INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic and progressive inflammatory respiratory disorder with clinical manifestation of airflow limitation that is not fully reversible.1,2 The pathogenesis of COPD lies in an abnormal, enhanced inflammatory response of the lungs invoked by noxious particles and gases, especially cigarette smoke.3,4 Lung macrophages and epithelial cells in response to insults such as cigarette smoke produce and release proinflammatory chemokines, such as tumor necrosis factor-α (TNF-α), interleukin 8 (IL-8), leukotriene B4, which induce activation and migration of neutrophils to the lungs.5-8 Activated neutrophils damage the lung tissue by producing and releasing proteinases and reactive oxygen species and by inactivating protective antiproteinases.9-11 Prolonged irritation of the respiratory tract with noxious particles in susceptible individu-
Simvastatin represents a class of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins), which decrease the blood cholesterol level by inhibiting its synthesis and increasing the expression of low-density lipoprotein (LDL) receptors in the liver. Apart from hypolipemic effect, the blocking of HMG CoA reductase inhibits the synthesis of isoprenoids, derivatives of mevalonic acid, which activate many cell signal pathways and are responsible for the so called pleiotropic properties of statins. Anti-inflammatory and antithrombotic effects of statins reduce cardiovascular event rates and mortality.

It has been observed that statin treatment has a beneficial effect in patients with COPD. Retrospective analysis of the mortality rate from various causes and the amount of statins sold in the Japanese population aged over 65 years demonstrated a correlation between statin use and reduction of mortality, not only from cardiovascular diseases, but also from COPD, pneumonia, as well as overall mortality. A prospective study with a 2-year follow-up conducted in Norway on patients hospitalized due to COPD exacerbation showed better survival of patients treated with statins. Retrospective analysis based on the registry of patients in Quebec, Canada, showed that statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers reduced the rate of hospitalizations due to COPD exacerbations, regardless of the treatment with inhaled corticosteroids.

Apart from observational studies, there has been experimental research on animals that provided relevant data on statin use. In rats exposed to cigarette smoke simvastatin treatment inhibited lung tissue damage as well as the development of pulmonary hypertension through decreasing the migration of inflammatory cells and synthesis of matrix metalloproteinase-9 (MMP-9) in the lung tissue. In another study, simvastatin-treated rats exposed to chronic hypoxia were protected from pulmonary hypertension by reducing smooth muscle cell proliferation in pulmonary vessels. Despite these data, the mechanisms of statin action in COPD are not fully understood. In our study, we aimed to evaluate whether simvastatin administration in patients with stable COPD can influence the levels of selected inflammatory markers measured in blood.

**Patients and Methods** Patients

We studied 56 patients, aged 44 to 80 years, with stable COPD (mean FEV₁: 55%, mean FEV₁/FVC: 57%), without exacerbations in the previous month. The exclusion criteria included the use of statin or oral corticosteroids within the past 3 months, unstable angina, myocardial infarction within the previous 6 months, congestive heart failure, chronic inflammatory diseases, liver dysfunction, renal failure, cancer, and inability to comply with study requirements. The study was approved by the local Ethics Committee and patients gave informed consent.

**Study protocol** After obtaining informed consent, all patients were asked to fill out the questionnaire concerning their symptoms, smoking status, and medical history. Then, they underwent physical examination, spirometry tests, electrocardiography, and echocardiography. The participants were randomized on an open-label basis into 2 groups: the statin group received simvastatin (Zocor, MSD) 40 mg daily for 3 months, and the control group did not receive simvastatin treatment. Randomization was based on the list randomly generated by the computer. Fasting blood was collected 3 times: at the beginning of the study, and during follow-up at 2 weeks and 3 months. Lipid profile, glucose, creatinine, alanine aminotransferase (ALT), and creatine kinase (CK) levels were measured using standard laboratory methods on the day of each visit. Blood samples for the assessment of inflammatory markers were centrifuged and stored at −80°C until analysis. Fibrinogen was determined in plasma using the von Clauss method. High-sensitivity CRP level in serum was measured by nephelometry (Dade Behring, Germany). TNF-α, IL-6, and MMP-9 levels in serum were assessed by immunoassay, using commercial kits (R&D Systems, Great Britain).

**Statistical analysis** Demographic data, clinical symptoms, comorbidities, treatment, spirometry and laboratory results were compared between groups using the t-test for independent variables and χ² Pearson test for qualitative variables. The results of inflammatory markers obtained during the 3 visits were analyzed, independently in each group, using the analysis of variance (ANOVA). Both groups were then divided into 2 subgroups according to the FEV₁ value and their results were analyzed (the cutoff value was 50%). The level of significance was set at P <0.05. All calculations were performed with the Statistica 6.0 software.
to 4.71 mmol/l, $P = 0.0018$) and LDL cholesterol (from 3.46 mmol/l to 2.47 mmol/l, $P = 0.000037$) after 3 months of statin treatment. The decrease occurred after 2 weeks and was maintained to the end of the study. The safety of simvastatin treatment was monitored by measuring CK and ALT levels. No increase in these markers that would indicate serious adverse events and would result in exclusion from the study and terminating the treatment was detected in any of the participants (Table 2).

Control spirometry at 3 months was comparable to the baseline results (Table 3).

### RESULTS

**Patient characteristics** The groups did not differ significantly in terms of demographic data. No significant differences were observed with regard to clinical symptoms, lipid profile, pharmacological treatment, and baseline spirometry results. Among comorbidities, only arterial hypertension was more frequent in the statin group (32.14% vs. 17.86%, $P = 0.032$) (Table 1).

**Lipid-lowering effect** The compliance in the statin group has been proven by a significant decrease in both total cholesterol (from 5.68 mmol/l to 4.71 mmol/l, $P = 0.0018$) and LDL cholesterol (from 3.46 mmol/l to 2.47 mmol/l, $P = 0.000037$) after 3 months of statin treatment. The decrease occurred after 2 weeks and was maintained to the end of the study. The safety of simvastatin treatment was monitored by measuring CK and ALT levels. No increase in these markers that would indicate serious adverse events and would result in exclusion from the study and terminating the treatment was detected in any of the participants (Table 2). Control spirometry at 3 months was comparable to the baseline results (Table 3).
There were no significant differences in the levels of inflammatory markers at baseline, 2 weeks, and 3 months, except for the IL-6 concentration, which was significantly lower in patients with mild and moderate COPD at the end of the study (\(P = 0.016\)) (FIGURE 2).

The levels of the measured markers in individual groups remained stable throughout the study (there were no significant differences between subsequent measurements). An insignificant decrease of CRP and IL-6 was observed in the subgroup of patients with mild and moderate COPD (TABLE 6).

**DISCUSSION**
The present study has been one of the first to investigate the effect of statin treatment on inflammation in COPD in a randomized study. Baseline concentrations of the inflammatory markers were comparable in both studied groups. CRP and IL-6 levels were similar to those observed by Garrod et al., and the meta-analysis of Gan et al. showed similar CRP and fibrinogen levels. After 3 months of simvastatin treatment no significant changes in the levels of the measured markers were observed. There was none of the participants reported drug intolerance or exacerbation of symptoms. No acute cardiovascular events were observed. Pharmacological treatment was not modified in any of the patients throughout the study.

**Inflammatory markers**
After 3 months of simvastatin treatment, there were no significant differences in the levels of any measured inflammatory markers (TABLE 4). Only an insignificant decrease in CRP was observed in the statin group (FIGURE 1). The statin group was divided into 2 subgroups: the subgroup of patients with FEV\(_1\) >50% (with mild and moderate COPD, according to the Global Initiative on Obstructive Lung Diseases classification) and the subgroup of patients with FEV\(_1\) <50% (with severe and very severe COPD). Such division was necessitated by a small number of patients with mild and very severe COPD – a division into 4 groups would make the statistical analysis unreliable. The subgroups were similar with regard to age, smoking status, hypercholesterolemia, and comorbidities (TABLE 5). Data analysis did not reveal significant between-group differences in the levels of inflammatory markers at baseline, 2 weeks, and 3 months, except for the IL-6 concentration, which was significantly lower in patients with mild and moderate COPD at the end of the study (\(P = 0.016\)) (FIGURE 2). The levels of the measured markers in individual groups remained stable throughout the study (there were no significant differences between subsequent measurements). An insignificant decrease of CRP and IL-6 was observed in the subgroup of patients with mild and moderate COPD (TABLE 6).

**TABLE 2** Compliance and safety measures during simvastatin administration at subsequent visits

<table>
<thead>
<tr>
<th></th>
<th>Statin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>visit 0</td>
<td>visit 1</td>
<td>visit 2</td>
</tr>
<tr>
<td>CK (U/l)</td>
<td>145 ± 25.82</td>
<td>114 ± 81.56</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>29 ± 8.4</td>
<td>33 ± 12.5</td>
</tr>
<tr>
<td>total cholesterol (mmol/l)</td>
<td>5.68 ± 1.39</td>
<td>4.44 ± 1.14</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.46 ± 0.98</td>
<td>2.34 ± 0.89</td>
</tr>
</tbody>
</table>

The results are shown as mean values with standard deviations.

**Abbreviations:** ALT – alanine aminotransferase, CK – creatine kinase, others – see TABLE 1

**TABLE 3** The comparison of spirometry values at the beginning and at the end of the study

<table>
<thead>
<tr>
<th></th>
<th>Statin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>visit 0</td>
<td>visit 1</td>
</tr>
<tr>
<td>FEV(_1) (%)</td>
<td>56.25 ± 16.2</td>
<td>56.03 ± 15.8</td>
</tr>
<tr>
<td>FEV(_1)/FVC (%)</td>
<td>57.8 ± 11.6</td>
<td>57.07 ± 11.5</td>
</tr>
</tbody>
</table>

The results are shown as mean values with standard deviations.

**Abbreviations:** see TABLE 1

**TABLE 4** Concentrations of inflammatory markers in both groups at subsequent visits

<table>
<thead>
<tr>
<th></th>
<th>Statin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>visit 0</td>
<td>visit 1</td>
<td>visit 2</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>6.3 ± 6</td>
<td>8.88 ± 16.03</td>
</tr>
<tr>
<td>fibrinogen (g/l)</td>
<td>4.06 ± 1.52</td>
<td>4.43 ± 1.59</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>2.01 ± 1.05</td>
<td>1.67 ± 0.41</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.1 ± 2.37</td>
<td>4.15 ± 3.16</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>599 ± 331</td>
<td>585 ± 221</td>
</tr>
</tbody>
</table>

The results are shown as mean values with standard deviations.

**Abbreviations:** CRP – C-reactive protein, IL – interleukin, MMP – matrix metalloproteinase, TNF-\(\alpha\) – tumor necrosis factor-\(\alpha\), others – see TABLE 1
In our study, an insignificant decrease in CRP was observed mainly in patients with mild and moderate COPD.

Our results, together with the data from retrospective studies, show the complex mechanisms involved in the pathogenesis of COPD. The beneficial properties of statins, associated with a reduction of risk of cardiovascular events as well as their influence on inflammatory markers measured in blood, may be related to statin-mediated effect on unstable atherosclerotic plaques, activated endothelium, and inflammatory cells circulating in blood. The inflammatory parameters in these disorders are measured exactly at the site affected by the disease. We measured blood levels of inflammatory markers, which may not be sufficient because the major site of inflammation in COPD is the respiratory tract, even though systemic inflammation is a feature of COPD. Samples obtained directly from the respiratory tract, e.g., bronchoalveolar lavage or exhaled air condensate, might be more appropriate for the assessment of statin induced effects in COPD.

Apart from the site of inflammation, other mechanisms by which statins operate should also be considered. Numerous experimental studies showed antithrombotic and immunomodulatory properties of statins. In ischemic heart disease statins reduce the risk of acute coronary events, and thus mortality, not only by stabilizing the plaque but also by inhibiting platelet aggregation and thrombin formation. Such prothrombotic and proinflammatory state occurs in exacerbated or unstable coronary heart disease. In COPD we also observe such intensification of inflammation during exacerbations. Polosa et al. revealed enhanced inflammatory state, increased activation of endothelial cells, hemostasis, and fibrinolysis during COPD exacerbations when compared with stable periods in the same patients. They measured the concentration of IL-6, expression of the von Willebrand factor, and levels of D-dimer and prothrombin fragment F1+2. Blamoun et al. conducted a 1-year follow-up of patients after COPD exacerbation. They found that patients receiving statins before exacerbation were significantly less likely to have another exacerbation and were at a lower risk of intubation during follow-up. Our study included only stable patients, therefore we did not observe an insignificant decrease in CRP level in the statin group and in IL-6 concentration in the subgroup of patients with mild and moderate COPD. Additionally, at 3 months, a significantly lower concentration was observed in patients with FEV1 >50% when compared with the other subgroup. Our results are not consistent with the study of Lee et al., in which a significant decrease in CRP and IL-6 levels was observed. However, their study lasted 6 months, the groups were much larger, and the subjects received pravastatin. Moreover, Lee et al., observed higher IL-6 values at baseline, and reduced CRP levels were demonstrated only in patients with elevated baseline CRP. In

**TABLE 5**

<table>
<thead>
<tr>
<th>Age, x ±SD</th>
<th>FEV1 &gt;50% (n = 18)</th>
<th>FEV1 &lt;50% (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking, n (%)</td>
<td>63.7 ± 12.42</td>
<td>67.5 ± 9.2</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>13 (72.2)</td>
<td>4 (40)</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>7 (38.9)</td>
<td>3 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>13 (72.2)</td>
<td>6 (60)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: see **TABLE 1**
TABLE 6  Concentrations of inflammatory markers in the subgroups with FEV₁ >50% and FEV₁ <50% of the statin group at subsequent visits

<table>
<thead>
<tr>
<th></th>
<th>visit 0</th>
<th>visit 1</th>
<th>visit 2</th>
<th>P</th>
<th>visit 0</th>
<th>visit 1</th>
<th>visit 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>6.3 ± 6</td>
<td>5.9 ± 9.62</td>
<td>3.18 ± 4.47</td>
<td>NS</td>
<td>6.3 ± 11.7</td>
<td>14.3 ± 32.2</td>
<td>4.42 ± 8.3</td>
<td>NS</td>
</tr>
<tr>
<td>fibrinogen (g/l)</td>
<td>4.09 ± 1.52</td>
<td>4.36 ± 1.79</td>
<td>4.37 ± 1.27</td>
<td>NS</td>
<td>4.01 ± 3.11</td>
<td>4.55 ± 2.68</td>
<td>4.12 ± 3.25</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.87 ± 1.08</td>
<td>1.60 ± 0.61</td>
<td>1.80 ± 0.93</td>
<td>NS</td>
<td>2.25 ± 1.94</td>
<td>1.79 ± 0.45</td>
<td>1.80 ± 0.63</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.82 ± 2.77</td>
<td>3.29 ± 2.65</td>
<td>2.60 ± 1.78</td>
<td>NS</td>
<td>4.57 ± 3.92</td>
<td>5.69 ± 5.76</td>
<td>5.34 ± 5.42</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>620 ± 434</td>
<td>587 ± 192</td>
<td>513 ± 294</td>
<td>NS</td>
<td>563 ± 362</td>
<td>581 ± 294</td>
<td>576 ± 438</td>
<td>NS</td>
</tr>
</tbody>
</table>

The results are shown as mean values with standard deviations.

Abbreviations: see TABLES 1 and 4

differences in inflammatory markers, even in relation to disease severity. The comparison of our results with those obtained during exacerbations might provide more information about the action of statins.

It has already been mentioned that both in COPD and cardiovascular diseases enhanced inflammation is accompanied by increased activation of blood coagulation. Such observations were reported by Alessandri et al. and by Ferroni et al. The beneficial effect of statins may be partly associated with their antithrombotic properties. Undas et al. assessed fibrin clots obtained in vitro from COPD patients before and after simvastatin treatment. They showed that fibrin clots from COPD patients are more dense and resistant to fibrinolysis than those obtained from healthy subjects. These properties were positively correlated with CRP levels. It is possible that during COPD exacerbation, when CRP level is elevated, coagulation is also activated. They also found that simvastatin administration improved the clot structure and susceptibility to fibrinolysis, so this mechanism might possibly reduce mortality in COPD patients treated with statins.

Voelkel et al. investigated the role of endothelial dysfunction in the development of pulmonary hypertension and emphysema in COPD. They emphasize a key role of the vascular endothelial growth factor (VEGF) in the proper functioning of pulmonary vessels and the surrounding lung tissue. VEGF deficiency may cause dysfunction of the pulmonary endothelium by reducing prostacyclin and nitric oxide synthesis. It may also account for lung damage and development of emphysema by decreasing superoxide dismutase expression in endothelial cells and increasing pulmonary endothelial and epithelial cell apoptosis. Nishimoto-Hazuku et al. discovered that simvastatin augmented VEGF synthesis in endothelial cells, and this mechanism may also be in part responsible for the beneficial effect of statins in COPD, such as prevention from pulmonary hypertension in rats exposed to hypoxia as observed by Girgis et al.

Limitations of the study  The group of patients was relatively small and heterogeneous, and the follow-up was short. The inflammatory markers were measured only in blood and we limited the study group only to stable patients. However, the negative results obtained in our study do not exclude the potential benefits of statin use in COPD patients. Given all publications that show clinical benefits of statin use in COPD, the current results show the complexity of inflammatory processes involved in this disease as well as other potential mechanisms of action of these drugs. Thus, further prospective studies with a longer follow-up should be planned to assess the action of statins both clinically and biochemically. The inflammatory markers should also be measured in the material collected from the respiratory tract, such as exhaled air condensate or bronchoalveolar lavage, both during stable periods and exacerbations.

Conclusions  In conclusion, simvastatin treatment in patients with stable COPD had no statistically significant influence on the inflammatory markers measured during the observation. A trend towards a decrease in CRP level was observed in the statin group, particularly in a subgroup with mild and moderate COPD and cardiovascular diseases enhanced was relatively small and heterogeneous, and the follow-up was short. The inflammatory processes involved in this disease as well as other potential mechanisms of action of these drugs. Thus, further prospective studies with a longer follow-up should be planned to assess the action of statins both clinically and biochemically. The inflammatory markers should also be measured in the material collected from the respiratory tract, such as exhaled air condensate or bronchoalveolar lavage, both during stable periods and exacerbations.

Acknowledgements  We would like to thank Professor Anetta Undas for valuable comments. This work was supported by the grant of the Polish Ministry of Science no. N40201332/0227 (K.S.).

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ARTYKUŁ ORYGINALNY

Wpływ simwastatyny na wybrane parametry stanu zapalnego u chorych z przewlekłą obturacyjną chorobą płuc

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STRESZCZENIE
WSTĘP Istnieją doniesienia o związku przewlekłej obturacyjnej choroby płuc (POChP) z rozwojem chorób układu krążenia. Simwastatyna to lek hipolipemizujący o udowodnionym działaniu w prewencji chorób układu sercowo-naczyniowego. W badaniach obserwacyjnych stwierdzono, że statyny mogą zmniejszać śmiertelność z powodu POChP, a w badaniach eksperymentalnych na zwierzętach wykazano działanie przeciwnapalne w tkance płucnej.

CELE Celem pracy była ocena wpływu simwastatyny na wybrane markery zapalne mierzone we krwi u chorych na POChP.

PACJENCI I METODY Do badania włączono 56 osób (w wieku 44–80 lat), ze stabilną POChP (średnia natężona objętość wydechowa pierwszosekundowa [forced expiratory volume in 1 second – FEV1] wynosiła 55%). Pacjentów podzielono losowo na grupę badaną otrzymującą simwastatynę 40 mg/d oraz grupę kontrolną nieotrzymującą statyny. Próbki krwi pobierano wyjściowo, po 2 tygodniach i 3 miesiącach od rozpoczęcia terapii simwastatyną. Oceniano stężenia fibrynogenu, białka C-reaktywnego (C-reactive protein – CRP), czynnika martwicy guza α (TNF-α), interleukiny-6 (IL-6) i metalloproteinazy macierzy zewnątrzkomórkowej-9 (matrix metalloproteinase-9 – MMP-9).

WYNIKI Obie grupy nie różniły się pod względem danych demograficznych, objawów klinicznych, stosowanego leczenia, parametrów spirometrycznych oraz profilu lipidowego przed leczeniem. Z chórów współistniejących jedynie nadciśnienie tętnicze występowało częściej w grupie otrzymującej simwastatynę (32,1% vs 17,9%, P = 0,03). Po 2 tygodniach oraz po 3 miesiącach stosowania simwastatyny nie zaobserwowano istotnego zmniejszenia stężeń badanych parametrów stanu zapalnego. W podgrupie chorych z FEV1 >50% zaobserwowano jednak nieznaczne zmniejszenie stężeń CRP i IL-6 w trakcie stosowania simwastatyny. Stwierdzono zmniejszenie stężenia całkowitego cholesterolu (z 5,68 do 4,71 mmol/l; P = 0,0018) oraz cholesterolu lipoprotein o małej gęstości (z 3,46 do 2,47 mmol/l; P = 0,000037) w grupie leczonej statyną.

WNIOSKI U chorych na POChP 3-miesięczna terapia simwastatyną nie prowadzi do zmniejszenia stężeń krążących we krwi parametrów stanu zapalnego.