ORIGINAL RESEARCH

Increased brachial intima-media thickness is associated with circulating levels of asymmetric dimethylarginine in patients with COPD

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Background: Chronic obstructive pulmonary disease (COPD) is associated with an increased cardiovascular risk. However, the mechanisms for this association are yet unclear. The aim of this study was to investigate the relationship between brachial intima-media thickness (B-IMT), an independent predictor of cardiovascular risk, systemic inflammation, and asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, in patients with COPD and respective controls.

Methods: The study sample consisted of 60 patients with stable COPD, free from overt cardiovascular disorders, as well as 20 smoking and 20 nonsmoking controls. Ultrasound assessment of B-IMT, spirometry, venous blood sampling for quantification of inflammatory markers and ADMA levels were carried out, and individual cardiovascular risk was calculated via the Framingham risk score.

Results: Patients with COPD showed significantly higher B-IMT compared to smoking (P=0.007) and nonsmoking controls (P=0.033). COPD patients with elevated B-IMT had a twofold increased calculated 10-year risk for cardiovascular events compared to those below the recommended cutoff (P=0.002). B-IMT was significantly associated with systemic inflammation (interleukin-6 [IL-6]; r=0.365, P=0.006) and ADMA (r=0.331, P=0.013) in COPD. Multivariate linear regression revealed male sex and ADMA as independent predictors of B-IMT in this study sample.

Conclusion: B-IMT is significantly increased in patients with COPD and is associated with systemic inflammation and ADMA levels.

Keywords: cardiovascular risk, chronic obstructive pulmonary disease, comorbidity, subclinical atherosclerosis, biomarker

Introduction

Chronic obstructive pulmonary disease (COPD) is associated with a two- to threefold increased risk of cardiovascular events.[1,2] In fact, autopsy studies revealed cardiovascular disease (CVD) as the most frequent, but undetected and potentially modifiable, cause of death in patients with COPD.[3] The underlying mechanisms for the relationship between COPD and CVD exceed the effects of traditional risk factors and remain largely unknown.

A long latency period between disease initiation and clinical manifestation allows the identification of subjects at risk at a subclinical, potentially reversible stage of CVD. For this purpose, several markers of subclinical atherosclerosis have been identified as predictors of cardiovascular risk.[4–6] Intima-media thickness of the brachial artery (B-IMT)[7] is a novel and easily accessible parameter for the quantification of

subclinical atherosclerosis. Recent studies validating markers of subclinical atherosclerosis against coronary angiography revealed B-IMT as an independent predictor of cardiovascular events.[8] In a 12-year follow-up study, B-IMT clearly outperformed the predictive power of flow-mediated dilation (FMD), the gold standard of noninvasive cardiovascular risk prediction via endothelial function.[9] B-IMT has not been studied in COPD so far.

Consequently, this study aimed to assess B-IMT in patients with COPD compared to healthy controls and to investigate potential associations between B-IMT and clinical as well as laboratory markers of COPD (ie, airflow limitation and systemic inflammation). In this context, the present analysis specifically aimed to assess the relationship between asymmetric dimethylarginine (ADMA) and COPD. ADMA is an endogenous inhibitor of nitric oxide synthase, providing a potential link between chronic inflammatory disorders and subclinical atherosclerosis.[10,11]

It was hypothesized that:

- B-IMT is significantly increased in patients with COPD compared to smoking and nonsmoking controls.
- B-IMT is significantly correlated with markers of systemic inflammation as well as ADMA levels in patients with COPD.

Materials and methods
Study sample

For this observational study, a sample of patients with COPD (n=60) previously recruited via the outpatient clinic database of the Otto Wagner Hospital, Vienna, was analyzed. Inclusion criteria comprised evidence of airflow limitation on spirometry according to current guidelines,[12] a smoking history of at least 20 pack-years and age >40 years. Subjects were excluded if they were on long-term oxygen therapy, had evidence of an acute exacerbation of COPD within the previous 4 months (requiring antibiotics, oral steroids, and/or hospitalization), or suffered from any other relevant lung disease. Other exclusion criteria comprised overt cardiovascular disorders (ie, coronary artery disease, chronic heart failure, cardiomyopathy, arterial hypertension, atrial fibrillation, and peripheral arterial disease), vasoactive medication, malignant diseases, diabetes mellitus, chronic renal failure, or chronic inflammatory conditions other than COPD. The control groups consisted of lifelong nonsmokers (n=20) as well as apparently healthy smokers (n=20). Exclusion criteria were the same as in the study group plus the absence of obstructive lung disease confirmed by clinical evaluation

and spirometry. Control subjects were recruited from the general population during a public awareness campaign on spirometry at the Otto Wagner Hospital.

The study was planned and conducted in accordance with the Declaration of Helsinki. The protocol was approved by the local ethics committee (Vienna City Council – EK-number: EK05-150-1205). Prior to enrollment, all the participants gave their written informed consent. Collected data were entirely anonymized. Data handling and reporting followed the STROBE-Statement (Strengthening the Reporting of Observational Studies in Epidemiology).[13]

Measurements
Ultrasound studies

Ultrasound studies of the brachial artery were conducted as previously reported.[8] Subjects were instructed to refrain from food, caffeine, tobacco, and vasoactive drugs for 4–6 h prior to the study visit. Measurements were performed at the antecubital fossa of the right arm after 10 min of rest with subjects placed in a supine position. Investigators blinded to clinical and spirometric results used high-resolution ultrasound (13 MHz) for depiction of the intimal and medial layer of the brachial artery in a longitudinal plane. Using a stereotactic device, the transducer position was fixed and images were stored at an adequate gain for subsequent offline analysis. B-IMT was assessed from the leading edge between lumen and intima to the leading edge between media and adventitia. A cutoff value ≥ 0.345 mm was regarded as elevated B-IMT[14] and was therefore used as a discriminator for cardiovascular risk using the Framingham risk score.[15] Lumen diameter was measured as the distance between the leading edge of the near and far intima–lumen interface. Finally, the cross-sectional intima-media area (B-IMA) was calculated following a previously described equation.[16]

Lung function testing

Lung function testing was performed according to the current guidelines[12] for the verification of COPD diagnosis, the classification of the GOLD stage, and the quantification of the distinct parameters of lung function (forced expiratory volume in 1 s [FEV_1], % predicted; forced vital capacity [FVC], % predicted; FEV_1/FVC ratio).

Physical examination

A physical examination was conducted in every participant for height, weight, heart rate, and arterial blood pressure measurement.

Laboratory analysis

Venous blood samples were collected and analyzed for inflammatory biomarkers (neutrophil count, interleukin [IL]-6) and levels of N-type pro-BNP (pro b-type natriuretic peptide). Circulating ADMA concentrations were measured via reversed-phase high-performance liquid chromatography following a previously described method.[17] The 10-year risk for cardiac events was calculated by means of the Framingham risk score[15] including the independent cardiovascular risk factors: age, sex, smoking history, arterial blood pressure, cholesterol (total and high-density lipoprotein [HDL]-cholesterol), and diabetes.

Data analysis

Variables of interest are expressed as counts (%) for categorical variables, as mean ± standard deviation or median and interquartile range for interval variables depending on the respective data distribution. Variables with non-normal distribution were logarithm transformed when possible. Between-group comparisons were performed using independent samples t-test or Mann–Whitney U-test depending on the respective data distribution. Univariate correlations were performed by means of Pearson's correlation coefficient or Kendall's tau depending on the respective data distribution. A multiple linear regression model was used to identify the strongest predictors of B-IMT. Through forward selection, the sequence of considered covariates was chosen by means of the highest increase in R^2. Variables remained in the model if they altered the coefficient by at least 10%. All calculations were two-tailed with a predefined significance level of $P < 0.05$. Analyses were performed using the Statistical Package for Social Sciences (SPSS [version 15 for Windows]; SPSS Inc., Chicago, IL, USA, 2006). Artwork was created using the GraphPad software, version 5 for Windows (GraphPad Inc., San Diego, CA, USA, 2005).

Results

Baseline characteristics

Sixty patients with stable COPD, free from overt cardiovascular disorders, were available for analysis. The sample of COPD patients comprised 32% GOLD stage II, 32% GOLD stage III, and 36% GOLD stage IV. The control groups comprised 20 apparently healthy smoking and nonsmoking subjects each. Clinical characteristics are listed in Table 1. The three groups were matched for age, sex, body mass

Table 1 Subject characteristics

Subject characteristics	COPD	Smokers	Never-smokers	P-value
Clinical characteristics				
Age, years	64±7.8	60±9.2	64±11.0	0.068[A], 0.881[B], 0.191[C]
Male sex, %	53	40	30	0.339[A], 0.083[B], 0.520[C]
Body mass index, kg/m^2	24±3.6	26±3.4	25±3.1	0.074[A], 0.406[B], 0.379[C]
Systolic blood pressure, mmHg	125±13	121±12	126±9.8	0.220[A], 0.649[B], 0.119[C]
Diastolic blood pressure, mmHg	76±6.6	73±8.5	75±5.5	0.223[A], 0.536[B], 0.598[C]
Heart rate, beats per minute	88±18	73±16	73±13	0.02[A], <0.001[B], 0.938[C]
Smoking history, pack-years	65±37	39±23	0±0	0.04[A], <0.001[B], <0.001[C]
Acute exacerbation rate, n/year	1.0 (0–1.6)			
Lung function testing				
FVC, % predicted	78±17	104±13	103±16	<0.001[A], <0.001[B], 0.880[C]
FEV$_1$, % predicted	41±18	99±12	101±16	<0.001[A], <0.001[B], 0.655[C]
FEV$_1$/FVC ratio	43±15	82±8.2	84±10	<0.001[A], <0.001[B], 0.503[C]
Laboratory parameters				
LDL/HDL ratio	1.6±0.80	1.7±0.72	1.4±0.69	0.543[A], 0.584[B], 0.269[C]
HbA1c, %	5.4±0.35	5.3±0.16	5.5±0.37	0.054[A], 0.946[B], 0.058[C]
Creatinine, mg/dL	0.8±0.19	0.82±0.20	0.81±0.18	0.415[A], 0.469[B], 0.923[C]
Neutrophils, ×10^3/mL	4.7±1.6	4.3±1.2	4.3±1.4	0.302[A], 0.208[B], 0.844[C]
Interleukin-6, pg/mL	2.7 (1.6–5.3)	2.2 (1.5–3.4)	0.97 (0.74–1.8)	0.051[A], <0.001[B], 0.123[C]
Cardiovascular parameters				
B-IMT, mm	0.37±0.08	0.32±0.06	0.33±0.06	0.007[A], 0.033[B], 0.559[C]
B-IMA, mm^2	4.7±1.4	3.9±1.2	3.9±1.3	0.027[A], 0.022[B], 0.910[C]
Pro-BNP, pg/mL	82 (47–161)	74 (45–166)	76 (36–177)	0.975[A], 0.771[B], 0.814[C]
ADMA, μmol/L	0.47 (0.42–0.54)	0.55 (0.52–0.62)	0.54 (0.43–0.59)	0.131[A], 0.817[B], 0.135[C]

Notes: P-values are not significant unless presented. [A]COPD versus smokers; [B]COPD versus nonsmokers; [C]nonsmokers versus smokers.
Abbreviations: COPD, chronic obstructive pulmonary disease; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin; B-IMT, brachial intima-media thickness; B-IMA, brachial intima-media area; Pro-BNP, pro b-type natriuretic peptide; ADMA, asymmetric dimethylarginine.

index, blood pressure, and lipid levels. There were significant differences in heart rate, smoking history, and lung function between patients with COPD and smoking, as well as non-smoking controls ($P<0.05$).

Brachial intima-media thickness

Results of B-IMT measurements for the different study groups are depicted in Figure 1. In patients with COPD, significantly higher B-IMT (0.37 ± 0.08 mm vs 0.32 ± 0.06 mm; $P=0.007$; vs 0.33 ± 0.06 mm; $P=0.033$) as well as B-IMA (4.7 ± 1.4 mm vs 3.9 ± 1.2 mm; $P=0.027$; vs 3.9 ± 1.3 mm; $P=0.022$) was observed compared to smoking and nonsmoking controls. Within the COPD group, male participants showed significantly higher B-IMT levels (0.41 ± 0.06 mm) than females (0.33 ± 0.07 mm) ($P<0.001$). Prediction of cardiovascular risk through the Framingham risk score revealed a twofold increased 10-year risk for cardiovascular events in COPD patients with elevated B-IMT (12 ± 7.6) compared to those with B-IMT levels below the recommended cutoff (5.9 ± 1.8) ($P=0.002$) (Figure 2). Univariate correlations between B-IMT and variables of interest are listed in Table 2.

Systemic Inflammation and ADMA

White blood cell count showed normal levels of neutrophils in the study sample without significant differences between COPD patients, smokers, and nonsmokers. IL-6 plasma levels were higher in patients with COPD compared to controls. There was a significant positive correlation between IL-6 plasma levels and B-IMT in patients with COPD (Figure 3).
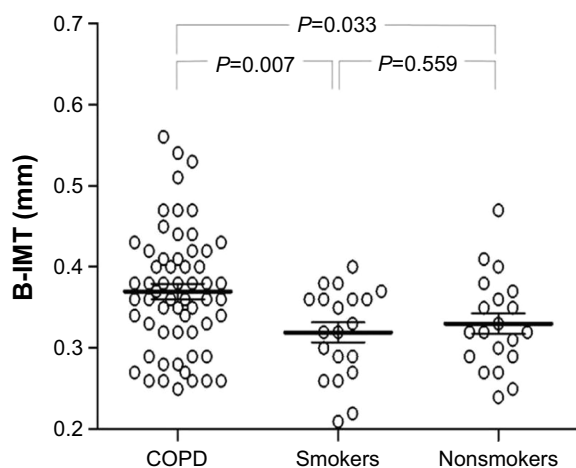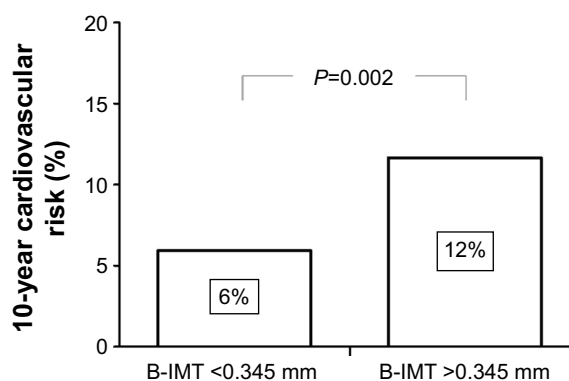
Figure 2 10-year risk for cardiovascular events expressed as Framingham risk score in COPD patients above (n=39) and below (n=21) the suggested reference value for B-IMT (0.345 mm).
Abbreviations: COPD, chronic obstructive pulmonary disease; B-IMT, brachial intima-media thickness.

There were no significant differences of circulating ADMA levels between the three study groups. In patients with COPD, B-IMT, however, was positively correlated with ADMA levels (Figure 4), an observation that was not observed in controls. In a multivariable linear regression model (Table 3) with B-IMT as the dependent variable, this study included age, male sex, systolic blood pressure, exacerbation rate, FEV_1/FVC ratio, Ln IL-6, Ln ADMA, and Ln pro-BNP as independent variables. Through forward selection, male sex, and ADMA remained independent predictors of B-IMT in the model.

Discussion

In the present study, significantly higher B-IMT was observed in patients with COPD compared to smoking and nonsmoking controls. Patients with elevated B-IMT had a twofold higher risk for future cardiovascular events calculated by means of the Framingham risk score. B-IMT was correlated with airflow limitation, systemic inflammation, and circulating levels of ADMA. The data support further evidence for an increased cardiovascular risk in patients with stable COPD, independent from traditional cardiovascular risk factors. Although ADMA plasma levels were not different between the groups, the correlation with B-IMT in COPD patients suggests an interaction with circulating ADMA levels as a determinant of subclinical atherosclerosis in COPD. Several markers of subclinical atherosclerosis were identified and previously validated in the general population. This is the first study to investigate B-IMT in patients with COPD free from overt cardiovascular disorders.

These findings support a previous study by Iwamoto et al, who assessed intima-media thickness of the carotid artery as a predictor of CVD risk in smokers with airflow limitation.[18]

Figure 1 B-IMT in patients with stable COPD (n=60) versus smoking (n=20) and nonsmoking controls (n=20).
Abbreviations: COPD, chronic obstructive pulmonary disease; B-IMT, brachial intima-media thickness.

Table 2 Univariate associations (Pearson's correlation coefficient) between brachial intima-media thickness and characteristics of patients with COPD and the complete sample

Subject characteristics	COPD		Smokers		Never-smokers	
	r-value	P-value	r-value	P-value	r-value	P-value
Age, years	0.421	<0.001	0.408	0.074	0.468	0.037
Body mass index, m/kg^2	0.131	0.321	−0.235	0.319	0.108	0.650
Smoking history, pack-years	0.046	0.732	0.331	0.154		
Systolic blood pressure, mmHg	0.314	0.016	−0.101	0.672	0.224	0.357
Acute exacerbation rate, n/year	0.251	0.014				
FEV$_1$, % predicted	−0.185	0.160	0.303	0.194	0.181	0.445
FVC, % predicted	0.034	0.799	0.355	0.125	0.094	0.693
FEV$_1$/FVC ratio	−0.286	0.028	0.115	0.631	−0.280	0.231
HbA1c, %	0.180	0.188	0.058	0.808	0.329	0.156
HDL/LDL ratio	0.047	0.69	0.376	0.151	0.440	0.115
Neutrophil count, ×10^3/mL	−0.056	0.681	0.018	0.943	0.278	0.235
Ln interleukin-6, pg/mL	0.365	0.006	0.229	0.346	−0.229	0.331
Ln pro-BNP, pg/mL	0.225	0.101	0.185	0.477	0.338	0.169
Ln ADMA, μmol/L	0.331	0.013	0.048	0.840	−0.283	0.226

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ln, natural logarithm; Pro-BNP, pro b-type natriuretic peptide; ADMA, asymmetric dimethylarginine.

The authors similarly observed a thickening of the arterial wall in COPD compared to healthy smoking and nonsmoking controls. Direct comparison of brachial versus carotid artery intima-media thickness in patients with coronary artery disease showed comparable diagnostic accuracy of both markers to detect diffuse coronary arteriosclerotic lesions.[19] Using the brachial artery, however, enables study of both structural and functional properties of systemic vasculature. Using brachial artery measurements of FMD, impaired endothelial function has been previously demonstrated in COPD compared to matched controls.[20–22] In a direct comparison, however, B-IMT appears to be a better and independent predictor of coronary artery disease when compared with FMD, in both cross-sectional[23] and prospective studies.[9]

Indeed, stratification of the COPD sample via a previously suggested cutoff[14] for B-IMT of 0.345 mm revealed a twofold higher risk of cardiac events in the Framingham risk score for COPD patients with elevated B-IMT compared to those below the recommended cutoff. The available literature suggests several potential mechanisms contributing to elevated subclinical atherosclerosis in COPD. In this study, male sex was identified as an independent predictor for B-IMT. This observation is consistent with findings from the epidemiological Gutenberg heart study.[24] In the absence of traditional cardiovascular risk factors, these findings suggest
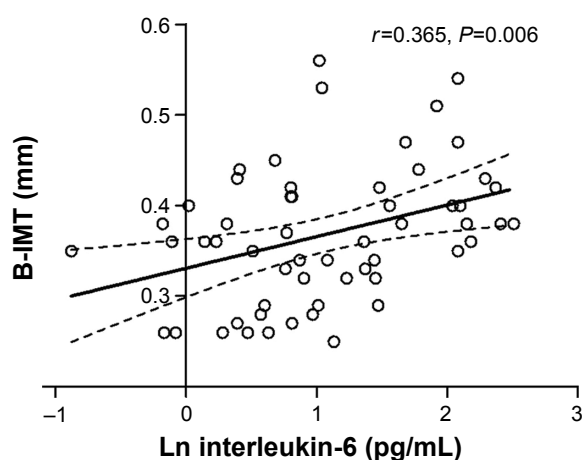
Figure 3 Linear regression analysis between B-IMT and interleukin-6 in patients with COPD (n=60).
Abbreviations: COPD, chronic obstructive pulmonary disease; B-IMT, brachial intima-media thickness; Ln, natural logarithm.
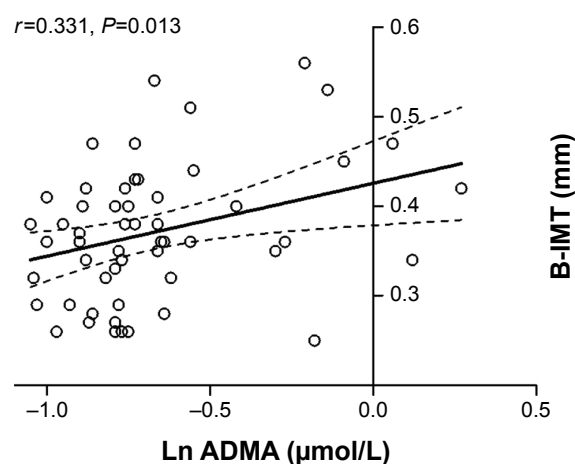
Figure 4 Linear regression analysis between ADMA and B-IMT in patients with COPD (n=60).
Abbreviations: COPD, chronic obstructive pulmonary disease; B-IMT, brachial intima-media thickness; Ln, natural logarithm; ADMA, asymmetric dimethylarginine.

Table 3 Independent predictors of brachial intima-media thickness in patients with COPD

Subject characteristics	β	Standardized β	P-value
Male sex	0.075	0.487	<0.001
Ln ADMA	0.084	0.311	0.011

Notes: Forward selection multiple linear regression including the following covariates: age, male sex, ln IL-6, ln ADMA, systolic blood pressure, FEV$_1$/FVC ratio, exacerbation rate, and ln pro-BNP. R^2=0.402.
Abbreviations: COPD, chronic obstructive pulmonary disease; Ln, natural logarithm; IL-6, interleukin-6; ADMA, asymmetric dimethylarginine; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; Pro-BNP, pro b-type natriuretic peptide.

potential vasoprotective effects of estrogen, a hypothesis that is supported by other studies.[25–27]

Tobacco smoke remains the major risk factor for both COPD and CVD. Patients with COPD, however, are at an increased risk of CVD, independent of smoking.[21,28,29] Indeed, analyses of the Framingham study identified airflow obstruction as an independent predictor of cardiac events, with an effect size stronger than traditional cardiovascular risk factors.[30,31] In line with these findings, the univariate analysis in this study showed an inverse correlation between B-IMT and FEV$_1$/FVC ratio in patients with COPD.

Both patients with airflow obstruction and those with CVD exhibit systemic inflammation.[28,32,33] Significantly higher levels of circulating IL-6 were observed in patients with COPD compared to controls. Importantly, IL-6 levels were strongly associated with B-IMT in univariate analysis. These observations are consistent with previous reports suggesting a link between systemic inflammation and subclinical atherosclerosis.[18,34] Systemic inflammation may drive the development of CVD via impairments of the nitric oxide pathway, a hypothesis that is supported by earlier findings of the relationship between circulating inflammatory markers and endothelial function in COPD.[21]

ADMA is an endogenous inhibitor of nitric oxide synthase, and implicated in the pathogenesis of CVD.[35] Indeed, a meta-analysis[10] of 22 original articles recently confirmed the relationship between ADMA and B-IMT. Furthermore, circulating ADMA levels predict clinical endpoints, such as myocardial infarction or stroke.[36,37] The lung has been identified as one of the most important sources of ADMA formation;[38] however, the role of ADMA in COPD remains unknown yet. Recent studies provide conflicting results, demonstrating either higher[39] or lower[40] circulating ADMA concentrations in patients with COPD compared with controls. Lower concentrations of ADMA were also found in smokers without COPD compared to nonsmokers.[41,42] In the present report, there were no statistically significant differences between COPD patients

and controls with respect to blood ADMA levels; however, a relationship of the latter with B-IMT exists exclusively in COPD. Thus, these findings may suggest a potential role for ADMA in the development of subclinical atherosclerosis in COPD, rather than a marker of lung function impairment as a consequence of smoking. The relationship between ADMA and impaired B-IMT in COPD in this report is novel and may extend earlier findings, providing a further potential interaction between structural, functional, and molecular properties of the systemic vasculature.

A number of potential limitations in the current study need to be acknowledged including but not limited to the small sample size and a highly selected patient population, free from overt CVDs. However, the present study aimed to investigate pathobiological associations by stringent exclusion of potentially confounding comorbid conditions. Nevertheless, it has to be admitted that the exclusion of coronary artery disease was based on patient surveys and medical history alone, as coronary angiography was not performed in this asymptomatic population. Finally, due to the observational nature of the study further data are needed to characterize the role of ADMA within the observed associations between intima-media thickness and cardiovascular risk in patients with COPD.

Conclusion

In conclusion, the present study reveals B-IMT as a promising and easily accessible tool for stratification of future cardiovascular risk in patients with COPD. Confirming previous observations, a significant association was found between subclinical atherosclerosis and systemic inflammation. Additionally, ADMA was characterized as a potential cofactor in the pathogenesis of COPD related cardiovascular comorbidity. These findings help in understanding the complex mechanisms linking COPD to the associated excess cardiovascular risk.

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on September 9, 2011, in Vienna, as a presentation with interim findings. The presentation's abstract was published in *Wien Klin Wochenschr* 2011;123(17–18):A31.

Author contributions

AV as the principal investigator is the guarantor for this study. He had full access to the data and will vouch for the integrity of the data analysis. He is responsible for conception of the study, analysis and interpretation of the data, and revision and approval of the final manuscript. MHU was responsible for data collection, analysis and interpretation of the data, drafting revision, and approval of the final manuscript. PE was responsible for patient recruitment, data collection, and revision and approval of the final manuscript. MW was responsible for laboratory analyses, interpretation and analysis of the data, and revision the final manuscript. GCF was responsible for analysis and interpretation of the data, and revision and approval of the final manuscript. OCB was responsible for analysis and interpretation of the data, revision and approval of the final manuscript, and provided support through the Ludwig Boltzmann Institute.

Disclosure

MHU, PE, OCB, and MW have nothing to disclose. GCF reports personal fees from GlaxoSmithKline, Boehringer Ingelheim, Astra Zeneca, and Menarini, during the conduct of the study. AV reports grants and personal fees from Boehringer Ingelheim, and personal fees from Novartis, Chiesi, GlaxoSmithKline, Pulmonx, PneumRx, and Olympus, outside the submitted work.

The authors report no other conflicts of interest in this work.

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