Original paper

Systemic corticosteroid therapy augments *ex vivo* release of sCD163 by peripheral blood monocytes of asthmatic patients

Paweł L Bernatowicz[1], Paweł Golec[2], Paweł Bielecki[3], Krzysztof Kowal[4,5]

[1]Department of Haematology, Medical University of Bialystok, Bialystok, Poland
[2]Department of Thoracic Surgery, Medical University of Bialystok, Bialystok, Poland
[3]Department of Otolaryngology, Medical University of Bialystok, Bialystok, Poland
[4]Department of Experimental Allergology and Immunology, Medical University of Bialystok, Bialystok, Poland
[5]Department of Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

Abstract

Introduction: The CD163 is exclusively expressed by mononuclear phagocytes as a transmembrane protein, which synthesis is regulated by anti- and pro-inflammatory signals. After shedding from the cell surface it exists in body fluids as a soluble protein (sCD163) which exerts anti-inflammatory effects.

Aim: To evaluate serum concentration and *ex vivo* production of sCD163 by peripheral blood mononuclear cells (PBMC) in asthmatic patients treated with inhaled (ICS) or oral corticosteroids (OCS).

Material and methods: The study was performed on 35 allergic asthma patients (AAs) including 15 treated with ICS (ICS-AAs), 10 with OCS (OCS-AAs), 10 during asthma exacerbation (EX-AAs) before OCS had been started and 13 non-atopic healthy subjects (HCs) as a control group. PBMC were cultured *in vitro* for up to 144 h. The concentration of sCD163 in serum and the culture supernatants was evaluated with ELISA.

Results: The greatest serum sCD163 concentration was demonstrated in EX-AAs, which was significantly greater than that in other studied subgroups. The concentration of sCD163 in PBMC culture supernatants was greater in AAs than in HCs ($p = 0.006$). Among individual asthma subgroups the greatest concentration of sCD163 was demonstrated in PBMC culture supernatants of OCS-AAs, which was significantly greater than in ICS-AAs ($p < 0.001$) and EX-AAs ($p < 0.001$), both being significantly greater than in HCs ($p < 0.001$).

Conclusions: In AAs, enhanced capability of PBMCs to release sCD163 may be at least partially responsible for the anti-inflammatory effects of systemic corticosteroid therapy.

Key words: CD163, asthma, inhaled corticosteroids, induced sputum.

Introduction

CD163 belongs to group B of the scavenger receptor cysteine-rich (SRCR) superfamily [1]. It is exclusively expressed by mononuclear phagocytes as a type I transmembrane protein which synthesis is regulated by anti- and pro-inflammatory signals [2]. Moreover, CD163 is also detected as a soluble protein in body fluids including plasma [3]. The main mechanism responsible for appearance of soluble CD163 in body fluids is thought to be shedding of CD163 from the cell surface of the mono-

nuclear phagocytes [4]. At least two enzymes have been implicated in this process: matrix metalloproteinase-9 (MMP-9) [5] and tumour necrosis factor α converting enzyme (TACE/ADAM17) [6]. Increased activity of MMP-9 has been associated with elevated levels of sCD163 in body fluids [7].

Expression of CD163 is strongly upregulated by anti-inflammatory mediators including corticosteroids (CS) and interleukin-10 (IL-10) [8]. Corticosteroids are very effective inducers of CD163 expression and the magnitude

of upregulation of CD163 expression depends on the potency of the CS [9]. Those having the greatest affinity for the CS receptor are the most potent for upregulating CD163 expression [10]. Simultaneous application of dexamethasone and IL-10 to macrophages cultured *in vitro* exerts an additive effect on CD163 expression [11]. Some studies demonstrated anti-inflammatory effects of CD163 [12–14]. Soluble CD163 inhibits in a dose-dependent manner phorbol ester-induced T cell proliferation *in vitro* [13]. This anti-inflammatory function of CD163 seems to be restricted to its soluble form as membrane-bound CD163 does not exert such an effect [14]. The associations between T cell proliferation and CD163 expression come also from *in vivo* studies in which expression of CD163 was inversely correlated with markers of T cell proliferation [15]. Macrophages from lymphoid follicles, which are located in a place where intensive lymphocyte proliferation occurs, express little or no CD163 at all [16]. Moreover, sCD163 modulates cytokine release induced by house dust mite allergens by peripheral blood mononuclear cells *in vitro* leading to augmented IL-10 secretion [17].

Our previous studies demonstrated that circulating monocytes of asthmatic patients, in particular those with severe asthma, have a greater expression of CD163 than those of healthy subjects [18, 19]. Moreover, systemic corticosteroid therapy leads to significant upregulation of CD163 expression on circulating monocytes [19]. Recently, the elevated serum sCD163 level has been linked to overweight/obesity and increased risk of asthma exacerbation in pregnant asthmatic women [20]. Therapy of mild-moderate asthmatic patients with inhaled corticosteroids leads to a dramatic increase in sCD163 concentration in induced sputum indicating a strong effect of corticosteroids on sCD163 production locally at the site of inflammatory response [21]. However, little is known on the effect of systemic corticosteroid therapy on CD163 production in asthmatic patients.

Aim

The aim of the current study was to evaluate the serum concentration of sCD163 and *ex vivo* release of this protein by peripheral blood mononuclear cells in patients with and without systemic corticosteroid therapy.

Material and methods

The study was performed in 35 allergic asthma (AAs) patients including 15 treated with inhaled corticosteroids (ICS), 10 treated with oral corticosteroids (OCS) and 10 during asthma exacerbation (EX) before OCS had been started. In addition, 13 non-atopic healthy subjects were included as a control group. After asthma diagnosis its severity was assessed according to the Global Initiative for Asthma (GINA) criteria. In all patients, forced expira-

tory volume within the 1st s (FEV_1) of less than 80% of the predicted value with at least 12% improvement 15 min after inhalation of 400 μg salbutamol was demonstrated. In OCS-AAs, a stable dose of oral corticosteroids had been used for at least 14 days before the study. Evaluation of EX-AAs was performed before systemic corticosteroid therapy was introduced. Asthma exacerbation was defined as progressive deterioration of lung function, which ultimately required therapy with systemic corticosteroids. Patients with any other systemic diseases or smoking history were not included in the study.

Venous blood was collected between 7 and 9 A.M. with heparin as an anticoagulant for cell isolation and without an anticoagulant for serum preparation. The study was approved by the local Ethics Committee. All participants provided written informed consent.

Cell isolation and culture

Peripheral blood mononuclear cells were isolated from 20 ml of heparin-anticoagulated venous blood by centrifugation with the use of Histopaque (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's protocol. The total number and viability of isolated cells were assessed using Fuchs Rosenthal chamber and trypan blue exclusion method. The cells were cultured in 24-well culture plates at a density of 1×10^6 cells/ml in RPMI-1640 medium (Sigma-Aldrich) supplemented with 5% heat inactivated foetal calf serum (Sigma-Aldrich), 25 mM L-glutamine (Sigma-Aldrich), 1% penicillin/streptomycin solution (Sigma-Aldrich).

After 24 (T_{24}) and 144 (T_{144}) h the supernatants were separated from the cells, aliquoted and stored frozen at –80°C until tested. In 6 HCs the supernatants were collected after 12, 24, 48, 72 and 144 h of culture to evaluate a time-dependent effect on release of sCD163 *ex vivo*.

Biochemical and immunologic assays

The concentration of sCD163 was evaluated using enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. All samples were tested in duplicates.

Statistical analysis

Continuous variables were compared using the *t*-Student test. For multiple comparisons Bonferroni correction was applied. Data for continuous variables were expressed as means with standard deviations. All computations were carried out using the Statistica software.

Results

There was no significant difference of demographic parameters between the studied groups (Table 1). The asthmatic patients ($n = 35$) had lower mean FEV_1 (70.6 ±19.3% predicted) than healthy subjects (HCs) (104.9

Table 1. Patients' characteristics

Parameter	HCs (n = 13)	ICS-AAs (n = 15)	OCS-AAs (n = 10)	EX-AAs (n = 10)	*P*-value
Age [years]	30.9 ±10.7	34.8 ±11.6	37.8 ±13.4	33.8 ±14.6	0.612
Sex (female/male)	6/7	6/9	4/6	5/5	0.95
FEV_1 (% predicted)	105±11.4	78.5 ±22.2*	72.1 ±16.4*	57.1 ±7.7*^#	< 0.01
BMI	23.8 ±2.7	24.6 ±3.8	25.6 ±4.7	26.1 ±3.9	0.484
ICS [µg/day]	–	560 ±241	1080 ±329	1400 ±283	–
OCS [mg/day]	–	–	24.5 ±10.7	–	–

ICS – the dose is presented as budesonide equivalent, OCS – the dose is presented as prednisone equivalent. *Significantly less than in HCs, ^significantly less than in ICS-AAs, #significantly less than in OCS-AAs.

±11.6% predicted; $p < 0.001$). Among asthmatics the greatest FEV_1 was demonstrated in ICS-AAs (78.5 ±22.2% predicted) which was significantly less than in HCs ($p < 0.001$) but greater than that in EX-AAs ($p = 0.008$). The daily dose of ICS differed significantly in individual subgroups of asthmatic patients. The greatest daily dose of ICS was received by EX-AAs (1400 ±283 µg), significantly greater than that in OCS-AAs (1080 ±329 µg; $p = 0.032$) and in ICS-AAs (560 ±241 µg; $p < 0.001$).

There was no significant difference among serum sCD163 concentrations between HCs, ICS-AAs and OCS-AAs (Figure 1). However, EX-AAs were characterized by a significantly greater serum sCD163 concentration in comparison to all other subgroups ($p < 0.001$). No significant association between the serum sCD163 concentration and age, sex or body mass index (BMI) of the studied patients could be demonstrated (not shown).

Analysis of sCD163 production in relation to time of cell culture was performed using peripheral blood mononuclear cells (PBMC) from 6 HCs. The concentration of sCD163 was evaluated in supernatants of cultures incubated for 12, 24, 48, 72 and 144 h (Figure 2). The concentration of sCD163 was already detected at T_{12} (mean 1.2 ±0.1 ng/ml) and increased with time in culture reaching the greatest concentration at T_{144} (mean 2.4 ±0.25 ng/ml) (Figure 2). For further studies only cultures of 24 or 144 h duration were used.

At T_{24} the mean concentration of sCD163 was greater in cell cultures of AAs (5.4 ±4.5 ng/ml) in comparison with HCs (1.8 ±0.35 ng/ml; $p = 0.006$) (Figure 3). The greatest concentration was demonstrated in OCS-AAs (11.3 ±4.52 ng/ml) which was significantly greater than that in ICS-AAs (2.77 ±0.7 ng/ml; $p < 0.001$) and in EX-AAs (3.52 ±1.44 ng/ml; $p < 0.001$). The mean sCD163 concentration at T_{24} was greater in each of the AAs subgroups than in HCs ($p < 0.001$ for all comparisons). Similar relations were demonstrated at T_{144}, the greatest mean concentration of sCD163 was observed in OCS-AAs (17.1 ±7.0 ng/ml) which was significantly greater than in ICS-AAs (6.2 ±4.4 ng/ml; $p < 0.001$), EX-AAs (6.94 ±4.1 ng/ml; $p < 0.001$) and in HCs (2.52 ±1.17 ng/ml; $p < 0.001$) (Figure 4).
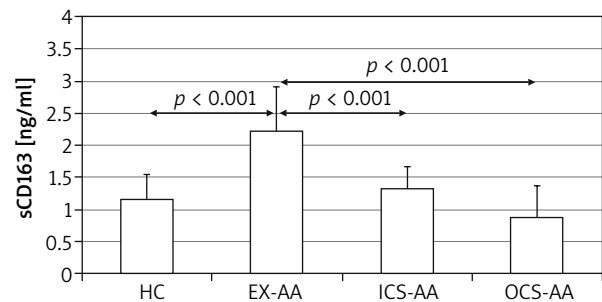

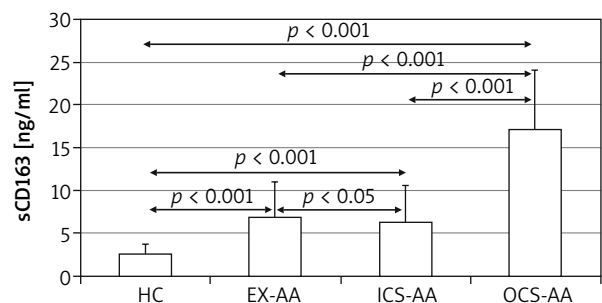**Figure 1.** Serum sCD163 concentration in individual groups

Figure 2. Concentration of sCD163 in supernatants of PBMC cultured for 12 to 144 h. The results present data obtained from 6 HCs
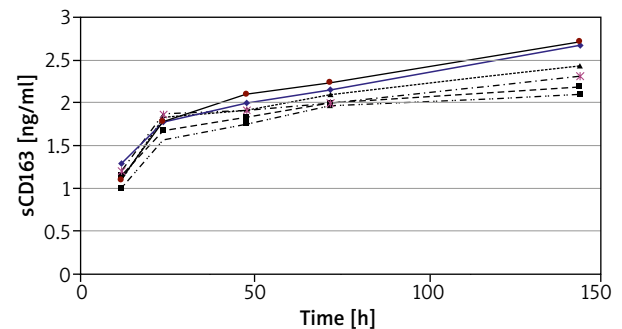

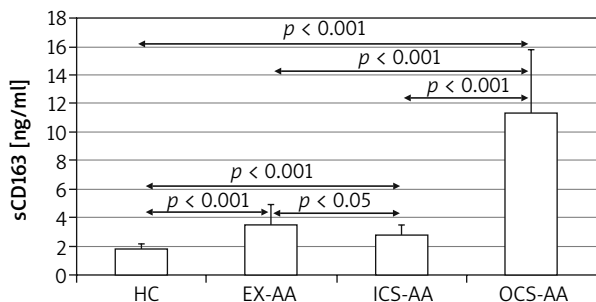**Figure 3.** Concentration of sCD163 in PBMC cultured for 24 h

Figure 4. Concentration of sCD163 in PBMC cultured for 144 h

Discussion

To the best of our knowledge, this is the first study which demonstrates the differences in *in vivo* and *ex vivo* sCD163 production in asthmatic patients depending on their clinical status.

CD163 is reported to demonstrate an anti-inflammatory function at least two ways. First, it can stimulate intracellular signalling leading to secretion of various anti-inflammatory cytokines and heme metabolites, which is triggered after CD163 mediated delivery of haemoglobin to the macrophage [12]. Second, sCD163 inhibits inflammatory response influencing T cell proliferation [13, 14]. The expression of CD163 is induced by corticosteroids [22] and anti-inflammatory cytokines such as IL-10 and IL-6 [23]. On the other hand, secreted by Th_2 type cells, IL-4 and IL-13 have been shown to downregulate the expression of CD163 influencing *de novo* CD163 synthesis [1]. A local expression of CD163 in the lungs during respiratory infection implies its role as an pulmonary defence element [24]. A reduced expression of CD163 on broncho-alveolar macrophages in patients with asthma suggests that its anti-inflammatory role in regulation of inflammatory responses in the lung of asthmatic patients may be impaired [25]. However, the reverse association of the serum sCD163 level with FEV_1 in patients in asthma was observed [25]. This implicates that increased CD163 shedding already within circulation is associated with the loss of lung function. A mouse model of allergic asthma provided further evidence for an important role of CD163 in regulation of inflammatory response in the airways [25]. Genetically modified mice, which do not express CD163, were characterized by a greater airway inflammation in response to a house dust mite allergen challenge [26]. The beneficial effect of CD163 was dependent on its direct binding to a major house dust mite allergen Der p 1, which led to attenuated production of CCL24 [26]. Exogenous sCD163 enhance Dermatophagoides pteronyssinus allergen extract induced IL-10 release by peripheral blood mononuclear cells [17].

Our study is consistent with previously published studies which demonstrate the increase in the serum sCD163 concentration in chronic inflammatory diseases such as asthma and rheumatoid arthritis [26, 27]. In ad-dition, it demonstrates that inflammation associated with asthma exacerbations leads to a systemic increase in sCD163, which is consistent with previous reports in pneumonia or chronic inflammatory diseases [28]. An increased activity of the enzymes participating in CD163 shedding in serum has been reported during asthma exacerbations [29]. In addition, altered fat metabolism significantly alters CD163 expression [27]. In some populations of obese patients, including obese asthmatic girls, an elevated serum sCD163 level was demonstrated [27]. However, in our patients we were not able to demonstrate any association between BMI or sex and sCD163 concentration, possibly due to a relatively homogenous population of our patients in terms of BMI and lack of truly obese patients included in our study. It seems that in our patients' inflammation was the dominant factor affecting CD163 expression and its serum concentration.

For the first time we have demonstrated in asthma that systemic corticosteroid therapy, which leads to the remission of the asthma exacerbation, is not associated with a significant increase in serum sCD163 concentration. This occurs despite the fact that corticosteroids are potent inducers of CD163 expression in monocytes/macrophages [8]. However, peripheral blood monocytes derived from patients treated with OCS are characterized by a strong production of sCD163 *ex vivo*. This in part can be related to the enhanced expression of membrane-bound CD163 on circulating monocytes of asthmatic patients treated with systemic corticosteroids [19]. The study indicates that systemic corticosteroid therapy of asthmatic patients leads to upregulation of monocyte CD163, which may be released as a soluble form at the site of monocyte recruitment. We have previously demonstrated that in asthmatic patients, the expression of CD163 on peripheral blood monocytes differs depending on the clinical status and monocyte subset [18, 19]. Asthma exacerbations, at least dependent on allergen exposure, seem to preferentially attract monocytes with a high CD163 expression [30]. This in turn may participate in downregulation of inflammatory response by releasing sCD163 in the airways. At least one enzyme, MMP-9, which participates in shedding of CD163 from monocyte/macrophage membranes is upregulated in the airways of asthmatic patients [31].

The elevated serum sCD163 concentration has also been reported in overweight subjects and the level correlates with BMI [20]. However, the mean BMI in our patients did not differ significantly in individual subgroups. Therefore, changes in the serum sCD163 concentration seem to reflect true relations that occur in asthmatic patients *in vivo*.

A tightly regulated expression of CD163 on peripheral blood monocytes and its release as a soluble form at the inflammatory site makes this molecule an attractive target which can act as a carrier for molecules which should be transferred to the particular site of the

body [32]. Our research supports the concept, which assumes that CD163 might be able to carry through anti-inflammatory agents such as dexamethasone to the site of inflammation. Moreover, monocytes that express a high level of CD163 seem to be an interesting target of anti-inflammatory therapies in allergic and inflammatory diseases. Therefore, further studies to evaluate and fully understand its role in inflammatory diseases, such as asthma, are still a necessity.

Conflict of interest

The authors declare no conflict of interest.

References

1. Van Gorp H, Delputte PL, Nauwynck HJ. Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. Mol Immunol 2010; 47: 1650-60.
2. Kowal K, Silver R, Sławińska E, et al. CD163 and its role in inflammation. Folia Histochem Cytobiol 2011; 49: 365-74.
3. Møller HJ. Soluble CD163. Scand J Clin Lab Invest 2012; 72: 1-13.
4. Timmermann M, Högger P. Oxidative stress and 8-iso-prostaglandin F2alpha induce ectodomain shedding of CD163 and release of tumor necrosis factor-alpha from human monocytes. Free Radic Biol Med 2005; 39: 98-107.
5. Hintz KA, Rassias AJ, Wardwell K, et al. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. J Leukoc Biol 2002; 72: 711-7.
6. Etzerodt A, Maniecki MB, Møller K, et al. Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. J Leukoc Biol 2010; 88: 1201-5.
7. Fabriek BO, Møller HJ, Vloet RPM, et al. Proteolytic shedding of the macrophage scavenger receptor CD163 in multiple sclerosis. J Neuroimmunol 2007; 187: 179-86.
8. Buechler C, Ritter M, Orsó E, et al. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. J Leukoc Biol 2000; 67: 97-103.
9. Schaer DJ, Boretti FS, Schoedon G, Schaffner A. Induction of the CD163-dependent haemoglobin uptake by macrophages as a novel anti-inflammatory action of glucocorticoids. Br J Haematol 2002; 119: 239-43.
10. Högger P, Erpenstein U, Rohdewald P, Sorg C. Biochemical characterization of a glucocorticoid-induced membrane protein (RM3/1) in human monocytes and its application as model system for ranking glucocorticoid potency. Pharm Res 1998; 15: 296-302.
11. Sulahian TH, Högger P, Wahner AE, et al. Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. Cytokine 2000; 12: 1312-21.
12. Moestrup SK, Moller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med 2004; 36: 347-54.
13. Frings W, Dreier J, Sorg C. Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T-lymphocytes, whereas membrane-bound protein has no effect. FEBS Lett 2002; 526: 93-6.
14. Timmermann M, Buck F, Sorg C, Högger P. Interaction of soluble CD163 with activated T lymphocytes involves its associa-
tion with non-muscle myosin heavy chain type A. Immunol Cell Biol 2004; 82: 479-87.
15. Fonseca JE, Edwards JCW, Blades S, Goulding NJ. Macrophage subpopulations in rheumatoid synovium: reduced CD163 expression in CD4+ T lymphocyte-rich microenvironments. Arthritis Rheum 2002; 46: 1210-6.
16. Van den Heuvel MM, Tensen CP, van As JH, et al. Regulation of CD 163 on human macrophages: cross-linking of CD163 induces signaling and activation. J Leukoc Biol 1999; 66: 858-66.
17. Bernatowicz P, Kowal K. Soluble CD163 modulates cytokine production by peripheral blood mononuclear cells stimulated by Dermatophagoides pteronyssinus allergens in vitro. Adv Med Sci 2016; 61: 249-54.
18. Kowal K, Møller HJ, DuBuske LM, et al. Differential expression of monocyte CD163 in single – and dual-asthmatic responders during allergen-induced bronchoconstriction. Clin Exp Allergy 2006; 36: 1584-91..
19. Moniuszko M, Bodzenta-Lukaszyk A, Kowal K, et al. Enhanced frequencies of CD14++CD16+, but not CD14+CD16+, peripheral blood monocytes in severe asthmatic patients. Clin Immunol 2009; 130: 338-46.
20. Murphy V. Influence of maternal body mass index and macrophage activation on asthma exacerbations in pregnancy – clinical key. J Allergy Clin Immunol Pract 2017; 5: 981-7.
21. Kowal K, Moniuszko M, Bodzenta-Lukaszyk A. The effect of inhaled corticosteroids on the concentration of soluble CD163 in induced sputum of allergic asthma patients. J Investig Allergol Clin Immunol 2014; 24: 49-55.
22. Buechler C, Eisinger K, Krautbauer S. Diagnostic and prognostic potential of the macrophage specific receptor CD163 in inflammatory diseases. Inflamm Allergy Drug Targets 2013; 12: 391-402.
23. Etzerodt A, Moestrup SK. CD163 and Inflammation: biological, diagnostic, and therapeutic aspects. Antioxid Redox Signal 2013; 18: 2352-63.
24. Abdullah M, Kähler D, Vock C, et al. Pulmonary haptoglobin and CD163 are functional immunoregulatory elements in the human lung. Respiration 2012; 83: 61-73.
25. Dai C, Yao X, Gordon EM, et al. A CCL24-dependent pathway augments eosinophilic airway inflammation in house dust mite-challenged Cd163$^{-/-}$ mice. Mucosal Immunol 2016; 9: 702-17..
26. Zhi Y, Gao P, Xin X, et al. Clinical significance of sCD163 and its possible role in asthma. Mol Med Rep 2017; 15: 2931-9.
27. Periyalil HA, Wood LG, Scott HA, et al. Macrophage activation, age and sex effects of immunometabolism in obese asthma. Eur Respir J 2015; 45: 388-95.
28. Suzuki Y, Enomoto Y, Yokomura K, et al. Soluble hemoglobin scavenger receptor CD163 (sCD163) predicts mortality of community-acquired pneumonia. J Infect 2016; 73: 375-92.
29. Oshita Y, Koga T, Kamimura T, et al. Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma. Thorax 2003; 58: 757-60.
30. Kowal K, Moniuszko M, Dabrowska M, Bodzenta-Lukaszyk A. Allergen challenge differentially affects the number of circulating monocyte subsets. Scand J Immunol 2012; 75: 531-9.
31. Gueders MM, Foidart JM, Noel A, Cataldo DD. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 2006; 533: 133-44.
32. Møller LNO, Knudsen AR, Andersen KJ, et al. Anti-CD163-dexamethasone protects against apoptosis after ischemia/reperfusion injuries in the rat liver. Ann Med Surg 2015; 4: 331-7.