ORIGINAL ARTICLE

Matrix metalloproteinase 9 in exhaled breath condensate in patients with stable chronic obstructive pulmonary disease: an observational study

Marta Maskey-Warzęchowska[1], Katarzyna Górska[1], Patrycja Nejman-Gryz[1], Magdalena Paplińska-Goryca[1], Tomasz Grzela[2], Alicja Krejner[2], Katarzyna Grzela[3], Rafał Krenke[1]

1 Department of Internal Medicine, Pulmonary Diseases and Allergy, Medical University of Warsaw, Warsaw, Poland
2 Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland
3 Department of Pediatrics, Pneumonology and Allergology, Medical University of Warsaw, Warsaw, Poland

KEY WORDS

chronic obstructive pulmonary disease, exhaled breath condensate, inflammation, inhaled corticosteroids, matrix metalloproteinase 9

Correspondence to:
Katarzyna Górska, MD, PhD, Department of Internal Medicine, Pulmonary Diseases and Allergy, Medical University of Warsaw, ul. Banacha 1a, 02-097 Warszawa, Poland, phone +48 22 599 25 62, email: drkpgorska@gmail.com
Received: May 1, 2018.
Revision accepted: June 29, 2018.
Published online: July 27, 2018.
Conflict of interest: RK reports receiving grants from National Science Centre, Poland, personal fees and nonfinancial support from Boehringer Ingelheim and Chiesi, and personal fees from Polpharma, without relevance to the submitted work. The other authors declare no conflict of interest.
Pol Arch Intern Med. 2018; 128 (7-8): 427-433
doi:10.20452/pamw.4302

ABSTRACT

INTRODUCTION Data on the measurement of matrix metalloproteinase 9 (MMP-9) in exhaled breath condensate (EBC) from patients with chronic obstructive pulmonary disease (COPD) are scarce and inconsistent.

OBJECTIVES We aimed to assess the usefulness of enzyme-linked immunosorbent assay (ELISA) and immunoenzymatic assay (IEA) for the measurement of MMP-9 in EBC, the agreement between the results of both methods, and the relationships between total and active MMP-9 in EBC and clinical and functional COPD characteristics.

PATIENTS AND METHODS Total (ELISA and IEA) and active (IEA) MMP-9 levels were assessed in EBC from 70 patients with stable COPD and 21 controls and correlated with pulmonary function and COPD symptom severity.

RESULTS MMP-9 levels did not reach the sensitivity threshold of the ELISA kit in any of the COPD patients and in 11 controls. Total and active MMP-9 (IEA) levels did not differ between COPD patients and controls. In COPD patients, total MMP-9 levels correlated positively with forced expiratory volume in 1 second (FEV_1) and FEV_1 to forced vital capacity ratio and inversely with residual volume to total lung capacity ratio. A weak positive correlation between active MMP-9 concentrations and COPD Assessment Test (CAT) score was found ($r = 0.31$, $P = 0.01$).

CONCLUSIONS The utility of ELISA in MMP-9 assessment in EBC is limited in COPD patients, while MMP-9 measurement in EBC by IEA is feasible. The positive correlation between active MMP-9 and CAT score in our patients and the inverse relationship between total MMP-9 concentration and the degree of airway obstruction reflect the complex role of MMP-9 in COPD.

INTRODUCTION Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes produced by different cell types that are involved in extracellular matrix component degradation and the regulation of the activity of other proteases and cytokines.[1] MMP-9 is one of the most abundant proteases in the alveolar space, and its role in the pathogenesis of various pulmonary diseases, particularly chronic obstructive pulmonary disease (COPD), has been documented.[1-4]

The precise role of MMP-9 in the physiopathology of COPD has not been fully elucidated. Elevated MMP-9 levels have been found in peripheral blood,[5-7] induced sputum (IS),[8-10] and bronchoalveolar lavage fluid (BALF)[11,12] from patients with COPD compared with healthy individuals. It is generally acknowledged that in patients with COPD, elevated concentrations of MMP-9 in respiratory samples correlate with the severity of local inflammation.[6,13] Moreover, higher serum

MMP-9 levels in comparison with healthy controls implies its role also in systemic inflammation. MMP-9 activity strongly depends on the activity of natural tissue inhibitors of metalloproteinases (TIMPs), and MMP-9/TIMP imbalance has been implicated in COPD development and progression.[6,8,12] There is a large body of literature documenting the correlation between MMP-9 and TIMP levels and the severity of airway obstruction and the degree of emphysema in patients with COPD.[5,7,9,13,14] On the other hand, Atkinson et al[15] showed that genetic MMP-9 deletion did not protect against cigarette smoke–induced inflammation and development of emphysema in murine models, indicating that MMP-9–independent pathways are also present in the physiopathology of COPD.

Exhaled breath condensate (EBC) analysis is a relatively simple method of airway sampling, which has been applied to evaluate and monitor airway inflammation in various respiratory diseases. Based on the analysis of volatile compounds detectable in droplets from cooled air exhaled during tidal breathing, in comparison with other methods of airway sampling (sputum induction, bronchoalveolar lavage), it is effortless for the patient, does not carry the potential risk of bronchoconstriction, and is noninvasive. EBC has been widely used in the research on different aspects of airway inflammation in asthma and COPD.[16-19] Reports on the concentrations of MMP-9 in EBC from patients with COPD are scarce. Kwiatkowska et al[8] found higher MMP-9 concentrations in patients with stable COPD when compared with healthy smokers, and these concentrations doubled during disease exacerbations. In contrast, Sng et al,[20] who also studied patients with stable COPD and during exacerbations as well as healthy never- and ex-smokers, failed to detect MMP-9 in EBC samples in any of the study participants. Both groups of investigators applied the enzyme-linked immunosorbent assay (ELISA) for MMP-9 measurement. Immunoenzymatic assay (IEA) is a relatively novel method that allows an assessment both at the level of a specific protein and its active form and that is characterized by high sensitivity and specificity.

There have been single reports on the use of IEA in MMP-9 detection in EBC from asthmatic children[21] and adults.[22] However, to our knowledge, there have been no reports so far on the use of IEA in studies involving assessment of MMP-9 in EBC from patients with COPD. Therefore, we conducted a study with the aim to assess the usefulness of ELISA and IEA for the measurement of MMP-9 levels in EBC from patients with COPD and to assess the agreement between the results obtained by the 2 methods. We also searched for potential relationships between the concentration of total and active MMP-9 in EBC and the clinical and functional characteristics of COPD.

PATIENTS AND METHODS

Study participants This prospective cross-sectional study was performed between March 2016 and July 2017 and included patients with COPD recruited from the outpatient pulmonary department of the Central Teaching Clinical Hospital in Warsaw. We enrolled consecutive COPD patients who were asked to participate in the study during a routine follow-up visit and had signed an informed consent. The study protocol was approved by the institutional review board (KB/133/2016).

The inclusion criteria for patients with COPD were as follows: 1) diagnosis of COPD in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) report[23]; and 2) absence of disease exacerbation within 6 weeks prior to enrollment. The exclusion criteria were respiratory infection or COPD exacerbation (or both) within 6 weeks prior to the study onset, and any other chronic respiratory disease (including asthma–COPD overlap).

The control group comprised of asymptomatic individuals who denied history of any chronic respiratory disease and had a normal spirometry result. The major exclusion criterion for this group was respiratory infection within 6 weeks before the study.

A separate analysis was performed for patients with COPD stratified according to treatment with inhaled corticosteroids (ICSs).

Functional assessment Spirometry with bronchial reversibility testing (Lung Test 1000, MES, Kraków, Poland) and body plethysmography (BodyBox 5500, Medi-soft S.A., Sorinnes, Belgium) were performed in accordance with the recommendations of the European Respiratory Society and American Thoracic Society.[24-26] Reference equations from the Global Lung Function Initiative were used for the calculation of the predicted values.[27] The degree of airway obstruction in patients with COPD was classified in accordance with the 2016 GOLD report.[23] For COPD patients, only postbronchodilator indices were considered for analysis.

Symptom severity in patients with COPD was assessed by the COPD Assessment Test (CAT).[28]

Exhaled breath condensate collection EBC was collected and processed according to the American Thoracic Society / European Respiratory Society guidelines,[29] using the TURBO-DECCS 09 system (Medivac, Parma, Italy) during tidal breathing for 20 minutes and at a condensation temperature of –5°C. The obtained samples were portioned into 500-µl aliquots, frozen and stored at –70°C until further analysis. The EBC samples were not lyophilized. None of the samples were repeatedly thawed and frozen for analysis.

Possible contamination with saliva was monitored by screening 5 randomly selected EBC samples for the presence of amylase.

Matrix metalloproteinase 9 measurement in exhaled breath condensate The EBC concentration of MMP-9 was determined by 2 different methods:

TABLE 1 Basic clinical and demographic data of patients with chronic obstructive pulmonary disease and controls

Variable	COPD (n = 70)	Controls (n = 21)	*P* value
Sex, male/female, n (%)	37 (53)/33 (47)	11 (52)/10 (48)	0.97
Age, y	67.5 (61.0–72.0)	36.0 (32.0–51.0)	<0.001
BMI, kg/m^2	26.8 (23.8–29.7)	25.9 (23.0–29.5)	0.63
Smoking status, current / ex-smokers / never-smokers, n (%)	28 (40)/42 (60)/0	5 (24)/5 (24)/11 (52)	<0.001
Smoking history, pack-years	45.0 (30.0–60.0)	0.0 (0.0–5.0)	<0.001
FEV_1, % of pred. value[a]	64.5 (46.8–76.0)	99.0 (93.0–109.0)	<0.001
FEV_1/FVC, %[a]	50.5 (40.0–56.0)	77.6 (75.0–80.7)	<0.001
RV, % of pred. value	181.5 (150.5–198.1)	110 (98.0–123.0)	<0.001
RV/TLC, %	53.8 (49.2–59.9)	30.0 (26.0–45.3)	<0.001
CAT score, points	16.0 (9.0–20.0)	NA	–

Data are presented as median and interquartile range (IQR) unless otherwise indicated.

a Postbronchodilator values were presented for the COPD group.

Abbreviations: BMI, body mass index; CAT, COPD Assessment Test; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; NA, not applicable; RV, residual volume; TLC, total lung capacity

TABLE 2 Matrix metalloproteinase 9 concentrations (total and active) in exhaled breath condensate measured by immunoenzymatic assay in patients with chronic obstructive pulmonary disease and controls

MMP-9	COPD	Controls	*P* value
Total MMP-9, ng/ml	3.95 (2.23–9.07)	5.24 (2.54–14.3)	0.25
Active MMP-9, ng/ml	3.26 (1.50–4.46)	4.29 (1.8–5.7)	0.14

Data presented as median (IQR).

Abbreviations: MMP-9, matrix metalloproteinase 9; others, see TABLE 1

ELISA for the total MMP-9 level and the immunoenzymatic assay (IEA) for total and active MMP-9 levels. The detection range and sensitivity of the applied ELISA kit (Human MMP-9 Quantikine ELISA kit, R&D Systems, Minneapolis, United States) was 0.3 to 20 ng/ml and 0.156 ng/ml, respectively. For the immunoenzymatic measurements, the Human MMP-9 Acivity Assay was used (QuickZyme Biosciences, Leiden, the Netherlands); the assay detection range was 0.016 to 16 ng/ml and the sensitivity was 0.01 ng/ml. Both assays were performed according to the detailed protocols provided by the manufacturer.

Statistical analysis Data were presented as median values and interquartile range (IQRs) or numbers and percentages. Statistical analysis was performed with Statistica 10.0 (StatSoft Inc, Tulsa, Oklahoma, United States) and MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium). The Shapiro–Wilk test was applied to assess quantitative data distribution. The differences between continuous variables in patients and controls were tested with the nonparametric Mann–Whitney test. Categorical variables were compared with the Fisher exact test and χ^2 test. The relationships between 2 variables were assessed by the Spearman rank correlation coefficient. The Bland–Altman plot was used to assess the agreement between the results of the ELISA and IEA. A *P* value of less than 0.05 was considered significant.

RESULTS **Patient characteristics** The study included 70 patients with COPD and 21 controls. The groups differed significantly in age, smoking history, and pulmonary function (TABLE 1). Nineteen patients (27.1%) with COPD were treated with ICS, with a mean (SD) daily dose of 1034 (399) μg of budesonide or equivalent.

Matrix metalloproteinase 9 measurement None of the randomly selected EBC samples for amylase measurement were contaminated by saliva. In all patients with COPD, the MMP-9 level measured with ELISA was below the lower detection limit. In the control group, the median MMP-9 level measured with ELISA was 0.35 ng/ml (IQR, 0.00–5.53 ng/ml); nevertheless, the MMP-9 level was below the lower detection limit in 11 subjects. For IEA, the MMP-9 level (total and active) reached detectable values both in COPD patients and controls and did not differ significantly between groups (TABLE 2).

Matrix metalloproteinase 9 levels and functional and clinical characteristics of patients We found weak but significant positive correlations between the total MMP-9 level and forced expiratory volume in 1 second (FEV_1) (% of predicted value) and FEV_1 to forced vital capacity ratio. Moreover, there was an inverse correlation between the total MMP-9 level and residual volume to total lung capacity ratio (FIGURE 1). There were no correlations between active MMP-9 levels and spirometry indices or lung volumes in patients with COPD. After adjustment for the ICS dose and the number of pack-years, total (but not active) MMP-9 was associated with FEV_1 (r = 0.31, P = 0.01 and r = 0.18, P = 0.15, respectively). Neither total nor active MMP-9 levels correlated with pulmonary function in the control group.

We did not find any associations between MMP-9 (total and active) and smoking status in any of the groups. However, in the group of patients with COPD, there was a weak positive correlation between active MMP-9 concentrations and CAT score (r = 0.31, P = 0.01). MMP-9 concentrations (both total and active) did not differ between patients with 1 or no exacerbations per year and those who experienced 2 or more exacerbations per year.

Total MMP-9 levels were lower in COPD patients treated with ICS when compared with those who did not receive ICS treatment (FIGURE 2). Such a difference was not observed for the concentration of the active MMP-9 form. There was no correlation between MMP-9 and the daily ICS dose.

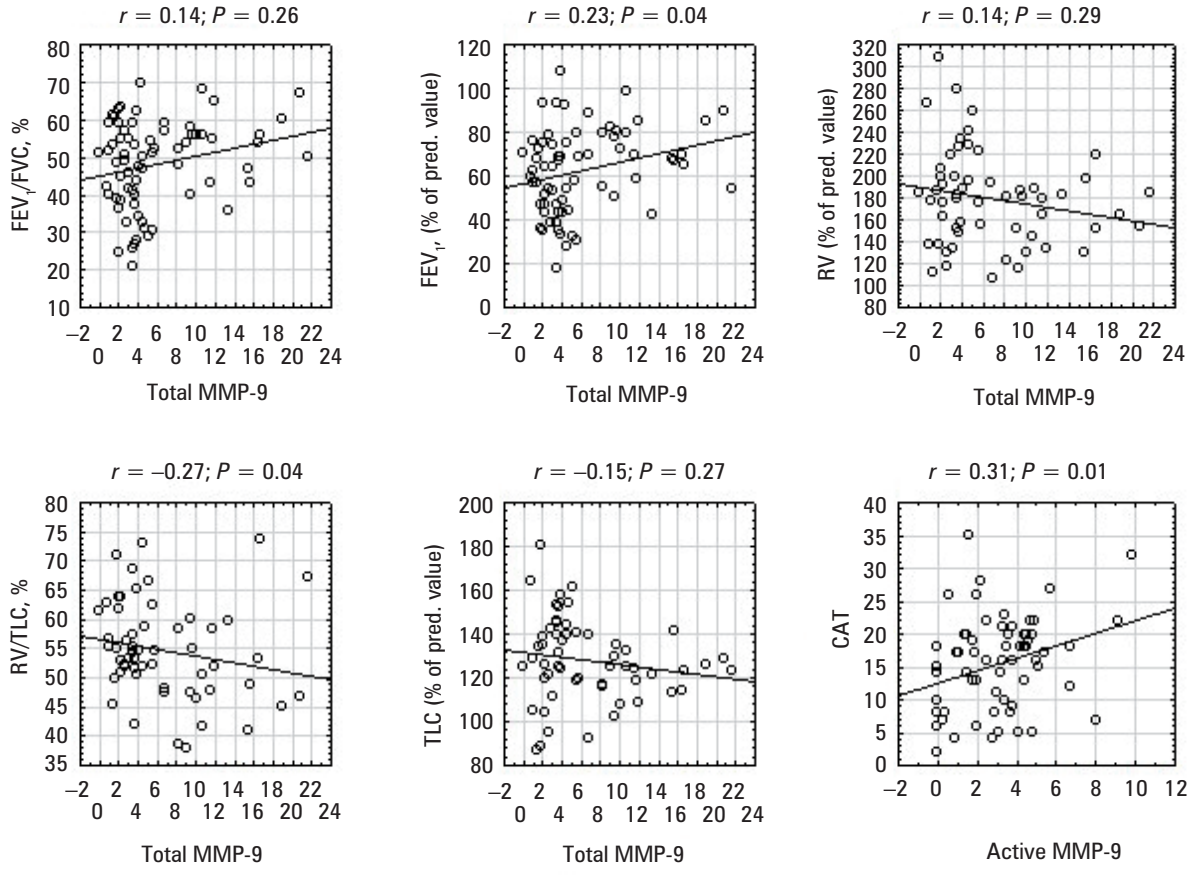

FIGURE 1 Correlations between pulmonary function (postbronchodilator value) and the COPD Assessment Test (CAT) score and the concentration of matrix metalloproteinase 9 (ng/ml) in exhaled breath condensate measured by immunoenzymatic assay (IEA) in patients with chronic obstructive pulmonary disease
Abbreviations: CAT, COPD Assessment Test; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; MMP-9, matrix metalloproteinase 9; RV, residual volume; TLC, total lung capacity

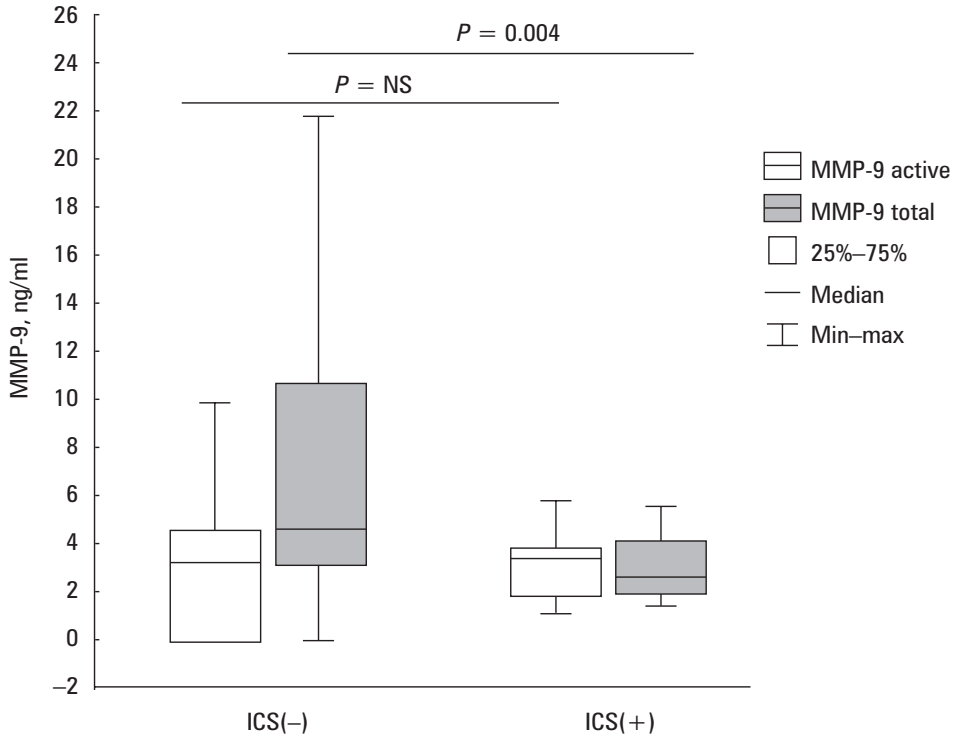

FIGURE 2 Matrix metalloproteinase 9 levels in exhaled breath condensate measured by immunoenzymatic assay (IEA) from patients with chronic obstructive pulmonary disease in relation to treatment with inhaled corticosteroids
Abbreviations: ICS(–), patients not treated with inhaled corticosteroids; ICS(+), patients treated with inhaled corticosteroids; NS, nonsignificant

DISCUSSION Our study is one of the very few reports on MMP-9 concentrations in EBC in patients with COPD. In our cohort, MMP-9 levels did not reach the lower detection limit of the applied ELISA kit. In contrast, we showed that the assessment of MMP-9 levels by IEA is feasible in these patients; nevertheless, MMP--9 concentrations (both total and active) did not differ between patients with COPD and controls. Although we found significantly lower total MMP-9 levels in COPD patients who received ICS when compared with those who did not, active MMP-9 levels were comparable between these subgroups. In COPD patients, higher MMP-9 levels correlated with a lower degree of airway obstruction.

Most studies on the role of MMP-9 in COPD were based on MMP-9 measurements in serum or IS, some also reported MMP-9 evaluation in BALF. A number of studies demonstrated higher serum MMP-9 levels in patients with COPD when compared with healthy controls[5,6,8,20,30]; however, comparable serum MMP-9 concentrations in COPD patients and healthy subjects were also reported.[7,10] Studies on MMP-9 levels in IS are more uniform and generally demonstrated higher MMP-9 concentrations in IS from COPD patients than from controls.[10,31-33] In our study, which involved a different biological material, EBC, MMP--9 levels (total and active) did not differ significantly between patients with stable COPD and controls. Despite a thorough literature search, we found only 2 reports on MMP-9 levels in EBC from patients with COPD.[8,20] Kwiatkowska et al[8] reported higher MMP-9 levels in patients with stable COPD when compared with asymptomatic smokers. These authors demonstrated an approximately 2-fold increase in exhaled MMP-9 during disease exacerbations, confirming its involvement not only in chronic but also in acute inflammation. As mentioned above, Sng et al[20] failed to find MMP-9 in any of the EBC samples, either from healthy controls or COPD patients. Both studies applied ELISA for MMP-9 measurement. Our results are in line with those reported by Sng et al,[20] as in EBC samples in the COPD group, the MMP--9 level did not reach the lower detection limit of the applied ELISA kit. There are at least 2 potential explanations for this observation. First, the EBC concentrations of various compounds tend to be low, mainly due to their high dilution as the main EBC content is water vapor.[16,29] Second, the low MMP-9 levels could have also resulted from the hindered diffusion of MMP-9 molecules to exhaled air due to their high molecular weight (~92 kD) and a lower mechanical force of the air stream secondary to airway obstruction in patients with COPD. On the other hand, we showed that MMP-9 is detectable in EBC from patients with COPD when IEA was used. This not only confirms that the MMP-9 measurement is feasible but also indicates that highly sensitive tests should be applied because those with lower sensitivity may not yield reliable results.

Data on the impact of elevated MMP-9 levels in respiratory samples on pulmonary function are equivocal. There is a large body of evidence that higher levels of MMP-9 are associated with a higher degree of airway obstruction and severity of emphysema, but this referred to biological samples other than EBC.[7,9,32,34] Other authors failed to show correlations between MMP--9 concentrations in airway specimens and pulmonary function.[30,35,36] Our finding that higher total MMP-9 levels in EBC are associated with better pulmonary function is in contrast to previous reports and is somewhat unexpected. However, this seems to be in agreement with the hypothesis on the worse MMP-9 diffusion from the airways to exhaled air due to airway obstruction as discussed above. Lowrey et al[32] found that lower MMP-9 activity in IS from patients with COPD was associated with a higher degree of airway obstruction. Matsumoto et al[37] reported an inverse correlation between airway wall thickening and the MMP-9 to TIMP ratio in patients with asthma. These findings were confirmed by Chaudhuri et al[38] who demonstrated that in asthmatic smokers, a low sputum MMP-9 to TIMP ratio was associated with a higher degree of airway obstruction and a smaller segmental airway lumen area assessed by computed tomography. In our opinion, this issue requires further studies involving patients with COPD.

Another plausible explanation for our results is the difference in the levels of inflammatory markers in different body compartments, which results from different sources contributing to the total concentration, varying dilutions, and a potential cross-reactivity with other sample contents. Ji et al[30] demonstrated that saliva, IS, BALF, and serum from COPD patients are characterized by a different inflammatory profile. Moreover, Cane et al,[36] who studied MMP-9 in sputum, serum, and urine from patients with COPD, found that MMP-9 levels were independent of one another and did not correlate with the degree of airway obstruction. In our previous study on a novel inflammatory marker, periostin, we showed that there were no correlations between periostin levels measured in EBC, IS, and BALF either in patients with COPD or those with asthma.[39] Therefore, we believe that the earlier reported correlations between MMP-9 in serum and IS and functional impairment in patients with COPD should not be extrapolated to EBC. Of the 2 studies on MMP-9 in EBC from patients with COPD, one showed no correlation between MMP-9 and spirometry parameters (either in stable disease or exacerbation),[8] while in the other, such an analysis could not have been performed because MMP--9 was not found in EBC from any of the study participants.[20]

We found lower total MMP-9 concentrations in patients with COPD who were treated with ICS when compared to those who were steroid-naive. Such a difference was not documented when active MMP-9 was compared; moreover, there was

no correlation between the daily ICS dose and MMP-9 levels. The impact of ICS on MMP-9 levels in respiratory samples has not been widely studied. Sohal et al,[40] in their pilot study on the effect of ICS on the markers of epithelial-mesenchymal transition in patients with COPD, demonstrated that the number of MMP-9–positive cells in the reticular basement membrane significantly decreased in patients treated with fluticasone. Moreover, it had reached values similar to those of normal controls. In an older study, Russell et al[11] reported that dexamethasone decreased the production of MMP-9 by BALF-derived alveolar macrophages from COPD patients. On the other hand, Grzela et al[21,41] found that ICSs do not decrease MMP-9 activity in EBC in asthma, either during prolonged treatment in patients with stable disease or during disease exacerbation. Nevertheless, these findings cannot be directly compared with our results, as they were observed in a different study population, that is, children with asthma.

Studies on the effect of ICS on MMP-9 in EBC in patients with COPD are lacking. We may speculate that the lower levels of exhaled MMP-9 due to ICSs may reflect a positive impact of this anti-inflammatory treatment in COPD. However, it should be noted that in our cohort, almost all patients who received ICS treatment presented with severe or very severe airway obstruction (FEV_1 below 50% of predicted value), and only 2 patients in this group had FEV_1 above 50%. With the lack of differences between groups with severe and very severe airway obstruction, a significant overlap of these groups with the groups treated and not treated with ICS, and similar levels of active MMP-9, we cannot exclude that the lower total MMP-9 levels in EBC in patients treated with ICS may be an incidental finding.

There are some limitations to our study which need to be acknowledged. One of the major limitations—a significant overlap between patients treated and not treated with ICS and those with severe and very severe airway obstruction—was mentioned above. However, our study was aimed at assessing MMP-9 levels in real-life conditions, hence ICS treatment was not an exclusion criterion. Considering the current recommendations for ICS use in patients with COPD, it could have been expected that most patients treated with ICS would present with a FEV_1 of less than 50%. Second, our analysis was limited to MMP-9 only; we did not assess other MMPs or TIMP. However, our study focused on the comparison of 2 different analytic methods and their feasibility in MMP-9 measurement in EBC, a material that had not been previously widely investigated in this context. The obtained MMP-9 values were low, and for all COPD patients and some controls, they were lower than the lower detection limit for the ELISA kit. Nevertheless, the values obtained by IEA were not significantly lower than in the control group, and despite these low numerical results we were able to demonstrate significant correlations between MMP-9 levels and some clinical features of the disease.

Another limitation that has to be considered are the differences in the number of the compared groups. Therefore, the results of the comparison between 70 COPD patients and 21 controls, as well as between patients treated and not treated with ICS (19 vs 51 patients), need to be interpreted with caution. The significant difference in age and smoking status between the group of patients with COPD and controls is yet another drawback. This was a result of the considerable difficulties in recruiting elderly healthy subjects who would meet the inclusion criteria for controls and would agree to participate in the study. On the other hand, Kwiatkowska et al[8] showed that age and the number of smoking pack-years do not correlate with EBC and serum MMP-9 levels.

In conclusion, the utility of ELISA in the assessment of MMP-9 in EBC may be limited in patients with COPD. The measurement of MMP-9 by IEA is feasible in these patients. The positive correlation between the active MMP-9 form and CAT score in our patients, as well as the inverse relationship between total MMP-9 concentration and the degree of airway obstruction, point to the technical and clinical considerations that need to be taken into account in MMP-9 evaluation in EBC.

ACKNOWLEDGMENTS We would like to thank Dr Piotr Korczyński for his assistance in the preparation of this manuscript.

CONTRIBUTION STATEMENT K. Górska, TG, MM-W, PN-G, and RK designed the study. K. Górska, MM-W, K. Grzela, and PN-G were responsible for literature search, patient recruitment, and data analysis. PN-G and MP-G carried out ELISA measurements. TG and AK carried out IEA measurements. MM-W wrote the first draft. All authors critically reviewed the manuscript and contributed to the final version.

REFERENCES

1 Grzela K, Litwiniuk M, Zagorska W, et al. Airway remodeling in chronic obstructive pulmonary disease and asthma: the role of matrix metalloproteinase-9. Arch Immunol Ther Exp. 2016; 64: 47-55.

2 Houghton AM. Matrix metalloproteinases in destructive lung disease. Matrix Biol. 2015; 44-46: 167-174.

3 Fingleton B. Matrix metalloproteinases as regulators of inflammatory processes. Biochim Biophys Acta. 2017; 1864: 2036-2042.

4 El-Badrawy MK, Yousef AM, Shaalan D, et al. Matrix metalloproteinase-9 expression in lung cancer patients and its relation to serum mmp-9 activity, pathologic type, and prognosis. J Bronchology Interv Pulmonol. 2014; 21: 327-334.

5 Brajer B, Batura-Gabryel H, Nowicka A, et al. Concentration of matrix metalloproteinase-9 in serum of patients with chronic obstructive pulmonary disease and a degree of airway obstruction and disease progression. J Physiol Pharmacol. 2008; 59 (Suppl 6): 145-152.

6 Li Y, Lu Y, Zhao Z, et al. Relationships of MMP-9 and TIMP-1 proteins with chronic obstructive pulmonary disease risk: a systematic review and meta-analysis. J Res Med Sci. 2016; 21: 12-20.

7 Koo HK, Hong Y, Lim MN, et al. Relationship between plasma matrix metalloproteinase levels, pulmonary function, bronchodilator response, and emphysema severity. Int J Chron Obstruct Pulmon Dis. 2016; 11: 1129-1137.

8 Kwiatkowska S, Noweta K, Zieba M, et al. Enhanced exhalation of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with COPD exacerbation: a prospective study. Respiration. 2012; 84: 231-241.

9 Chaudhuri R, McSharry C, Spears M, et al. Sputum matrix metalloproteinase-9 is associated with the degree of emphysema on computed tomography in COPD. Transl Respir Med. 2013; 1: 11-15.

10 Kleniewska A, Walusiak-Skorupa J, Piotrowski W, et al. Comparison of biomarkers in serum and induced sputum of patients with occupational asthma and chronic obstructive pulmonary disease. J Occup Health. 2016; 58: 333-339.

11 Russell RE, Culpitt SV, DeMatos C, et al. 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: 602-609.

12 Papakonstantinou E, Karakiulakis G, Batzios S, et al. Acute exacerbations of COPD are associated with significant activation of matrix metalloproteinase 9 irrespectively of airway obstruction, emphysema and infection. Respir Res. 2015; 16: 78-89.

13 Górka K, Soja J, Jakieła B, et al. Relationship between the thickness of bronchial wall layers, emphysema score, and markers of remodeling in bronchoalveolar lavage fluid in patients with chronic obstructive pulmonary disease. Pol Arch Med Wewn. 2016; 126: 402-410.

14 Ostridge K, Williams N, Kim V, et al. Relationship between pulmonary matrix metalloproteinases and quantitative CT markers of small airways disease and emphysema in COPD. Thorax. 2016; 71: 126-132.

15 Atkinson JJ, Lutey BA, Suzuki Y, et al. The role of matrix--metalloproteinase-9 in cigarette smoke-induced emphysema. Am J Respir Crit Care Med. 2011; 183: 876-884.

16 Konstantinidi EM, Lappas AS, Tzortzi AS, et al. Exhaled breath condensate: technical and diagnostic aspects. ScientificWorldJournal. 2015; 2015: 435160.

17 Górska K, Nejman-Gryz P, Paplińska-Goryca M, et al. Comparison of thymic stromal lymphopoietin concentration in various human biospecimens from asthma and COPD patients measured with two different ELISA kits. Adv Exp Med Biol. 2017; 955: 19-27.

18 Maskey-Warzęchowska M, Nejman-Gryz P, Osinka K, et al. Acute response to cigarette smoking assessed in exhaled breath condensate in patients with chronic obstructive pulmonary disease and healthy smokers. Adv Exp Med Biol. 2017; 944: 73-80.

19 Santini G, Mores N, Shohreh R, et al. Exhaled and non-exhaled non--invasive markers for assessment of respiratory inflammation in patients with stable COPD and healthy smokers. J Breath Res. 2016; 10: 017102.

20 Sng JJ, Prazakova S, Thomas PS, et al. MMP-8, MMP-9 and neutrophil elastase in peripheral blood and exhaled breath condensate in COPD. COPD. 2017; 14: 238-244.

21 Grzela K, Zagorska W, Krejner A, et al. Prolonged treatment with inhaled corticosteroids does not normalize high activity of matrix metalloproteinase-9 in exhaled breath condensates of children with asthma. Arch Immunol Ther Exp. 2015; 63: 231-237.

22 Barbaro MP, Spanevello A, Palladino GP, et al. Exhaled matrix metalloproteinase-9 (MMP-9) in different biological phenotypes of asthma. Eur J Intern Med. 2014; 25: 92-96.

23 Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for Diagnosis, Management, and Prevention of COPD – 2016. http://goldcopd.org/global-strategy-diagnosis-management-prevention--copd-2016. Published 2016. Accessed February 15, 2018.

24 Miller MR, Hankinson J, Brusasco V, et al; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005; 26: 319-338.

25 Wanger J, Clausen JL, Coates A, et al; ATS/ERS Task Force. Standardisation of the measurement of lung volumes. Eur Respir J. 2005; 26: 511-522.

26 Pellegrino R, Viegi G, Brusasco V, et al; ATS/ERS Task Force. Interpretative strategies for lung function tests. Eur Respir J. 2005; 26: 948-968.

27 Quanjer PH, Stanojevic S, Cole TJ, et al; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012; 40: 1324-1343.

28 Jones PW, Harding G, Berry P, et al. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009; 34: 648-654.

29 Horváth I, Hunt J, Barnes PJ, et al; ATS/ERS Task Force on Exhaled Breath Condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005; 26: 523-548.

30 Ji J, von Schéele I, Bergström J, et al. Compartment differences of inflammatory activity in chronic obstructive pulmonary disease. Respir Res. 2014; 15: 104.

31 Paone G, Conti V, Vestri A, et al. Analysis of sputum markers in the evaluation of lung inflammation and functional impairment in symptomatic smokers and COPD patients. Dis Markers. 2011; 31: 91-100.

32 Lowrey GE, Henderson N, Blakey JD, et al. MMP-9 protein level does not reflect overall MMP activity in the airways of patients with COPD. Respir Med. 2008; 102: 845-851.

33 Louhelainen N, Stark H, Mazur W, et al. Elevation of sputum matrix metalloproteinase-9 persists up to 6 months after smoking cessation: a research study. BMC Pulm Med. 2010; 10: 13-21.

34 Boschetto P, Quintavalle S, Zeni E, et al. Association between markers of emphysema and more severe chronic obstructive pulmonary disease. Thorax. 2006; 61: 1037-1042.

35 D'Armiento JM, Goldklang MP, Hardigan AA, et al. Increased matrix metalloproteinase (MMPs) levels do not predict disease severity or progression in emphysema. PLoS One. 2013; 8: e56352.

36 Cane JL, Mallia-Millanes B, Forrester DL, et al. Matrix metalloproteinases -8 and -9 in the airways, blood and urine during exacerbations of COPD. COPD. 2016; 13: 26-34.

37 Matsumoto H, Niimi A, Takemura M, et al. Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. Thorax. 2005; 60: 277-281.

38 Chaudhuri R, McSharry C, Brady J, et al. Low sputum MMP-9/TIMP ratio is associated with airway narrowing in smokers with asthma. Eur Respir J. 2014; 44: 895-904.

39 Górska K, Maskey-Warzęchowska M, Nejman-Gryz P, et al. Comparative study of periostin expression in different respiratory samples in patients with asthma and chronic obstructive pulmonary disease. Pol Arch Med Wewn. 2016; 126: 124-137.

40 Sohal SS, Soltani A, Reid D, et al. A randomized controlled trial of inhaled corticosteroids (ICS) on markers of epithelial-mesenchymal transition (EMT) in large airway samples in COPD: an exploratory proof of concept study. Int J Chron Obstruct Pulmon Dis. 2014; 9: 533-542.

41 Grzela K, Zagórska W, Krejner A, et al. Inhaled corticosteroids do not reduce initial high activity of matrix metalloproteinase (MMP)-9 in exhaled breath condensates of children with asthma exacerbation: a proof of concept study. Cent Eur J Immunol. 2016; 41: 221-227.