Boyars, M.; Grant, J.A.; Lee, J.; Forsythe, P. et al. Increased MCP-1, RANTES, and MIP-1alpha in bronchoalveolar lavage fluid of allergic asthmatic patients. Am J Respir Crit Care Med, 1996, 153, 1398–1404, DOI 10.1164/ajrccm.153.4.8616572.
12. McAllister, F.; Henry, A.; Kreindler, J.L. et al. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol, 2005, 175, 404–12, DOI