Assessment of remodeling in chronic obstructive pulmonary disease using imaging methods

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INTRODUCTION
Chronic obstructive pulmonary disease (COPD) is characterized by progressive and not fully reversible airflow limitation in the airway. 1, 2 It results from an abnormal inflammatory reaction of the respiratory tract to harmful substances and dust, predominantly tobacco smoke, and leads to different degrees of airway obstruction. 3 In COPD, like in bronchial asthma, remodeling of the bronchial wall is observed; however, the remodeling itself differs significantly between these 2 diseases. 4, 5 In COPD, thickening of the basement membrane is minimal, while hypertrophy of the mucosal glands is prominent. 5 Epithelial damage, smooth muscle hypertrophy and hyperplasia, angiogenesis, and deposition of elastin and proteoglycans are more pronounced in asthma than in COPD. 6 Most data regarding remodeling in COPD are derived from studies of histopathological specimens obtained during bronchoscopy or after surgical lung resection. 8, 9

KEY WORDS
chronic obstructive pulmonary disease, computed tomography, endobronchial ultrasound, remodeling

ABSTRACT
INTRODUCTION
While spirometry plays a key role in diagnosing chronic obstructive pulmonary disease (COPD), imaging methods including endobronchial ultrasound (EBUS) and chest computed tomography (CT) appear to be useful for investigating structural changes in the lungs.

OBJECTIVES
The aim of this study was to evaluate remodeling in COPD patients using EBUS and chest CT.

PATIENTS AND METHODS
The study included 33 patients with COPD, 15 patients with severe asthma, and 15 control subjects. All subjects underwent pulmonary function tests and bronchoscopy with EBUS to measure the total thickness of the bronchial wall and its layers. Additionally, in COPD patients, a chest CT was performed to measure total bronchial wall thickness.

RESULTS
The total bronchial wall thickness measured by EBUS in patients with COPD (1.192 ±0.079 mm) was significantly smaller than that in asthmatic patients (1.433 ±0.230 mm, \( P = 0.001 \)) and significantly greater than in control subjects (1.099 ±0.095 mm, \( P = 0.04 \)), and was positively correlated with residual volume (RV) / total lung capacity (\( r = 0.5, P = 0.02 \)), RV (\( r = 0.6, P = 0.007 \)), and RV (%) (\( r = 0.5, P = 0.05 \)). The thickness of the bronchial wall layers in patients with COPD were as follows: \( L_1 = 0.135 ±0.018 \) mm, \( L_2 = 0.151 ±0.026 \) mm, and \( L_3–5 = 0.906 ±0.065 \) mm. There was no correlation between the thickness of the bronchial wall layers and forced expiratory volume in 1 second.

CONCLUSIONS
The results of this study show that EBUS is a useful method for evaluating bronchial wall layers not only in asthma but also in COPD, and suggest that the pattern of remodeling differs in each of these diseases.
However, imaging methods such as endobronchial ultrasound (EBUS) and computed tomography (CT) of the chest are emerging as possible alternative techniques for assessing remodeling in these patients. EBUS allows to distinguish 5 layers of the bronchial wall in the trachea and cartilaginous bronchi. Our previous study performed in asthmatics showed the utility of EBUS for the assessment of bronchial wall remodeling. It has been shown that bronchial wall thickness correlates with disease severity. In a study by Kita et al., the relationship between airway wall structure and bronchial hyperresponsiveness in asthmatic patients was evaluated. Percentage wall thickness measured by EBUS and the thickness of the second layer were significantly greater in asthmatic patients when compared with nonasthmatic subjects. The provocative concentration of methacholine causing the forced expiratory volume in 1 second (FEV1) to drop by 20% or more (PC20) was negatively correlated with the thickness of the second layer.

The aim of this study was to evaluate remodeling in COPD based on EBUS and chest CT.

PATIENTS AND METHODS The study included 33 patients with COPD (9 women, 24 men; mean age, 66.8 ±8.8 years; mean FEV1, 57.8% ±22.3%), 15 control subjects (4 women, 11 men; mean age, 46.1 ±11.9 years; mean FEV1, 105.0% ±14.6%), and 15 patients with severe asthma (12 women, 3 men; mean age, 48.1 ±12.2 years; mean FEV1, 63.3% ±21.6%). All patients were enrolled in the study between September 2010 and July 2013.

The diagnosis of COPD and asthma was established according to the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and Global Initiative for Asthma (GINA), respectively. In the control group, bronchofiberoscopy was performed to diagnose hemoptysis and persistent cough, or to exclude a neoplastic process. Our study was a prospective controlled trial. The protocol was approved by the Bioethics Committee of Jagiellonian University (Kraków, Poland). Informed consent was obtained from all patients.

In all patients, spirometry (Jaeger MasterLab, Jaeger-Toennies GmbH, Höchberg, Germany) was performed before and after a short-acting β2-agonist was administered to assess bronchial reversibility. Each patient with COPD had a body plethysmography (Jaeger Master Screen PFT, Höchberg, Germany) performed to measure total lung capacity (TLC), residual volume (RV), and RV/TLC, and a chest CT to measure total bronchial wall thickness in the posterior basal segment (B10).

All tomographic examinations were performed with a 64-row multidetector computed tomography scanner (Aquilion TSX-101A, Toshiba Medical Systems Corporation, Japan) in the helical scanning mode, without an intravenous administration of a contrast medium. After taking a deep breath by a patient, the scanning was performed in a caudocranial direction. CT parameters were as follows: collimation of 64 × 0.5, helical pitch of 53, and 0.5-second per rotation with a standard radiation dose (150 ±50 mAs and 120 kVp).

A free open-source software (Slicer 3D, Boston, Massachusetts, United States) was used to measure the total bronchial wall thickness in B10, and 1-mm images were obtained with a high-resolution reconstruction algorithm that were subsequently analyzed with a lung window setting with a width of 1500 Hounsfield units (HU) and a level of –500 HU. A semiautomatic method was used in which an observer chose an appropriate bronchus, and the bronchial wall thickness was measured by a computer. To detect the bronchial wall, we chose a recommended method of phase congruency. It is believed that this method is more accurate than the traditionally used “full-width at half-max” method in detecting bronchial boundaries, and especially in differentiating the bronchial wall from adjacent vessels.

Finally, bronchoscopy with EBUS was performed in all patients to measure bronchial wall thickness and its layers. Bronchofiberoscopy was performed under local anaesthesia (lidocaine, 2%) and mild sedation with fentanyl (0.05 to 0.1 mg IV) and midazolam (2.5 to 5 mg IV). Ultrasonography was performed with a bronchofiberscope (BF-1T180; Olympus; Tokyo, Japan), a 20-MHz ultrasonographic probe, and a processor (EU-ME1, Olympus, Tokyo, Japan). The ultrasound examination prolonged bronchoscopy by approximately 4 minutes. No complications after bronchoscopy with EBUS were observed. The ultrasound probe was placed in the lumen of segment 10 of the right bronchus (B10). Our method allowed us to discriminate 5 bronchial wall layers in COPD. Similarly to our previous study, we analyzed the inner layers of the bronchial wall (layer 1 [L1] and layer 2 [L2]) separately, including the mucosa, submucosa, and smooth muscle, while the outer layers (layers 3 to 5 [L3–5]) that corresponded to cartilage were analyzed together (Figures 1 and 2).

The images selected from the video recorded during bronchoscopy were saved as bitmaps. Subsequently, the selected digital sequences of frames were imported to a dedicated software (Feature Extraction Software [FES], AGH, Kraków, Poland) for further analysis. FES was designed to process images, especially to convert data from the raster to vector format using the subpixel precision method. The FES software allowed us to measure the distance between 2 points on an image and convert it to millimeters. The borders of the layers were marked manually.

An independent researcher chose 5 frames on which the laminar structure of the bronchial wall was best visualized. The 5 measurements of each layer (L1, L2, L3–5) were performed, and the mean values were treated as the final result.

All calculations were performed with a StatSoft, Inc. (2011) STATISTICA 10 data analysis software system. Categorical variables were presented as numbers and percentages. Continuous
The mean age of patients with COPD was significantly higher than in patients with asthma and controls (P < 0.001). As regards sex distribution, men predominated in the COPD group (72.7%), while women predominated in the severe-asthma group (80%), which reflects the distribution in the general population. In COPD patients, the mean duration of the disease was 9.3 ±7.8 years and the mean pack-years was 39.5 ±31.1. The proportion of smokers was significantly greater in the COPD group (97%) than in the severe-asthma group (13.3%, P <0.001). There was no significant difference in the number of patients with COPD using inhaled corticosteroids compared with those with severe asthma (90.9% vs 100%, P = 0.576). The mean daily dose of fluticasone was comparable between patients with COPD and those with severe asthma (738.3 ±397.9 µg and 924 ±280.7 µg, respectively, P = 0.105). Additionally, 11 patients with COPD (33.3%) and 8 patients with severe asthma (53.3%, P = 0.32) were taking oral corticosteroids. There was no significant difference between the mean daily dose of methylprednisolone in patients with COPD and those with severe asthma (8.4 ±4.2 mg and 7.5 ±4 mg, respectively, P = 0.657).

The analysis of the EBUS and CT measurements did not show significant differences (P = 0.1), although the total bronchial wall thickness assessed using EBUS was slightly greater than that assessed with CT (1.192 ±0.079 mm vs 1.173 ±0.064 mm).

In our study, the total bronchial wall thickness in patients with COPD (1.192 ±0.079 mm) was significantly smaller than in patients with severe asthma (1.433 ±0.230 mm, P = 0.001) and significantly greater than in control subjects (1.099 ±0.095 mm, P = 0.04). Total bronchial wall thickness in patients with COPD positively correlated with RV (r = 0.6, P = 0.007), RV (%) (r = 0.5, P = 0.05), and RV/TLC (r = 0.5, P = 0.02), as measured with EBUS. Also in chest CT, total bronchial wall thickness positively correlated with RV (r = 0.5, P = 0.04).

We also used the general linear model for response variables, including qualitative and quantitative variables (covariates) and their interactions. This approach allowed us to eliminate the effect of age and sex differences among the studied groups on the results of the statistical analysis. The correlations between variables were estimated with the Spearman rank-order correlation. A P value of less than 0.05 was considered statistically significant.

RESULTS  The study was performed in 35 patients with COPD; however, only 33 patients were included in the final analysis because the ultrasound images for 2 patients were not adequate for a reliable assessment of the bronchial wall. Bronchoscopy with EBUS was additionally performed in 15 control subjects and 15 patients with severe asthma. The severity of COPD and severe asthma was comparable (P = 0.8). The FEV₁ values in these 2 groups were statistically lower than in control subjects (P <0.001). The mean age of patients with COPD was significantly higher than in patients with asthma and controls (P <0.001). As regards sex distribution, men predominated in the COPD group (72.7%), while women predominated in the severe-asthma group (80%), which reflects the distribution in the general population. In COPD patients, the mean duration of the disease was 9.3 ±7.8 years and the mean pack-years was 39.5 ±31.1. The proportion of smokers was significantly greater in the COPD group (97%) than in the severe-asthma group (13.3%, P <0.001). There was no significant difference in the number of patients with COPD using inhaled corticosteroids compared with those with severe asthma (90.9% vs 100%, P = 0.576). The mean daily dose of fluticasone was comparable between patients with COPD and those with severe asthma (738.3 ±397.9 µg and 924 ±280.7 µg, respectively, P = 0.105). Additionally, 11 patients with COPD (33.3%) and 8 patients with severe asthma (53.3%, P = 0.32) were taking oral corticosteroids. There was no significant difference between the mean daily dose of methylprednisolone in patients with COPD and those with severe asthma (8.4 ±4.2 mg and 7.5 ±4 mg, respectively, P = 0.657).

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- L₁ = 0.135 ±0.018 mm
- L₂ = 0.151 ±0.026 mm
- L₃–5 = 0.906 ±0.065 mm

The thickness of L₁ and L₂ in patients with COPD was significantly smaller than that in asthmatic patients (P = 0.05 and P = 0.003, respectively) and comparable to control subjects (FIGURES 3 and 4). The thickness of L₃–5, corresponding to cartilage, was significantly greater in patients with severe asthma than in patients with COPD (P = 0.002) and control subjects (P <0.001). The difference between the thickness
**FIGURE 3** Thickness of L₁ in patients with chronic obstructive pulmonary disease (COPD), patients with severe asthma, and controls.

**FIGURE 4** Thickness of L₂ in patients with chronic obstructive pulmonary disease (COPD), patients with severe asthma, and controls.
of \( L_{25} \) in patients with COPD and in control subjects showed borderline significance \((P = 0.06)\). There was no correlation between bronchial wall layers and FEV1.

**DISCUSSION** COPD is a heterogeneous disease characterized by pathological changes in distal and proximal bronchi, lung parenchyma, and bronchial vessels.\(^{17,18}\)

The diagnosis of COPD according to the GOLD guidelines is established on the basis of spirometry but this approach has some limitations, particularly when it comes to early diagnosis.\(^{19}\) Thus, there is an ongoing search for new methods that would enable a more precise evaluation of the severity of obstructive diseases. An interesting alternative seems to be the use of imaging methods, notably chest CT.\(^{20-22}\)

So far, none of the imaging methods have enabled a direct evaluation of small bronchi in COPD. An indirect method is the evaluation of air trapping in CT scans. However, in COPD, the evaluation of air trapping and its clinical impact is limited due to the coexistence of emphysema, unlike in asthma.\(^{23}\)

Our study was the first attempt to use the ultrasound method to assess total bronchial wall thickness and its particular layers in COPD. Earlier, we showed the utility of EBUS in the assessment of remodeling in asthma.\(^{24}\) In patients with COPD, bronchial wall thickness was significantly smaller than in asthmatics and significantly greater than in controls. A similar tendency was observed in a study by Kościuch et al\(^{25}\) using chest CT. Also, Shimizu et al,\(^{26}\) who tested groups of patients with comparable severity of lung disorders, identified significantly thicker bronchial walls at the third to fifth generation in patients with asthma as compared with patients with COPD and the control group. Bronchial wall thickness in COPD patients and in the control group did not differ significantly.

In patients with COPD, there was no correlation between total bronchial wall thickness and FEV1. However, other studies yielded different results. Nakano et al\(^{27}\) reported that a decreased FEV1 (% predicted) was associated with an increased airway wall area. Also, the study of Patel et al\(^{28}\) showed that FEV1 (% predicted) was independently associated with airway wall thickness at a lumen perimeter of 10 and 20 mm.

In our study, total bronchial wall thickness in EBUS positively correlated with RV and RV (%), which are higher in patients with COPD. Also, a positive correlation was observed between total bronchial wall thickness in EBUS and the RV/TLC ratio, which is an indicator of hyperinflation. Our findings are confirmed by the study of Nakano et al,\(^{29}\) who showed that airway thickening expressed as wall area percentage correlates with FEV1 and RV/TLC, but not with diffusing capacity of the lung for carbon monoxide.

In our study, bronchial wall layers were assessed in patients with COPD for the first time.

The thickness of \( L_{25} \) and \( L_3 \) in patients with COPD was significantly smaller than in patients with severe asthma and did not differ when compared to control subjects. The thickness of \( L_{25} \), corresponding to cartilage, was significantly greater in patients with severe asthma than in patients with COPD and the control group. No correlation between bronchial wall layers and FEV1, was found, contrary to the results in asthmatic patients.

Our study has several limitations. Firstly, the study was limited to measuring the bronchial wall thickness in segmental and subsegmental airways (greater than 2 mm) rather than in small bronchi. However, Nakano et al\(^{26}\) showed that bronchial wall thickening observed in CT closely correlates with small airway dimensions in histological specimens and thus may indirectly indicate small airway disease. Secondly, the number of patients enrolled in the study was small, but this was due to the lack of consent for CT with high radiation exposure or for invasive techniques such as bronchoscopy with EBUS.

In conclusion, the results of our study showed for the first time that EBUS can be used to evaluate remodeling in COPD. A smaller total thickness of the bronchial wall and its layers and a lack of correlation with FEV1 was found in COPD patients, which is in contrast with the results observed earlier in asthmatics. This discrepancy confirms there is a difference in the pattern of remodeling that occurs in these obstructive diseases.

**Contribution statement** JS and KS conceived the idea for the study and contributed to the design of the research project. IGS, KG, ŁK, AA, GP, and MR were involved in data acquisition. JS and KS performed the bronchoscopy with EBUS. SM measured the total bronchial wall thickness and its layers from the images obtained during bronchoscopy. PL measured the total bronchial wall thickness in chest CT scans. AC analyzed the data. All authors edited and approved the final version of the manuscript.

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Ocena remodelingu w przewlekłej obturacyjnej chorobie płuc przy wykorzystaniu metod obrazowych

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SŁOWA KLUCZE
przebudowa ściany oskrzeli, przewlekła obturacyjna choroba płuc, tomografia komputerowa, ultrasonografia wewnątrzoskrzelowa

STRESZCZENIE

WPROWADZENIE
Spirometria odgrywa kluczową rolę w diagnostyce przewlekłej obturacyjnej choroby płuc (POChP), podczas gdy metody obrazowe, w tym ultrasonografia wewnątrzoskrzeloowa (endobronchial ultrasound – EBUS) i tomografia komputerowa (TK) klatki piersiowej, wydają się przydatne w badaniu zmian strukturalnych w płucach.

CELE
Celem badania była ocena przebudowy ściany oskrzeli (remodeling) u chorych na POChP w oparciu o EBUS i TK klatki piersiowej.

PACJENTI I METODY
Do badania włączono 33 chorych na POChP, 15 pacjentów z ciężką astmą i 15 osób z grupy kontrolnej. U wszystkich chorych zostały wykonane badania czynności płuc oraz bronchoskopia z EBUS w celu pomiaru całkowitej grubości ściany oskrzeli i jej poszczególnych warstw. Dodatkowo u chorych na POChP została wykonana TK klatki piersiowej z pomiarem całkowitej grubości ściany oskrzeli.

WYNiki
Całkowita grubość ściany oskrzeli oceniana w badaniu EBUS była znacznie mniejsza u chorych na POChP (1,192 ±0,079 mm) niż u pacjentów z astmą (1,433 ±0,230 mm; p = 0,001) i znacznie większa niż w grupie kontrolnej (1,099 ±0,095 mm; p = 0,04) oraz dodatnio korelowała ze stosunkiem objętości zalegającej (residual volume – RV) do całkowitej pojemności płuc (r = 0,6; p = 0,02), RV (r = 0,6; p = 0,007) i RV (%) (r = 0,5; p = 0,05). U chorych na POChP grubość warstw ściany oskrzeli wyniosła: L\textsubscript{1} = 0,135 ±0,018 mm, L\textsubscript{2} = 0,151 ±0,026 mm, L\textsubscript{3–5} = 0,906 ±0,065 mm. Nie stwierdzono korelacji pomiędzy grubością warstw ściany oskrzeli a natężoną objętością wydechową pierwszosekundową.

WNIOSKI
Wyniki badania wykazały, że EBUS jest przydatną metodą w ocenie warstw ściany oskrzeli nie tylko u chorych na astmę oskrzelową, ale także na POChP, i sugeruje odmienny charakter przebudowy ściany oskrzeli w obu chorobach.