

Effect of omalizumab on bronchoalveolar lavage matrix metalloproteinases in severe allergic asthma

Weronika Zastrzeżyńska, Stanisława Bazan-Socha, Marek Przybyszowski, Agnieszka Gawlewicz-Mroccka, Bogdan Jakiela, Hanna Plutecka, Lech Zaręba, Jacek Musiał, Krzysztof Okoń, Krzysztof Śladek & Jerzy Soja

To cite this article: Weronika Zastrzeżyńska, Stanisława Bazan-Socha, Marek Przybyszowski, Agnieszka Gawlewicz-Mroccka, Bogdan Jakiela, Hanna Plutecka, Lech Zaręba, Jacek Musiał, Krzysztof Okoń, Krzysztof Śladek & Jerzy Soja (2021): Effect of omalizumab on bronchoalveolar lavage matrix metalloproteinases in severe allergic asthma, Journal of Asthma, DOI: [10.1080/02770903.2021.1903917](https://doi.org/10.1080/02770903.2021.1903917)

To link to this article: <https://doi.org/10.1080/02770903.2021.1903917>



Accepted author version posted online: 25 Mar 2021.



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



Article views: 2



View related articles [↗](#)



View Crossmark data [↗](#)

Effect of omalizumab on bronchoalveolar lavage matrix metalloproteinases in severe allergic asthma

Weronika Zastrzeżyńska^{1ABCEFG}, Stanisława Bazan-Socha^{2BCDEF}, Marek Przybyszowski^{12B}, Agnieszka Gawlewicz-Mroccka^{12B}, Bogdan Jakiela^{2J}, Hanna Plutecka^{2J}, Lech Zaręba^{3C}, Jacek Musiał^{2E}, Krzysztof Okoń^{4HI}, Krzysztof Śladek^{12ADEG*}, and Jerzy Soja^{12ABCDEFG*}

¹Department of Pulmonology, University Hospital, Krakow, Poland

²Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland

³Interdisciplinary Centre for Computational Modelling, College of Natural Sciences, University of Rzeszow, Poland

⁴Department of Pathology, Jagiellonian University Medical College, Krakow, Poland

*Equal senior-author contribution

Corresponding author: Jerzy Soja, MD, PhD, 2nd Chair of Internal Medicine, Jagiellonian University Medical College, 8 Skawińska St., 31-066 Krakow, Poland, phone: +48 -12-430 -51 -47, e-mail: jerzysoja@op.pl

Authors' Contribution

^AStudy Design

^BData Collection

^CStatistical Analysis

^DData Interpretation

^EManuscript Preparation

^FLiterature Search

^GBronchofiberscopy

^HHistology and immunohistochemistry

^ICell count

^JBAL analysis

Abstract

Introduction: Airway inflammation in asthma is accompanied by reconstruction of the bronchial wall extracellular matrix that most likely occurs with a contribution of matrix metalloproteinases (MMPs). Recently we have reported that omalizumab may decrease reticular basement membrane (RBM) thickness together with fibronectin deposits in asthmatic airways, although mechanisms involved are unknown. **Objective:** In the present study, we have investigated the impact of omalizumab on MMPs concentrations in bronchoalveolar lavage fluid (BAL) of asthmatic subjects in relation to airway remodeling changes in histology. **Patients and methods:** The study group consisted of 13 severe allergic asthmatics treated with omalizumab for at least 12 months. In each subject, clinical and laboratory parameters, bronchoscopy with BAL, and endobronchial biopsy were evaluated before and after the biologic therapy. RBM thickness, fibronectin, and collagen deposits in bronchial mucosa specimens were analyzed in histology. The investigations also included BAL cytology and BAL concentrations of MMP-2, -3, and -9. **Results:** Omalizumab was related to a decrease in all measured MMPs in BAL ($p < 0.001$, each), although such declines were not observed in each patient. The depletions were associated with a lower asthma exacerbation rate and better asthma control. Interestingly, patients who showed a decline in at least one MMP ($n = 10$, 77%) were characterized by a higher decrease in the RBM thickness ($-1.61 [-2.02 \text{ to } -0.6]$ vs. $-0.06 [-0.09 \text{ to } +3.3]$, $p = 0.03$). Likewise, individuals with lower concentrations of MMP-9 after omalizumab ($n = 7$, 58%) had a greater reduction in the RBM layer as compared to those with steady MMP-9 levels ($-1.8 [-2.4 \text{ to } -1.14]$ vs. $-0.13 [-0.6 \text{ to } -0.06]$ μm , $p = 0.03$). Moreover, the latter group also had unfavorable higher collagen I accumulation after biologic ($42 [20 \text{ to } 55]$ vs. $0 [-10 \text{ to } 20]\%$, respectively, $p = 0.03$). Higher concentrations of MMPs in BAL at baseline were related to the lower systemic steroid dose and better omalizumab response concerning the decline in RBM thickness. **Conclusion:** Our data suggest that omalizumab therapy is associated with decreased BAL MMPs concentration in the subgroup of asthma patients. The decline was linked with a reduction in the RBM thickness what might play a beneficial role in airway remodeling.

Keywords asthma, omalizumab, remodeling, matrix metalloproteinases

Introduction

Asthma is a chronic inflammatory disease of the airways. The ongoing inflammation is accompanied by structural and functional changes referred to as airway remodeling. The process encompasses thickening of the reticular basement membrane (RBM), airway epithelium damage, excessive production of growth factors, such as transforming growth factor (TGF) β , which are involved in fibroblast proliferation, smooth muscle hypertrophy, and neovascularization [1–3].

It has been demonstrated that in asthmatic airways, accumulation of extracellular matrix (ECM) proteins, such as fibrin collagen types I, III, and V, laminin, tenascin, and fibronectin, occurs in parallel with decreased elastin deposition and abnormal composition and thickness of collagen fibers [2]. The critical role in that bronchial ECM reorganization is likely played by matrix metalloproteinases (MMPs) and their inhibitors, particularly tissue inhibitors of metalloproteinases (TIMPs).

MMPs play an essential role in the degradation of all ECM proteins [4]. Thus, they are involved in numerous immunological and physiological processes, such as apoptosis, angiogenesis, tissue growth, wound healing, and inflammatory response [5]. One of the essential functions of MMPs, particularly MMP-2 and MMP-9, is collagen type IV degradation, a primary component of the vascular basement membrane. Hence, they facilitate leukocyte migration into the inflammatory loci, local tumor growth, and cancer metastases [5]. It has been demonstrated that MMP-2 and MMP-9 participate in asthma pathologies, such as airway inflammation, hyperresponsiveness, and remodeling [2]. Increased MMP-9 concentrations have been reported in the subepithelial basement membrane, and bronchoalveolar lavage fluid (BAL) of severe asthmatics and airway allergen challenge results in its further release [6]. Noteworthy, the concentration of MMP-9 was related to the number of neutrophils and macrophages in BAL [7].

MMPs are also involved in the inflammatory response to viral respiratory tract infections. Elevated concentrations of MMP-8, -9, and to a lesser extent MMP-2, -3, -11, and -12, have been identified in airways of pediatric patients with acute respiratory tract infections, as compared to healthy controls [8].

It is worth noting that in the type-2 airway inflammation, MMP-9 is produced by eosinophils [9], neutrophils [10], and alveolar macrophages [10], while immunoglobulin (Ig)E plays a critical role in the initiation of that process [11]. Binding to its high-affinity receptor, IgE activates various airway cells (e.g. mast cells and eosinophils) and thus participate in allergen-induced airway response and inflammation in asthma. Furthermore, it has been suggested that IgE is also involved in airway remodeling pathology [12, 13]. Omalizumab, an anti-IgE therapeutic monoclonal antibody, binds free IgE in the circulation and prevents its attachment to the surface of inflammatory cells. It is useful in treating severe allergic asthma [14, 15] and may decrease unfavorable bronchial remodeling changes [16, 17]. We have recently reported that omalizumab may decrease RBM thickness together with reducing fibronectin deposits in asthmatic airways [18]. Furthermore, anti-IgE therapy was related to improved lung computed tomography airway geometry parameters referred to as the bronchial wall thickening [19], which also corresponded to the subepithelial fibrosis in mucosal histology [20]. However, the exact mechanisms of how anti-IgE therapy influences airway structural changes are unknown.

To date, the impact of omalizumab on MMPs in asthmatic airways has not been analyzed. Furthermore, the mechanisms of matrix homeostasis are complex and need further investigations. Thus, in the current project, we aimed to investigate how anti-IgE therapy influences lower airway matrix metalloproteinase homeostasis in asthma. We measured BAL concentrations of MMP-2, -3, and -9 in severe asthma patients before and after omalizumab therapy. We have also analyzed their associations with clinical asthma features, airway inflammatory cell composition, and bronchial remodeling parameters described in histopathological examination of bronchial mucosa specimens. To the best of our knowledge, such studies have not been performed to date.

Patients and methods

Patients

The study included 13 patients with severe allergic asthma treated with omalizumab for at least 12 months. Severe asthma was defined according to the GINA guidelines [21] as a severe persistent disease despite treatment with a high daily dose of inhaled corticosteroids (ICS) (>500 µg of fluticasone propionate [dry powder inhaler] or its equivalent). According to the clinical recommendation, the dosage and frequency of omalizumab administration were based on the IgE concentration and the patient's body weight.

Spirometry was performed using a Jaeger, Master Screen spirometer (Jaeger, Wurzburg, Germany) following the American Thoracic Society standards.

Clinical asthma assessment and basic laboratory tests

Before the initiation of omalizumab therapy, the serum IgE level was analyzed by latex nephelometry (Siemens, Marburg, Germany), and the patient's atopy status was assessed by measurement of allergen-specific IgE (CAP system, Siemens, Marburg, Germany) and/or by skin prick testing (Allergopharma, Reinbeck, Germany).

Moreover, we collected results of the Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ) and asthma treatment mode before and after biologic, together with severe asthma exacerbation episodes during the entire period of omalizumab administration. Severe asthma exacerbation was defined according to the American Thoracic Society/European Respiratory Society Statement [23] as an episode characterized by a progressive increase in asthma symptoms which required the use of systemic corticosteroids (tablets, suspension or injections), or an increase of the usually administered stable maintenance dose, at the physician's

discretion, for at least 3 days, and/or hospitalization or an emergency visit because of asthma requiring systemic corticosteroids. The effectiveness of omalizumab therapy was assessed using the Global Evaluation of Treatment Effectiveness (GETE) scale. All the enrolled patients demonstrated at least a good clinical response to the treatment.

Bronchofiberoscopy

Bronchofiberoscopy with BAL collection and endobronchial biopsy was performed twice: up to two weeks before starting omalizumab therapy, and the second time after at least 12 months of a constant drug administration.

Bronchofiberoscopy was performed according to the guidelines of the American Thoracic Society [22] using a BF 1T180 bronchofiberscope (Olympus, Tokyo, Japan), with the local anesthesia (2% lidocaine) and in mild sedation (0.05–0.1 mg fentanyl and 2.5–5 mg midazolam, both given intravenously). During the procedure, BAL was performed with 200 ml of 0.9% saline administered to the right middle lobe bronchus, and 2–3 bronchial biopsy specimens were collected from the right segmental basal bronchi (the carina between B9 and B10) for further histological investigations.

Bronchoalveolar lavage fluid analysis

The cytospin preparations (Thermo Scientific, Waltham, MA, USA) were made from BAL fluid samples and stained with the May-Grunwald Giemsa dye. One thousand cells in each preparation were counted; the results are presented as the percentage of all the inflammatory cells (except the epithelial cells).

The levels of MMP-2, MMP-3, and MMP-9 were determined using the MMP 3-Plex Human ProcartaPlex™ Panel 2 assay (Invitrogen™, Thermo Fisher Scientific, MA, USA), according to the manufacturer's instructions. The plates were read using the MAGPIX magnetic bead reader (Luminex Corp., Austin, TX, USA) based on the xPONENT® 4.2 software (Luminex). The results were analyzed using the MILLIPLEX® Analyst 5.1 program (Millipore, Burlington, MA, USA).

Histology and immunohistochemistry

Mucosa specimens were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. The 2 µm sections were prepared and stained by the hematoxylin-eosin method. The images were recorded using an Axioscop microscope (Zeiss, Germany), a 100x oil-immersion lens, and a Nikon D5100 digital camera. The RBM thickness was measured with an imaging system (AnalySIS imaging system; Soft Imaging System GmbH; Germany) along the available basement membrane and an arbitrary-distance software tool. For each patient, at least 30 individual measurements were performed, and the harmonic mean was calculated.

To evaluate collagen type I, III, and fibronectin deposits, 2 µm sections were prepared, using the routine laboratory manner. UltraVision Quanto Detection system with HRP DAB (Thermo Fisher Scientific, Waltham, MA, USA) and hematoxylin as counterstaining were applied for color detection. The employed primary antibodies were: for collagen I - monoclonal rabbit antibody clone EPR7785 (Abcam, UK), for collagen IV - monoclonal mouse antibody clone FH-7A (Abcam, UK), for fibronectin - polyclonal rabbit antibody A025 (Dako, Denmark).

The results were assessed semiquantitatively and scored: 0 (lack of staining), 1 (very faint staining), 2 (evident stain), 3 (strong reaction), or estimating the surface area of the section percentage that showed reactivity for the selected antibodies (ECM deposition).

Statistical analysis

Data management was performed with STATISTICA 13.1 software (StatSoft Inc., Tulsa, OK, USA). The Shapiro-Wilk test was employed to identify the data distribution. An extensive variability characterized BAL MMPs levels. Thus, they were Box-Cox transformed before the analysis. Continuous variables in Tables and the main manuscript's text were presented as a mean and standard deviation or median and interquartile range, as appropriate. Categorical variables were presented as numbers and percentages and were compared by the χ^2 test. The Mann-Whitney U-test, Kruskal-Wallis, multiple repetition tests, and the Student's t-test were applied for data comparison, accordingly. To eliminate the impact of potential confounders, including gender, age, and body mass index (BMI), the covariance analysis (ANCOVA) was performed on Box-Cox-transformed data. The Pearson or the Spearman rank correlation tests were calculated for the correlation of continuous variables.

The Cox-Mantel test and Kaplan-Meier curves analyzed the relationship of BAL MMPs with asthma exacerbation rate during follow-up.

The results that presented a p values of less than 0.05 were considered statistically significant.

Results

Patient characteristics

The clinical and laboratory characteristics of the subjects studied are provided in Table 1. All patients but one were females with the median age of 48 (39–56) years. The median asthma duration was 24 (15–36) years, while the median omalizumab therapy, i.e. an interval between both bronchoscopies, was 22 (20–26) months. All the patients received a high daily dose of ICS, long-acting β_2 agonists, and oral corticosteroids at the recruitment time.

Omalizumab improves asthma control and course

As it has been shown in Table 1, omalizumab resulted in a significant reduction in the asthma exacerbation rate and improvement in asthma control and asthma-related quality of life ($p < 0.001$, all), together with a median 15% increase in FEV₁ ($p = 0.03$) (Table 1). The biologic was also related to the significant reduction in systemic steroid use (Table 1).

Omalizumab leads to a decline in the concentration of metalloproteinases in bronchoalveolar lavage fluid

As expected, MMPs at enrollment were related to the percentage of BAL neutrophils (MMP-2: $r = 0.65$, $p = 0.02$; MMP-3: $r = 0.62$, $p = 0.02$; and MMP-9: $r = 0.74$, $p = 0.01$) and lymphocytes (MMP-2: $r = 0.67$; $p = 0.02$ and MMP-3: $r = 0.61$; $p = 0.03$). Furthermore, baseline MMP-3 was linked with BAL eosinophilia ($r = 0.71$, $p = 0.005$).

Baseline MMPs BAL levels were associated with one another ($r > 0.95$, $p < 0.001$, all), similarly to their changes after therapy ($r > 0.73$, $p < 0.004$, all).

In Figure 1, we have depicted BAL MMPs concentrations before vs. after omalizumab therapy. The biologic led to the decrease in all the three MMPs concentrations ($p < 0.001$, each). However, a decline in all of them was recorded only in five (38%) patients. In comparison, three (23%) subjects demonstrated a decrease in two of MMPs (MMP-2 and MMP-3 in one; MMP-3 and

MMP-9 in two), and two (15%) patients had a depletion in only one MMP (MMP-2 or MMP-3). The remaining three (23%) individuals had unchanged BAL MMPs after biologic.

Concentrations of BAL MMP-3 and MMP-9 after omalizumab correlated with the percentage of BAL lymphocytes ($r = 0.58$, $p = 0.03$, and $r = 0.77$, $p = 0.02$, respectively), while a decline in MMP-3 levels was related to decreased circulating eosinophil count ($r = 0.67$, $p = 0.01$). In contrast, patients who experienced a decrease in BAL MMP-2 had a higher percent of BAL lymphocytes at enrollment (15 [7–21] vs. 4 [2–4]%, $p = 0.01$).

Surprisingly, subjects with lower ICS and systemic steroid doses at baseline (median values as cut-off points, respectively 2000 μg and 10 mg) were characterized by higher baseline MMPs (MMP-2: $p = 0.02$, MMP-3: $p = 0.003$, and MMP-9: $p = 0.008$, concerning ICS, and MMP-2: $p = 0.07$, MMP-3: $p = 0.01$, and MMP-9: $p = 0.02$, concerning systemic steroid) and a greater decrease in MMP-3 and MMP-9 after therapy ($p < 0.01$, all). Thus, in conclusion, asthma patients with a lower dose of inhaled and systemic steroids at baseline had higher BAL MMPs concentrations at enrollment and responded better in MMPs decline after omalizumab.

Interestingly, MMPs before biologic remained in a positive relationship with the number of asthma exacerbations during the preceding year (MMP-2: $r = 0.73$, $p = 0.02$; MMP-3: $r = 0.72$, $p = 0.01$; and MMP-9: $r = 0.77$, $p = 0.006$). In turn, a decrease in concentration of MMPs after omalizumab correlated positively with the reduction in asthma exacerbation rate (MMP-2: $r = 0.55$, $p = 0.049$; MMP-3: $r = 0.7$, $p = 0.007$; and MMP-9: $r = 0.79$, $p = 0.001$) and better asthma control (MMP-3: $r = 0.59$, $p = 0.03$ and MMP-9: $r = 0.6$, $p = 0.03$) in a follow-up period. Furthermore, patients with reported declines in MMP-2 and/or MMP-9 ($n = 7$, 53% both) showed a decreased asthma exacerbation rate while on omalizumab compared to those with steady BAL MMPs levels (MMP-2: $p = 0.03$ and MMP-9: $p = 0.048$). That relationship was also documented in the Kaplan-Meier curves, indicating that a probability of severe asthma exacerbation during anti-IgE therapy was significantly lower in patients who showed a concurrent decrease of MMPs in the airways (Figure 2). For MMP-3, such association was not recorded.

Declines in bronchoalveolar lavage fluid metalloproteinases are linked with reductions in reticular basement membrane thickness

In our previous report [18], we have shown a significant reduction in the median RBM thickness (22%, $p = 0.002$) and fibronectin deposits (33%, $p = 0.02$) in the submucosa after omalizumab therapy (as summarized in Table 1). Here, we additionally analyzed whether this effect might be associated with a change in MMPs. We have found that patients with a documented decrease in at least one MMP after biologic ($n = 10$, 77%) were characterized by a higher decline in the RBM thickening as compared to the remainder (-1.61 [-2.02 to -0.6] vs. -0.06 [-0.09 to $+3.3$], respectively, $p = 0.03$). Likewise, individuals with lower concentrations of MMP-9 ($n = 7$, 58%) had a greater decrease in RBM thickness (-1.8 [-2.4 to -1.14] vs. -0.13 [-0.6 to -0.06] μm , $p = 0.03$) and stable collagen I accumulation (0 [-10 to 20] vs. 42 [20 to 55]%, respectively, $p = 0.03$) after omalizumab. Moreover, the declines in RBM thickness correlated positively with MMP-2, MMP-3, and MMP-9 concentrations before therapy (MMP-2: $r = 0.56$, $p = 0.048$; MMP-3: $r = 0.66$, $p = 0.01$; and MMP-9: $r = 0.55$, $p = 0.049$). In conclusion, higher concentrations of BAL MMPs at baseline and decline in those levels during anti-IgE therapy may be considered predictors of a more pronounced reduction in RBM thickness after omalizumab.

In Figure 3, we have depicted representative histology images of significant reduction of the RBM thickness in a patient with documented declines in all three BAL MMPs after omalizumab therapy (A-B) and lack of such reduction in a patient with no decrease in MMPs (C-D)

Interestingly, asthma duration before omalizumab administration and length of biological therapy, i.e. the period between both bronchoscopies, had no impact on our results.

Anti-IgE treatment also did not change the other studied ECM proteins, such as fibronectin and collagen III.

Discussion

In the present study, we have demonstrated for the first time that at least one-year omalizumab therapy of severe allergic asthmatics leads to the reduction in MMP-2, MMP-3, and MMP-9 concentrations in BAL. Furthermore, those declines were related to the decrease in RBM thickness. Moreover, all reported changes occur during the first 12 months of omalizumab administration since the more extended therapy had no additional impact on our outcomes.

MMPs play an essential role in normal lung biology; however, their overexpression might indicate enhanced turnover of ECM proteins, suggesting ongoing processes of advanced tissue degradation and reconstruction [24]. Therefore, decreased levels of those enzymes might be associated with lower susceptibility to the likely unfavorable airway remodeling pathology in asthma. We previously reported in the same group of patients that omalizumab was associated with a decrease in RBM thickness and lower fibronectin deposit in the bronchial mucosa specimens [18]. In the current study, we have presently extended our knowledge concerning the beneficial effect of omalizumab, this time regarding airway MMPs concentrations. Furthermore, we have demonstrated that higher MMPs at baseline may predict better omalizumab response concerning decreases in RBM thickness. That is a novel finding confirming that subepithelial fibrosis is, to some extent, reversible during anti-IgE therapy, especially in asthma patients with increased airway MMPs production.

Interestingly, a decline in MMP-9 after omalizumab has been associated with a higher degree of RBM thinning. This observation is parallel to the data presented by Hoshino et al. [25], who showed that collagen and tenascin deposits are increased in RBM of asthma patients with higher MMP-9 expression in the basal lamina. In our study, a decline in MMPs has not been related to reduced collagen or fibronectin accumulation. However, individuals who did not show a reduction in airway MMP-9 during the anti-IgE therapy demonstrated a further increase in subepithelial deposition of collagen I. This observation complements results of previous studies, which suggests ongoing processes of tissue degradation and reconstruction in asthma airways [24]

Concentrations of MMPs in BAL at enrollment were positively associated with the percentage of neutrophils and lymphocytes in BAL, indicating these cells as the leading producers of MMPs in the airway. Indeed, neutrophils were demonstrated to be a primary source of MMPs [26], along with macrophages, fibroblasts, endothelial cells, and eosinophils [8, 27]. However, it is worth noting that MMP-3 was shown to be overexpressed in mast cells, eosinophils, and neutrophils, while MMP-9 overexpression was recorded mainly in neutrophils [28]. Our data confirm these findings, as in our study, BAL eosinophilia was related only to the MMP-3 concentration. It needs to be stressed that active MMP-3 can convert MMP-9 from its inactive to active form [29], thus might regulate the MMP-9 activity.

Another important finding of our study is the relationship between the concentration of BAL MMPs and clinical asthma course. At enrollment, MMPs positively correlated with the number of asthma exacerbation episodes during the preceding year. Additionally, the decline in BAL MMPs observed during anti-IgE therapy was associated with a lower asthma exacerbation rate. Therefore, the decrease in the airway MMPs during biological treatment might be a novel biomarker of a positive response to the treatment, also concerning the reduction in the RBM layer.

The final issue which deserves comment is the impact of steroid doses on MMPs response to anti-IgE therapy. Interestingly, lower doses of inhaled and systemic steroids were related to the higher MMPs in BAL at enrollment and, surprisingly, a better response to omalizumab therapy. That observation suggests that more aggressive steroid therapy might reduce MMPs production in the lungs. Alternatively, in some asthma patients, steroid therapy could still be suboptimal to control airway inflammation and MMPs responses, despite satisfactory disease symptoms management. These patients likely may benefit best from the biological treatment. Indeed, MMP-9 has been shown to increase in severe asthma [30] and during disease exacerbations [31, 32]. However, data presented by other investigators do not mirror our outcomes. Mattos et al. [31] showed no impact of increased budesonide on blood MMP-9 in mild asthmatics. Likewise, MMP-9 in exhaled breath condensates of patients with allergic asthma did not change after a substantial ICS increase. However, the study mentioned above [31] included newly exacerbated patients, which might influence the study results. In turn, Russel et al. [33] showed that dexamethasone might prevent *in vitro* the release of MMP-9 in the alveolar macrophage culture. Discrepancies between our results and the cited publications require additional research on a larger number of patients.

Study limitations

Our study has several limitations. Firstly, the analyzed group is small, and subgroup analyzes should be treated with caution. The time elapsed between the first and the second bronchoscopy differed among patients, although it had no impact on our results. We analyzed MMPs only twice, which does not fully reflect possible fluctuations over time. Nevertheless, bronchoscopy and BAL analysis performed at enrolment and follow-up visit allowed us to show the dynamics of MMPs response in anti-IgE therapy at the very site of asthmatic inflammation.

Conclusion

In the present study, we report for the first time that omalizumab therapy in severe allergic asthma is related to the decrease in the concentration of BAL MMPs. Furthermore, the decline in MMPs was associated with reduced asthma exacerbation rate and improvement in symptom control, albeit the biological therapy's beneficial role has not been observed in all subjects studied. Higher concentrations of MMPs in BAL at baseline were related to the better omalizumab response concerning the decline in RBM thickness. However, the clinical relevance and impact of MMPs decline on asthma severity and airway remodeling pathology require further investigations.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Bibliography:

1. Bazan-Socha S, Buregwa-Czuma S, Jakiela B, Zareba L, Zawlik I, Myszk A, et al. Reticular Basement Membrane Thickness Is Associated with Growth- and Fibrosis-Promoting Airway Transcriptome Profile-Study in Asthma Patients. *Int J Mol Sci* [Internet]. 2021 Jan 20 [cited 2021 Jan 21];22(3):998. Available from: <https://www.mdpi.com/1422-0067/22/3/998>.
2. Kraus-Filarska M, Kosińska M, Tomkiewicz A. Metalloproteinases and Airway Remodeling

in Asthma. *Advances in Clinical and Experimental Medicine* 2007, 16, 417–423.

3. Bazan-Socha S, Bukiej A, Marcinkiewicz C, Musial J. Integrins in pulmonary inflammatory diseases. *Curr Pharm Des.* 2005; 11: 893–901.
4. McMillan SJ, Kearley J, Campbell JD, Zhu XW, Larbi KY, Shipley JM, Senior RM, Nourshargh S, Lloyd CM. Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation. *J Immunol.* 2004 15; 172: 2586-94.
5. Lipka D, Boratyński J. Metalloproteinases. Structure and function. *Postepy Hig Med Dosw (Online).* 2008; 62: 328-36.
6. Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunol.* 2003; 111: 1345-52.
7. Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway after allergen challenge. *Am J Respir Crit Care Med.* 2000; 162: 1157-61.
8. Kong M, Gaggar A, Li Y, Winkler M, Blalock JE, Clancy JP. Matrix Metalloproteinase Activity in Pediatric Acute Lung Injury. *Int J Med Sci.* 2009; 6: 9–17.
9. Ohno IH, Ohtani, Y. Nitta, J. Suzuki, H. Hoshi, M. Honma, S. Isoyama, Y. Tanno, G. Tamura, K. Yamauchi, et al. 1997. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am. J. Respir. Cell Mol. Biol.* 1997; 16: 212-9.
10. Cataldo D, Munaut C, Noel A, Frankenne F, Bartsch P, Foidart JM, and Louis R. Matrix metalloproteinases and TIMP-1 production by peripheral blood granulocytes from COPD patients and asthmatics. *Allergy* 2001; 56: 145-51.
11. Borish L. The immunology of asthma: Asthma phenotypes and their implications for personalized treatment. *Ann Allergy Asthma Immunol.* 2016; 117: 108-14.
12. Presta L, Shields R, O’Connell L, Lahr S, Porter J, Gorman C, Jardieu P. The binding site on human immunoglobulin E for its high affinity receptor. *J Biol Chem.* 1994 21; 269: 26368-73.
13. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature.* 1999 25; 402: B24-30.

14. Zastrzeżyńska W, Przybyszowski M, Gawlewicz-Mrocza A. Efficacy of omalizumab in severe allergic asthma - preliminary experience from the University Hospital in Cracow, Poland. *Przegląd Lekarski*; 2018;6: 270-274.
15. Zastrzeżyńska W, Gawlewicz-Mrocza A, Soja J, et al. Omalizumab is effective and available for treatment of patients with severe allergic asthma in Poland. *Pol Arch Intern Med*. 2017 31; 127: 715-717/
16. Hoshino M, Ohtawa J. Effects of adding omalizumab, an anti-immunoglobulin E antibody, on airway wall thickening in asthma. *Respiration* 2012; 83: 520-8.
17. Riccio AM, Dal Negro RW, Micheletto C, et al. Omalizumab modulates bronchial reticular basement membrane thickness and eosinophil infiltration in severe persistent allergic asthma patients. *Int J Immunopathol Pharmacol* 2012; 25: 475-84.
18. Zastrzeżyńska W, Przybyszowski M, Bazan-Socha S, et al. Omalizumab may decrease the thickness of the reticular basement membrane and fibronectin deposit in the bronchial mucosa of severe allergic asthmatics. *J Asthma*. 2019, 23: 1-10.
19. Przybyszowski M, Paciorek K, Zastrzeżyńska W, et al. Influence of omalizumab therapy on airway remodeling assessed with high-resolution computed tomography (HRCT) in severe allergic asthma patients. *Adv Respir Med*. 2018; 30.
20. Koźlik P, Żuk J, Bartyzel S, Zarychta J, Okoń K, Zareba L, Bazan JG, Kosalka J, Soja J, Musiał J, Bazan-Socha S. The relationship of airway structural changes to blood and bronchoalveolar lavage biomarkers, and lung function abnormalities in asthma. *Clin. Exp. Allergy* 2019; doi: 10.1111/cea.13501.
21. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention 2020. Available from: www.ginasthma.org.
22. Guidelines for fiberoptic bronchoscopy in adults. American Thoracic Society. Medical Section of the American Lung Association. *Am Rev Respir Dis*. 1987; 136:1066.
23. Reddel HK, Taylor DR, Bateman ED, et al. on behalf of the American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An Official

American Thoracic Society/European Respiratory Society Statement: Asthma Control and Exacerbations Standardizing Endpoints for Clinical Asthma Trials and Clinical Practice. *Am J Respir Crit Care Med* 2009; 180: 59–99.

24. Hendrix AY, Kheradmand F. The Role of Matrix Metalloproteinases in Development, Repair, and Destruction of the Lungs. *Prog Mol Biol Transl Sci*. 2017; 148: 1-29.
25. Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol*. 1998; 102: 783-8.
26. Ventura I, Vega A, Chacón P, Chamorro C, Aroca R, Gómez E, Bellido V, Puente Y, Blanca M, Monteseirín J. Neutrophils from allergic asthmatic patients produce and release metalloproteinase-9 upon direct exposure to allergens. *Allergy*. 2014; 69: 898-905.
27. Mori S, Pawankar R, Ozu C, et al. Expression and Roles of MMP-2, MMP-9, MMP-13, TIMP-1, and TIMP-2 in Allergic Nasal Mucosa. *Allergy Asthma Immunol Res*. 2012;4: 231–239.
28. Dahlen B, Shute J, Howarth P. Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax*. 1999; 54: 590-6.
29. Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. *J Biol Chem* 1992; 267: 3581–4.
30. Ko FW, Diba C, Roth M, McKay K, Johnson PR, Salome C, King GG. A comparison of airway and serum matrix metalloproteinase-9 activity among normal subjects, asthmatic patients, and patients with asthmatic mucus hypersecretion. *Chest*. 2005; 127: 1919-27.
31. Mattos W, Lim S, Russell R, Jatakanon A, Chung KF, Barnes PJ. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. *Chest*. 2002; 122: 1543-52.
32. Oshita Y, Koga T, Kamimura T, Matsuo K, Rikimaru T, Aizawa H. Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma. *Thorax*. 2003; 58: 757-60.

33. Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and Activity of Matrix metalloproteinase-9 and Tissue Inhibitor of metalloproteinase-1 by Alveolar Macrophages from Patients with Chronic Obstructive Pulmonary Disease. *Am J Respir Cell Mol Biol.* 2002; 26: 602-9.

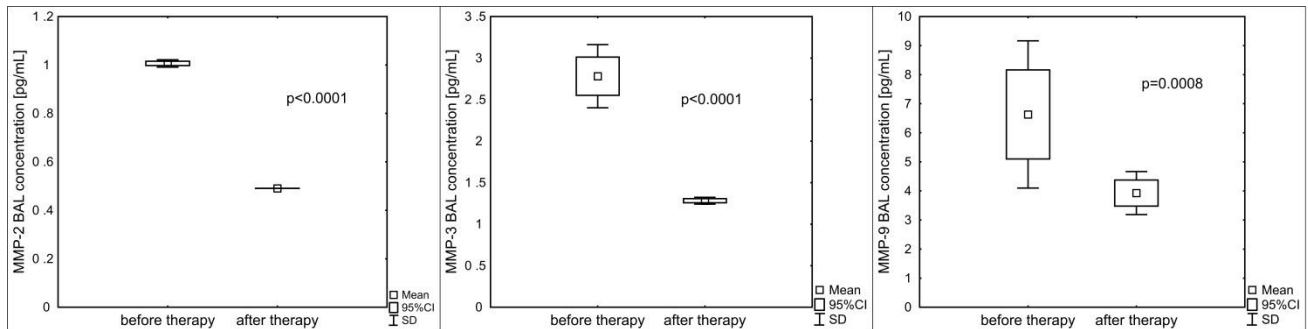


Figure 1. Changes in the concentration of metalloproteinases (MMPs) in bronchoalveolar lavage fluid (BAL) before vs. after omalizumab therapy.

Data are presented as mean with 95% confidence interval (CI) of the mean and standard deviation (SD).

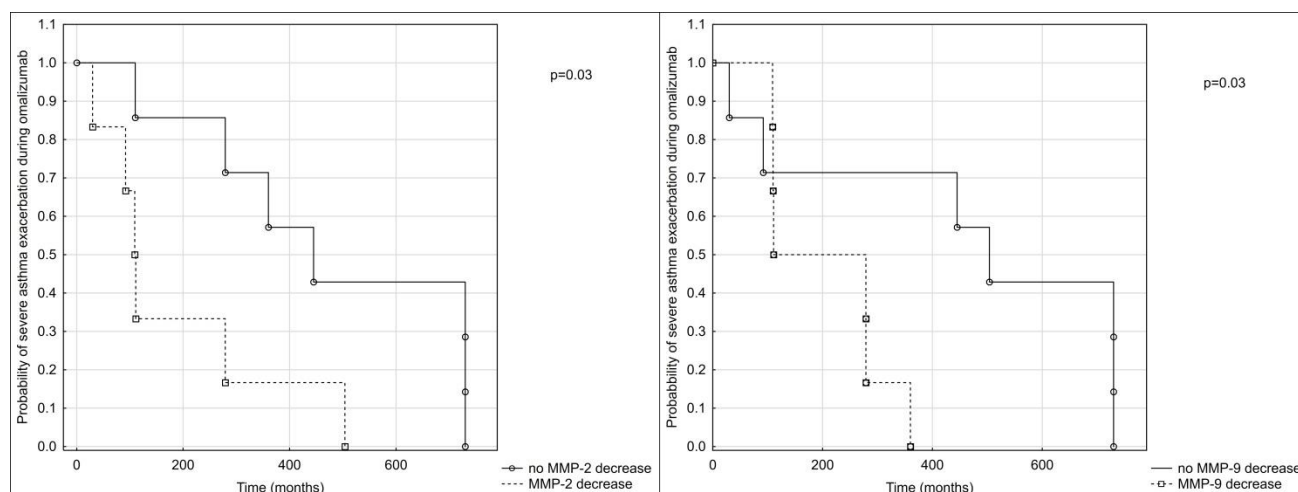


Figure 2. Kaplan-Meier curves presenting a risk of severe asthma exacerbation during omalizumab therapy in patients characterized by decline vs. no-decline in the concentration of metalloproteinase 2 (MMP-2) and 9 (MMP-9) in bronchoalveolar lavage fluid.

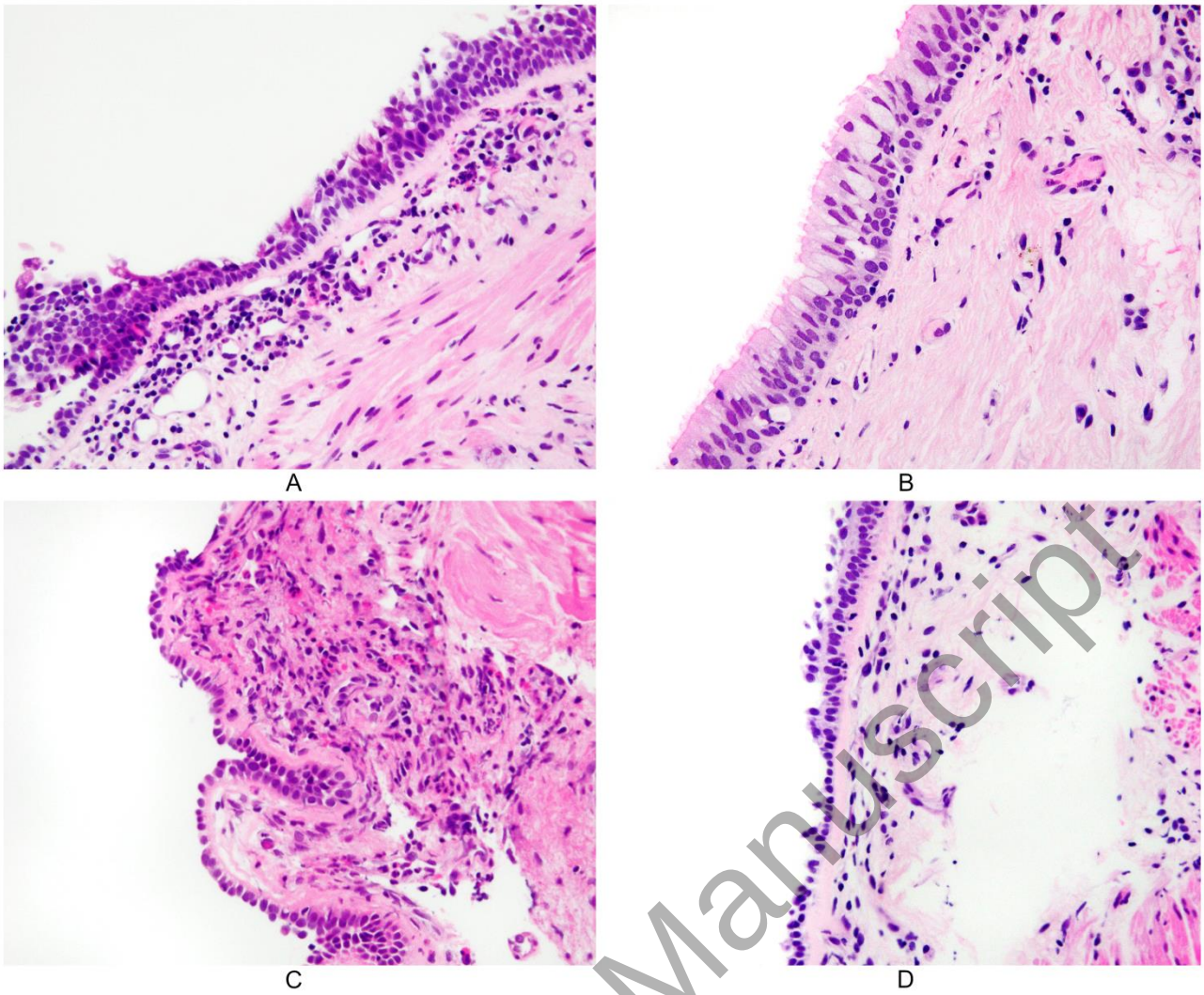


Figure 3. Representative histology images of significant reduction of the reticular basement membrane thickness in a patient with documented declines in all three bronchoalveolar lavage metalloproteinases after omalizumab therapy (A-B) and lack of such reduction in a patient with no decrease in metalloproteinases (C-D) - hematoxylin and eosin, original magnification 400 x.

Table 1: Clinical and laboratory characteristics of asthma patients at the baseline and after omalizumab therapy. Data are presented as median and interquartile range.

	Baseline, <i>n</i> = 13	After therapy, <i>n</i> = 13	p-value
Clinical characteristics and lung function tests			
Inhaled corticosteroid dose, µg (recalculated to fluticasone propionate)	2000 (1000-2750)	2000 (1000-3500)	0.45
Oral corticosteroid dose, mg (recalculated to methylprednisolone)	8 (4-15)	1 (0-8)	<0.001
Asthma Control Questionnaire, number of points	2.9 (2.8-3.9)	2.10 (1.14-2.30)	<0.001
Asthma Quality of Life Questionnaire, number of points	3.8 (3.0-4.0)	5.06 (4.30-5.80)	<0.001
Exacerbation episodes, number in the last year	4 (3-6)	0 (0-1)	<0.001
Forced expiratory volume in 1 s, % of predicted value	61 (56-70)	76 (60-79)	0.03
Blood eosinophilia and airway mucosa histology			
Blood eosinophilia, number/µl	200 (130-300)	250 (120-370)	0.26
Reticular basement membrane thickness, µm	6.54 (5.35-7.15)	5.12 (4.9-5.9)	0.002
Collagen type III (extracellular matrix deposition)	50 (40-75)	65 (50-75)	0.31
Collagen type I (extracellular matrix deposition)	25 (5-40)	30 (25-50)	0.07
Fibronectin (extracellular matrix deposition)	75 (50-90)	50 (25-75)	0.02
Eosinophils (infiltration into bronchial mucosa, number of cells per 10 microscopic high-power fields)	0.47 (0.17-0.65)	0.24 (0-0.56)	0.44
Bronchoalveolar lavage cytology (percentage of cells)			
Macrophages, %	88 (74-92)	80 (79-88)	0.75
Neutrophils, %	2 (0.9-3.6)	1.5 (1-3.2)	0.51
Eosinophils, %	1 (0.5-2.9)	0.5 (0.4-1)	0.25
Lymphocytes, %	7 (4-16)	7 (4-12)	0.73