NON-INVASIVE OSCILLOMETRICALLY MEASURED AORTIC PULSE WAVE VELOCITY IN HEALTHY INDIVIDUALS AND COPD PATIENTS

Ph.D. Thesis

by

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2020

1. Introduction

The importance of subclinical target organ damage has been demonstrated in several studies, as part of cardiovascular (CV) risk assessment. Aortic stiffness, in particular aortic pulse wave velocity (PWVao), has become an increasingly recognized biomarker in CV risk assessment. Early onset of asymptomatic subclinical atherosclerosis commonly manifests at an early age, as shown by studies of young soldiers killed in both the Korean War and the Vietnam War. Carotid ultrasound can directly detect the presence and extent of asymptomatic carotid atherosclerosis, which plays a very important role in the recognition of the asymptomatic patients at risk of heart attack and stroke. Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death worldwide. Small airway obstruction, chronic inflammation, frequent exacerbation and progressive outcome are the main features of the disease. The arterial rigidity (arterial stiffness) became into focus of the interest of the experts in the field of cardiovascular prevention in the last two decades. Decreased compliance of the arterial wall can be identified with functional methods at the earliest stage of atherosclerosis before morphologic changes occurs. PWVao, one of the most characteristic variables of arterial stiffness is a strong predictor of adverse cardiovascular outcome including mortality, independently from the traditional risk factors. Measurement of arterial stiffness beyond routine cardiovascular risk assessment can help in reclassification of the risk of asymptomatic individuals. It can provide further benefit in COPD because it can help to confirm the occurrence of cardiovascular comorbidities at early stage of atherosclerosis and identify individuals at high risk.

2. Objectives

The aim of our study was to determine the subclinical atherosclerosis with a functional oscillometric technic in a healthy, middle-aged population in the association of asymptomatic carotid atherosclerosis and investigate the early cardiovascular risk at COPD patients which is a public health problem worldwide by detecting non-invasively determinable arterial stiffness parameters in inflammation.

2.1 The Measurement of Aortic Pulse Wave Velocity (PWVao) and Asymptomatic Carotid Atherosclerosis in a Middle-Aged, Healthy Population

1. Investigate the association between subclinical carotid atherosclerosis (ACA) and aortic pulse wave velocity in healthy individuals. What is the difference in stiffness parameters observed in the presence of ACA compared to ACA negative individuals?
2. Determine the independent markers of asymptomatic carotid atherosclerosis.
3. Determine the sensitivity, specificity and predictive values of PWVao for the detection of asymptomatic carotid atherosclerosis in healthy individuals.

2.2 The measurement of Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) and Arterial Stiffness parameters in Patients with COPD and healthy individuals.

1. Compare the inflammatory markers in COPD and healthy individuals and a new marker predicting both inflammation and atherosclerosis between two groups of suPAR levels to investigate the association between low-level inflammation in COPD and early atherosclerosis.
2. Determine the arterial stiffness parameters in COPD and healthy population and determine the differences between the two groups.
3. Measure the correlations of FEV1 loss and COPD symptom scores and plasma suPAR level.
4. Investigate the association between smoking and early atherosclerosis in the light of arterial stiffness parameters and inflammatory markers, suPAR levels in the groups of healthy individuals and healthy smokers.

3. The Measurement of Aortic Pulse Wave Velocity (PWVao) and Asymptomatic Carotid Atherosclerosis in a Middle-Aged, Healthy Population

3.1. Materials and methods

3.1.1. Materials

In this observational cross-sectional study, we measured a relatively big cohort, 781 subjects, (aged 57 ± 12 years) who attended our day care center of Heart Institute, Medical School, University of Pécs, Hungary by their own initiative for a cardiovascular check-up. The study has been approved by the local Institutional Ethics Committee of the University of Pécs, Hungary (PTE KK RIKEB - 5111/2013), and all participants gave their informed consent.

In this study 236 middle-aged, 30-60 years, healthy individuals were included, without any complaints or case history for coronary heart disease or stroke. Exclusion criteria of this cross-sectional study were: coronary heart disease, hypertension, atrial fibrillation, tachycardia (heart rate above 90/min), stroke, peripheral arterial disease, chronic renal disease, diabetes and statin use. Those patients who previously were diagnosed with coronary heart disease (e.g. positive case history, or who had experienced for angina pectoris or who had positive coronary angiography / positive coronary CT scan) were excluded. Hypertension was defined as systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥ 90 mmHg or current use of antihypertensive medication. Diabetes mellitus was defined as a current use of oral antidiabetic drugs, insulin, or self-report of the diagnosis. Chronic renal disease was excluded by anamnestic data and according to laboratory parameters (GFR lower than 60 ml/min/1.73m^2). Figure 1 displays the enrollment of participants into the study.

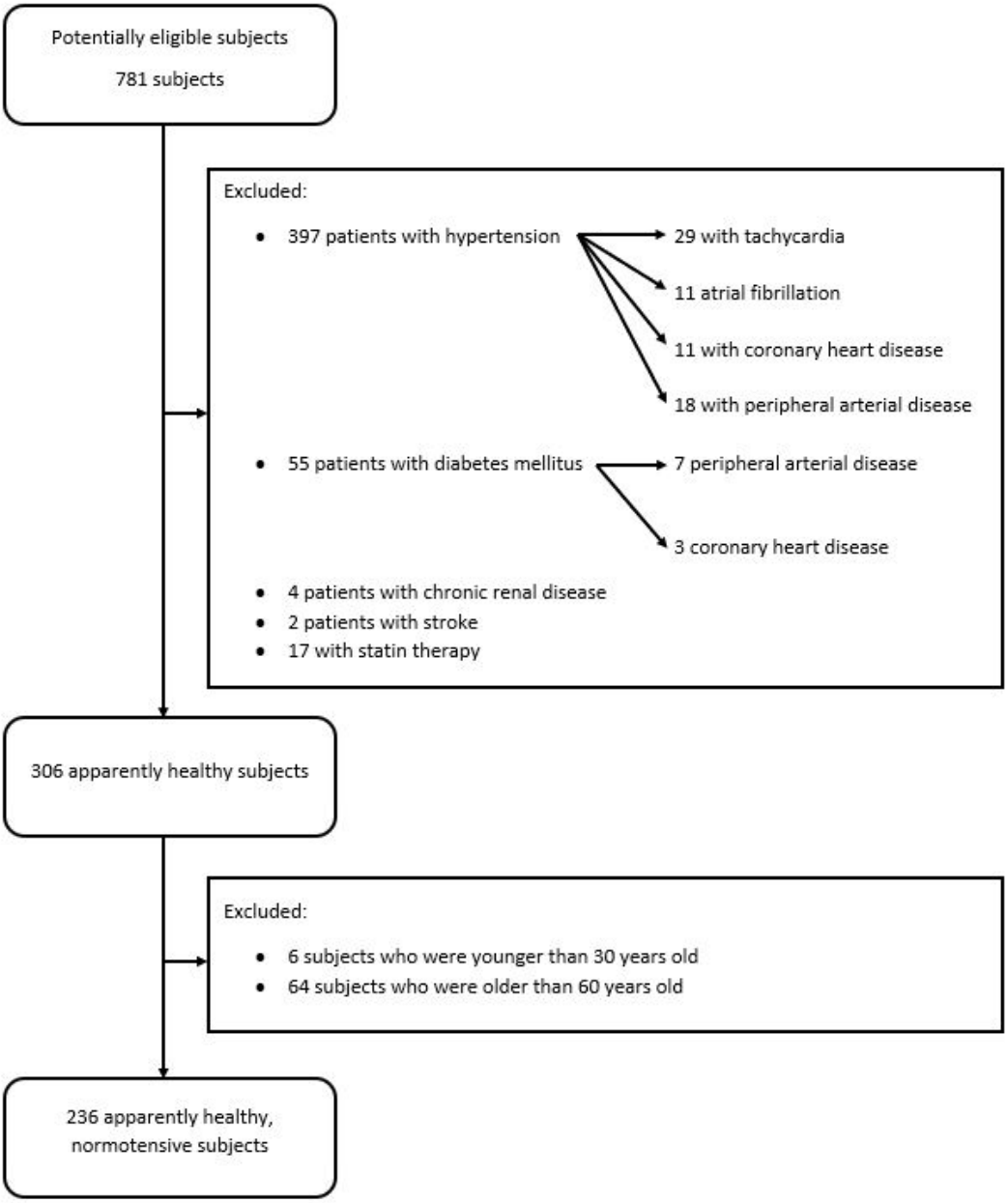

Figure 1. Flow chart of the number and selection of individuals in the study population

3.1.2. Detection of the arterial stiffness parameters

The simultaneous measurements of PWVao and blood pressure were performed in the supine position after 10 minutes of rest in a separate room at constant room temperature, by an invasively validated oscillometric, occlusive, non-invasive technique (Arteriograph, TensioMed Ltd., Budapest, Hungary). This method allows very simple, fast and user independent measurement of aortic PWV using only a single upper arm cuff. This upper arm cuff arteriography simultaneously detects systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) and aortic pulse wave velocity (PWVao) and return time (RT).

3.1.3. Carotid artery ultrasound examination

All carotid artery ultrasound examinations were performed by a single examiner using a HP Sonos 2000 device with 7.5 MHz linear probe (Hewlett Packard Ltd. Andover, Massachusetts, U.S.A.) on the same day and within 1 hour of the measurement of arterial stiffness. The whole extracranial carotid artery system was examined bilaterally, and we followed the American Society of Echocardiography guidelines regarding standard screening methods of carotid plaques. Asymptomatic carotid atherosclerosis (ACA) was defined according to the European Mannheim consensus. The presence of plaque in the carotid artery was defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness > 1.5 mm as measured from the media-adventitia interface to the intima lumen interface.

3.1.4. Statistical analysis

Data are reported as mean values ± SD. Group means of continuous variables were compared with independent samples Student's t–test, whereas groups of categorical variables were analyzed with the $\chi 2$ test. Stepwise logistic regression was used to define predictor variables for the binary outcome of the presence of ACA. Receiver operating characteristics (ROC) analysis was performed to assess threshold values for PWVao relative to ACA. A probability (p) <0.05 was considered to be significant. Data were analyzed using SPSS 16.0 statistical package (SPSS Inc., Chicago, Illinois, USA).

3.2. Results

236 asymptomatic normotensive subjects (age 47 ± 8 years; 52% women) comprised the study group. The participants' characteristics are presented in Table 1. ACA was present in 51 subjects (22%). There were no differences between subjects with and without ACA in gender, BMI, total cholesterol, or HR. However, age, smoking, SBP, and PWVao were higher in the ACA group compared to ACA negative subjects (50 ± 8 vs. 47 ± 8 years, p = 0.009; 29% vs. 8.3%, p < 0.001; 128 ± 9 mmHg vs. 125 ± 10 mmHg, p = 0.048; 9 ± 2 m/s vs. 8 ± 1 m/s, p < 0.001, respectively).

	N	All	Carotid atherosclerosis		P
			Negative	Positive	
N		236	185	51	
Age (years)	236	47 ± 8	47 ± 8	50 ± 8	0.009
Weight (kg)	236	76.0 ± 16.0	75.5 ± 15.7	77.9 ± 17.1	0.344
Height (cm)	236	172 ± 10	171 ± 9	173 ± 11	0.295
BMI (kg/m^2)	236	25.7 ± 4.1	25.6 ± 4.1	25.8 ± 3.9	0.735
Serum total cholesterol (mmol/l)	134	5.6 ± 1.3	5.6 ± 1.3	5.9 ± 1.5	0.240
SBP (mmHg)	236	125 ± 10	125 ± 10	128 ±9	0.048
DBP (mmHg)	236	75 ± 8	75 ± 8	76 ± 7	0.671
MAP (mmHg)	236	92 ± 7	92 ± 8	93 ± 7	0.228
PP (mmHg)	236	50 ± 8	50 ± 8	52 ± 8	0.049
HR (beats per min)	236	70 ± 9	70 ± 9	71 ± 10	0.380
PWVao (m/s)	236	8.2 ± 1.5	7.9 ± 1.3	9.3 ± 1.6	<0.001
Female / Male	236	122 / 114	98 / 87	24 / 27	0.45
Smoker / Nonsmoker	236	30 / 206	15 / 170 8.3%	15 / 36 29%	<0.001

Table 1. Descriptive data of the study population according to carotid artery atherosclerosis
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; PWVao, aortic pulse wave velocity. Values are presented as mean ± standard deviation.

Independent markers of asymptomatic atherosclerosis were determined using Stepwise logistic regression, as shown in Table 2.

	Odds ratio and 95% confidence limits for 1unit change	P
PWVao (m/s)	1.88 [1.44; 2.50]	<0.001
Smoker (yes/no)	3.79 [1.56; 9.22]	0.003
SBP (mmHg)	1.05 [1.001; 1.10]	0.046
DBP (mmHg)	0.94 [0.89; 0.99]	0.038

Table 2. Independent markers of asymptomatic carotid artery atherosclerosis by stepwise logistic regression analysis

A stepwise logistic regression including age, gender, body mass index, smoking habits, systolic and diastolic blood pressure, heart rate and PWVao showed aortic PWV, smoking, systolic and diastolic blood pressure to be significant independent markers.

PWVao, aortic pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure.

A stepwise logistic regression including age, gender, BMI, smoking habit, SBP and DBP, HR, and PWVao was used to reveal parameters independently related to ACA. PWVao, smoking habit, and SBP, DBP were independently associated to ACA (Table 2). Age was not an independent marker of ACA. Based on the calculated odds ratio (1.88) and the SD (1.6 m/s) of PWVao, 1 SD increase of PWVao almost duplicated (98%) the risk of ACA, supposing that other parameters remained unchanged.

A receiver operating characteristic (ROC) curve analysis was performed to determine the optimal threshold value of PWVao for detecting ACA. This value proved to be 8.3 m/s. (Figure 2). The area under the curve was 0.751 and the optimal PWVao threshold proved to be 8.3 m/s, with a sensitivity of 0.71 and a specificity of 0.65.

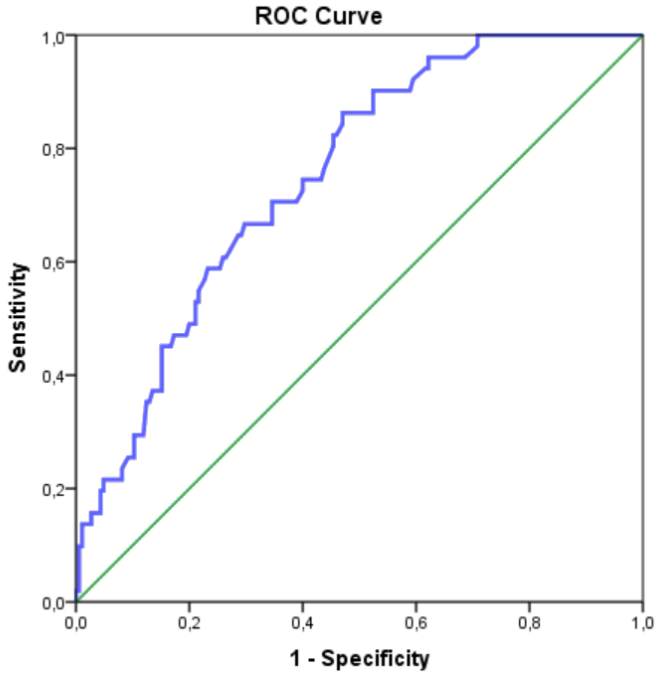

Figure 2. Receiver operating characteristic curve determining asymptomatic carotid atherosclerosis by measuring aortic pulse wave analysis

Using this cutoff value of PWVao, the sensitivity, specificity, positive and negative predictive values, relative risk, and odds ratio for ACA were 0.71, 0.65, 0.36, 0.89, 2.04 and 4.54, respectively. (Table 3).

The prevalence of asymptomatic carotid atherosclerosis is 22% in a young, apparently healthy population.

	Value	95% CI
Sensitivity	0.71	0.56 – 0.83
Specificity	0.65	0.58 – 0.72
Positive predictive value	0.36	0.27 – 0.46
Negative predictive value	0.89	0.82 – 0.94
Relative risk	2.04	1.56 – 2.66
Odds Ratio	4.54	2.31 – 8.91

Table 3. Sensitivity and specificity of using 8.3 m/s as a cutoff value of aortic pulse wave velocity to reveal asymptomatic carotid atherosclerosis.
CI, indicates confidence interval

3.3. Discussion

The most important finding of our study was that the oscillometrically, Arteriograph measured PWVao proved to be an independent marker of ACA. It is important to emphasize that age did not turn to be an independent factor of asymptomatic atherosclerosis in this

population. Although subjects with ACA were older compared to those without ACA, the difference (3.3 years) was modest. More importantly, the prevalence of ACA was 22% which is surprisingly high in this relatively young and apparently healthy population.

However, other authors also found similar ACA prevalence in almost identical population.

In our study, the age independency of PWVao to reveal ACA maybe of paramount importance since most of cardiovascular risk scores are age-driven. Consequently, many older people are considered to have high CV risk, despite of their normal cardiovascular status. However, subjects with low or moderate risk could be missed from the cardiovascular screening program because of their lower risk score driven by their younger age. Consequently, therapeutic intervention cannot be performed in time and fatal consequences may occur. However, several results confirmed that atherosclerotic process begins even at a very young age. Furthermore, it has to be emphasized that almost 60% of cardiovascular and cerebrovascular events occur among low-risk patients involving three-quarters of the population, where traditional risk factors do not identify the majority of patients who will develop cardiovascular disease in the next 10 years. Consequently, measuring PWVao oscillometrically in an apparently healthy, middle-aged population to find individuals with asymptomatic atherosclerosis, who will take advantage from early intervention and aggressive treatment, may have great clinical importance.

We found 71% sensitivity for ACA by using the PWVao threshold value of 8.3 m/s based on the ROC curve in this middle-aged, apparently healthy, normotensive population, which sensitivity seems to be acceptable. Our opinion could be supported by the fact that the sensitivity of the commonly used exercise ECG as a routine procedure to diagnose the presence of coronary artery disease in only 45-50% according to the 2013 ESC guidelines on the management of stable coronary artery disease. Consequently, based on the fast, user-independent, easy application of the Arteriograph, this higher sensitivity seems to be acceptable for screening of asymptomatic atherosclerosis assuming that atherosclerosis is the systemic disease of the intimal layer of middle and large arteries potentially having long latent subclinical phase and can affect the carotid arteries as well as the coronary arteries. The observed relatively low positive predictive value of 36% may result from the low prevalence of ACA positive cases in this study population. However, the high negative predictive value means that the majority of patients with PWVao lower than 8.3 m/s are unaffected from ACA. The relative risk and the odds ratio of the test proved to be 2.04 and 4.54, respectively, referring that the PWVao measurement seems to be a suitable screening tool for ACA.

The 8.3 m/s PWVao cut-off value for ACA in our study with middle-aged hypertensive patients was lower than it was recommended (10 m/s) by the ESC/ESH guidelines for the management of arterial hypertension as a marker of aortic stiffness and underlying arteriosclerosis. However, it must be emphasized that this higher cut-off value suggested by the Guidelines does not refer only to ACA.

The relatively low age of our study population may point out that asymptomatic carotid atherosclerosis can be present even when PWVao is moderately elevated. Consequently, the 10 m/s general cut-off value may not be used for all the patients of different ages to estimate cardiovascular outcomes.

Our results were obtained by applying an ease of use, oscillometric method for measuring PWVao, which simplifies the PWVao measurement in the routine clinical work. This might be particularly useful for improvement of cardiovascular risk factor assessment in

the primary care setting, where simplicity, user independency and swiftness are mandatory requirements.

4. The measurement of Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) and Arterial Stiffness parameters in Patients with COPD and healthy individuals.

4.1. Materials and Methods

4.1.1. Study subjects

A total of 42 middle aged individuals (n = 19 males, mean age: 59 ± 11 years), were included in the study. Figure 3 displays the enrollment of participants into the study.
COPD patients (n = 24) and 18 healthy control individuals were recruited at stable state at the outpatient clinic of the Department of Pulmonology, Semmelweis University. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria based on symptoms, suggestive history and postbronchodilator FEV1/ FVC < 0.70. Patients were categorized into ABCD subgroups according to the 2017 GOLD criteria. Symptom scores were determined (CAT, mMRC). Exacerbations in the last 12 months were defined as episodes requiring an increase in inhaler use or need for addition of antibiotics and/or systemic steroids. Frequent exacerbator phenotype was defined as having ≥ 2 exacerbations last year. None of the patients had suffered from acute exacerbation in the last 3 months. Control volunteers were recruited among co-workers at the Department of Pulmonology. Subjects with known cardiovascular disease, including coronary artery disease, cerebrovascular disease, or peripheral arterial disease and those who had diabetes mellitus were excluded. None of the study participants had an ongoing infection during the study. The study has been approved by the Semmelweis University Ethics Committee (TUKEB 131/2017), and all participants gave their informed consent.

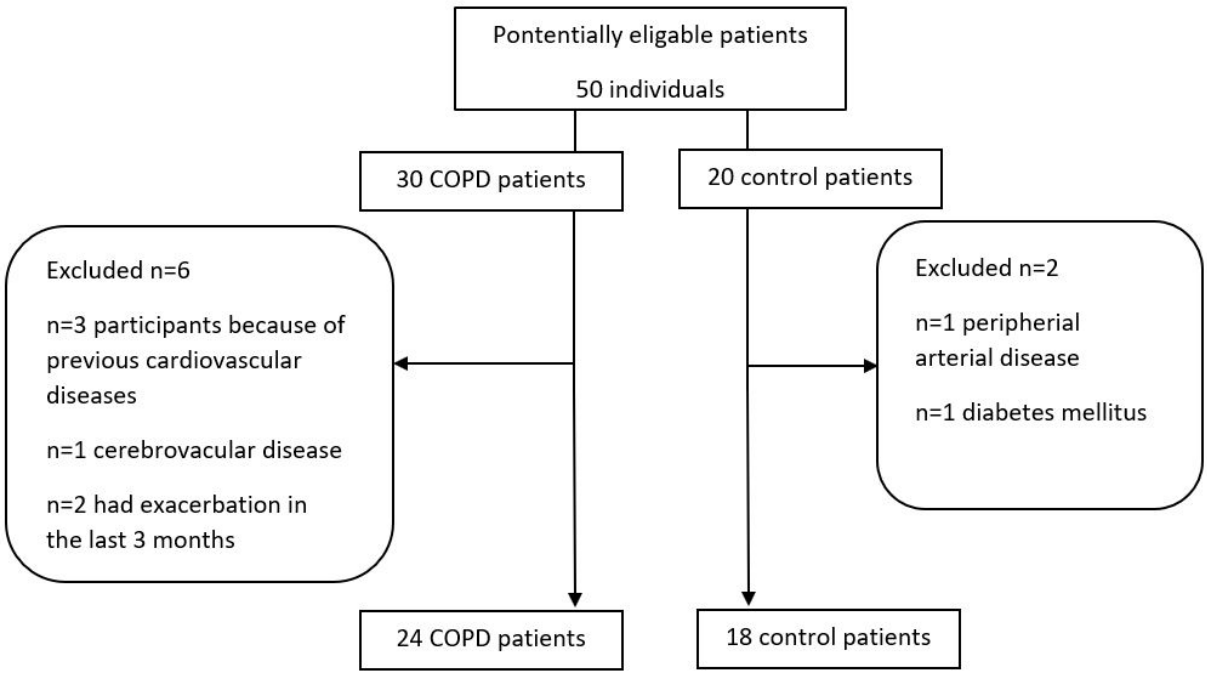

Figure 3. Flow chart of the number and selection of individuals in the study population.

4.1.2. Detection of arterial stiffness parameters and blood pressure

The simultaneous measurements of PWVao and blood pressure were performed in the supine position after 10 minutes of rest in a separate room at constant room temperature, by an invasively validated oscillometric, occlusive, non-invasive technique (Arteriograph, TensioMed Ltd., Budapest, Hungary). This method allows very simple, fast and user independent measurement of aortic PWV using only a single upper arm cuff. This upper arm cuff arteriography simultaneously detects systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), central arterial pressure (SBPao), aortic pulse wave velocity (PWVao), augmentation index (Aix) and return time (RT). We calculated the pressure amplification, that is the difference between the central and peripheral systolic pressure (SBPao–SBPbr).

4.1.3. Body Plethysmography

Lung function tests and body plethysmography were carried out with the PDD-301/s device (Piston Ltd., Budapest, Hungary) according to the American Thoracic Society guidelines. Lung function indices were calculated using the best of three technically acceptable measurements. We determined the patients FVC, FEV1, FEV1/FVC values and arterial blood gas test.

4.1.4. Circulating Biomarkers

Plasma was isolated from EDTA anticoagulated fasting blood samples and stored at − 80 °C until measurement. Plasma suPAR concentrations were measured with the suPARnostic Flex ELISA assay (ViroGates A/S, Birkerřd, Denmark) according to the manufacturers' instructions. Plasma endothelin-1 levels were determined with the Endothelin (1–21) ELISA Kit (Biomedica, Medizinprodukte GmbH & Co KG, Wien). The hsCRP levels were measured using commercially available tests (Roche Diagnostics GmbH, Mannheim, Germany). Interleukin-6 levels were analyzed by the Immulite 2000 immunoassay system (Siemens Healthcare GmbH, Erlangen, Germany).

4.1.4. Statistical Analysis

GraphPad Prism 5.03 (GraphPad Software, La Jolla, CA, US) was used for statistical analysis. Data normality has been assessed with the Shapiro–Wilk test. COPD and control groups were compared with un-paired *t* test, Mann–Whitney and Chi square tests. The relationships between plasma suPAR levels and clinical variables as well as circulating biomarkers were assessed with Pearson's and Spearman's tests. Data are expressed as mean ± standard deviation for parametric and median/range/for non-parametric variables. A p value < 0.05 was considered significant. The sample size was calculated to find a difference between COPD and control group with an effect size of 0.90, power of 0.80 and an alpha of 0.05. These numbers were based on a distribution of plasma suPAR values. Post hoc sensitivity analyses ensured it was possible to detect correlations between suPAR and clinical variables as well as other plasma biomarkers with an effect size of 0.54 (− 0.40 and 0.40, minimal and maximal critical r values), statistical power of 0.80 and alpha of 0.05.

4.2. Results

In our cross-sectional study, we enrolled 24 COPD patients and 18 control individuals in our study. Arterial stiffness parameters, plasma suPAR levels, endothelin-1, hsCRP, IL-6 levels, respiratory function parameters, smoking habits, exacerbations, and COPD symptoms scores (Medical Research Council Dyspnoea Scale: mMRC, COPD Assessment Test: CAT) were measured. Characteristics of the study population are presented in Table 4.

	COPD (n=24)	Controls (n=18)	p value
Age (years)	60.9±5.3	58.4±6.5	0.16
Gender (males%)	54%	33%	0.18
Smoker (Ever/never)	23/1	9/9	<0.01
Smoker (current/ex/never)	9/14/1	8/1/9	<0.01
Cigarette pack years	33.9±18.2	11.4±15.2	<0.01
Number of frequent exacerbators	12	NA	NA
FEV_1 (L)	1.43±0.67	2.81±0.67	<0.01
FEV_1 (% pred.)	47.8±22.4	101±19.9	<0.01
FVC (L)	2.7±0.83	3.6±0.9	<0.01
FVC (% pred.)	69.7±23.3	107.6±18.2	<0.01
FEV_1/FVC (%)	51.9±12.7	78.2±3.9	<0.01
RV (L)	4.2±1.6	2.2±0.8	<0.01
TLC (L)	7.3±1.8	5.9±1.6	0.018
RV/TLC (%)	57.3±11.9	36.7±8.4	<0.01
Raw (kPa*s/l)	0.48±0.2	0.28±0.1	<0.01
pO_2 (mmHg)	65.1±7.4	76.8±8.1	<0.01
pCO_2 (mmHg)	41.1±4.7	38.9±2.7	0.13
CAT	18.5±7.2	7.8±2.7	<0.01
mMRC	1.8±0.8	0.2±0.4	<0.01
Total cholesterol (mmol/L)	5.4±0.8	5.1±0.8	0.42
Triglyceride (mmol/L)	1.3±1.0	1.9±1.5	0.18
HDL-C (mmol/L)	1.7±0.3	1.4±0.25	0.04
LDL-C (mmol/L)	2.9±1.0	3.0±0.5	0.67
hsCRP (mg/l)	2.50 /0.50-7.80/	1.65 /0.5-4.9/	0.14
IL-6 (pg/ml)	4.29 /2.61-13.63/	3.47 /1.65-5.75/	0.03
suPAR (ng/ml)	2.8±0.7	2.4±0.6	0.03
ED-1 (fmol/ml)	1.3 /0.0-10.1/	0.8 /0.0-6.1/	0.18

Table 4. Subjects' characteristics

COPD: Chronic obstructive pulmonary disease, FEV_1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, RV: Residual Volume, TLC: Total lung capacity, Raw: airway resistance, CAT: COPD Assessment Test, mMRC: Modified Medical Research Council Dyspnea Scale, HDL-C: *high-density lipoprotein* cholesterol, LDL: low-*density lipoprotein* cholesterol, hsCRP: high sensitivity C-reactive protein, IL-6: interleukin-6, suPAR: soluble urokinase-type plasminogen activator receptor, ED-1: endothelin-1. Data are expressed as mean ± standard deviation or median /range/ or percentage.

The levels of plasma suPAR were significantly higher in patients with COPD (2.84 ± 0.67 ng/ml vs. 2.41 ± 0.57 ng/ml, p = 0.03, Figure 4).

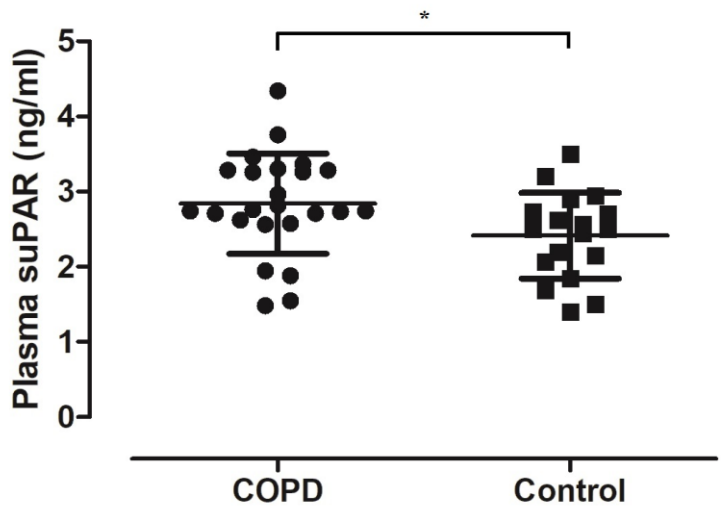

Figure 4. Plasma suPAR levels in COPD and controls
Significantly higher plasma suPAR levels were detected in COPD (* p=0.03). Individual data are presented with mean ± standard deviation.

We assessed the relationship between circulating suPAR and measures of COPD severity and activity. There was a significant relationship between circulating suPAR levels and FEV_1 % (r = -0.65, p<0.01, Figure 5).

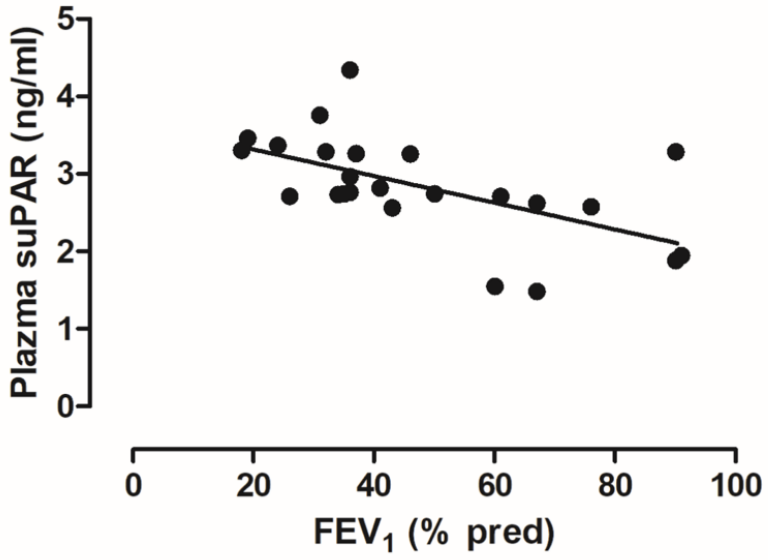

Figure 5. Relationship between plasma suPAR levels and lung function
A significant relationship was detected between plasma suPAR levels and FEV_1 (r=-0.65, p<0.01).

There was a significant relationship between FEV_1/FVC (r = -0.46, p=0.02) and mMRC (r = 0.55, p < 0.01, Figure 6).

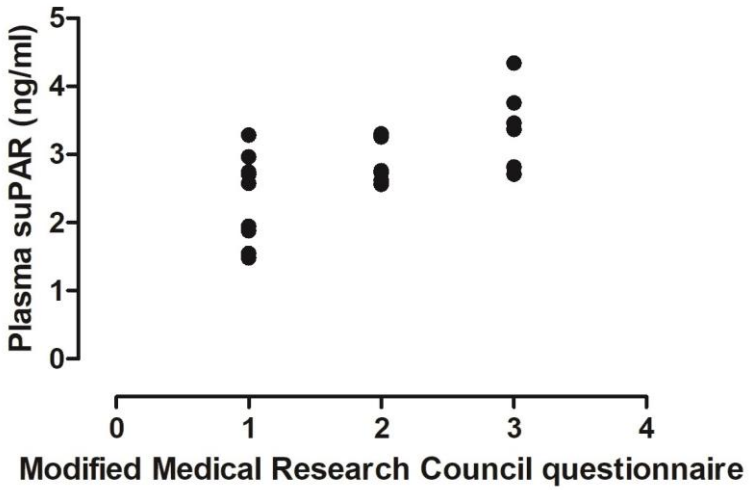

Figure 6. Relationship between plasma suPAR levels and symptoms burden
A significant relationship was detected between plasma suPAR levels and mMRC score (r=0.55, p<0.01).

In addition, plasma suPAR levels tended to be elevated in patients with frequent exacerbations (3.09±0.39 ng/ml vs. 2.58±0.79 ng/ml, p=0.058). In contrast, there was no relationship between plasma suPAR levels and FVC, pO_2, pCO_2, R_{aw}, RV, TLC, RV/TLC or CAT (all p>0.05).

We measured the relationship between circulating suPAR and markers of arterial stiffness parameters between the COPD and Control group. Comparison of the two groups in terms of arterial stiffness is found in Table 5. There was a significant difference in SBPao, SBPao-SBPbr, PWVao and RT (all p<0.05), suggesting increased arterial stiffness in COPD.

	COPD (n=24)	**Controls (n=18)**	**p value**
SBPbr (mmHg)	135±12.4	130±9.6	0.18
DBP (mmHg)	85±10.9	77±7.3	0.02
HR (min)	72±16	69±13.7	0.59
PP (mmHg)	53.5±10.5	51.6±6.4	0.50
SBPao (mmHg)	143 /106-156/	123 /98-148/	<0.01
SBPao-SBPbr (mmHg)	4 /-16-11/	-4 /-11-7/	<0.01
Aix%	10.1±30.6	-7.4±20.3	0.06
ED (ms)	308.5±36.5	320.6±28.7	0.25
PWVao (m/s)	10.6±1.9	8.8±1.3	<0.01
RT (ms)	99.6±20.6	115.2±20.2	<0.01

Table 5. Comparison of measures of blood pressure and arterial stiffness between COPD and controls
SBPbr: brachial systolic blood pressure, DBP: brachial diastolic blood pressure, HR: heart rate, PP: pulse pressure, SBPao: the central blood pressure, SBPao-SBPbr pressure amplification, Aix: augmentation index, ED: ejection duration, PWVao: aortic pulse wave velocity, RT: return time. Data are expressed as mean ± standard deviation or median /range/ or percentage.

When all subjects were investigated together, a significant correlation was seen between plasma suPAR concentrations and ejection duration (r = -0.31, p = 0.04), PWVao (r = 0.38, p = 0.01) and RT (r = -0.31, p = 0.04), but there was no correlation with Aix%, PP or SBPao (all

p>0.05). When only the COPD subjects were analysed, only ejection duration correlated with plasma suPAR levels (r = -0.44, p = 0.03).

We examined the association between smoking and early atherosclerosis by determining arterial stiffness parameters and inflammatory markers and suPAR levels in healthy non-smokers, healthy smokers. There was a significant correlation between cigarette pack years and plasma suPAR levels in controls (r = 0.68, p<0.01).

Comparison of inflammatory markers and clinical characteristics of smoker and non-smoker controls are presented in Table 6.

	Control non-smoker (n=9)	**Control smoker (n=9)**	**p value**
Age (years)	56.9±4.7	59.9±7.8	0.19
Gender (males%)	33%	33%	
FEV_1 (L)	3.1±0.68	2.5±0.54	0.05
FEV_1 (% pred.)	103.8±21.5	99.3±19.9	0.28
FVC (L)	4.1±0.9	3.2±0.7	0.02
FVC (% pred.)	112.3±19.1	102.9±17.0	0.3
FEV_1/FVC (%)	76.8±3.8	79.6±3.7	0.13
RV (L)	2.56±0.9	1.9±0.67	0.17
TLC (L)	6.8±1.8	5.1±0.8	0.11
RV/TLC (%)	37.1±6.3	36.4±10.22	0.74
Raw (kPa*s/l)	0.26±0.8	0.31±0.4	0.27
hsCRP (mg/l)	1.4 /0.50-3.8/	1.9 /0.7-4.9/	0.18
IL-6 (pg/ml)	3.3 /2.55-4.13/	3.7 /1.65-5.75/	0.29
suPAR (ng/ml)	2.1±0.5	2.7±0.5	0.08
ED-1 (fmol/ml)	0.29 /0.0-3.1/	1.17 /0.3-6.1/	0.11

Table 6. Comparison of non-smoker and smoker control

FEV_1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, RV: Residual Volume, TLC: Total lung capacity, Raw: airway resistance, hsCRP: high sensitivity C-reactive protein, IL-6: interleukin-6, suPAR: soluble urokinase-type plasminogen activator receptor, ED-1: endothelin-1, Data are expressed as mean ± standard deviation or median /range/ or percentage.

Healthy control smokers (*n* = 9) tended to have elevated plasma suPAR levels, and significantly increased PWVao as well as decreased RT, FEV1 and FVC compared to non-smokers (Table 6 and Table 7).

	Control non-smoker (n=9)	Control smoker (n=9)	p value
SBPbr (mmHg)	129±11,1	131±8,4	0,76
DBP (mmHg)	78±8,6	76±6,4	0,71
HR (min)	67±8,6	71±17,8	0,52
PP (mmHg)	51,1±6,9	52,0±6,1	0,78
SBPao (mmHg)	122 /98-142/	129 /113-148/	0,71
SBPao-SBPbr (mmHg)	-5 /-10-2/	-1,1 /-11-7/	0,18
Aix%	-12,2±23,6	-2,6±33,0	0,49
ED (ms)	320,0±25,9	321,1±33,1	0,94
PWVao (m/s)	8,1±0,9	9,5±1,3	0,03
RT (ms)	127,7±15,8	102,7±16,3	<0,01

Table 7. Descriptive statistics on blood pressure and arterial stiffness parameters in non-smoker and smoker control groups

SBPbr: brachial systolic blood pressure, DBP: brachial diastolic blood pressure, HR: heart rate, PP: pulse pressure, SBPao: the central blood pressure, SBPao-SBPbr pressure amplification, Aix: augmentation index, ED: ejection duration

Relationship between circulating suPAR and IL-6, endothelin and hsCRP

There was a significant direct relationship between circulating suPAR concentrations and IL-6 ($r=0.45$, $p<0.01$), hsCRP ($r=0.47$, $p<0.01$) and endothelin-1 ($r=0.48$, $p<0.01$) levels in all subjects. When the COPD subjects were analysed separately, plasma suPAR related to hsCRP ($r=0.53$, $p<0.01$) and endothelin ($r=0.54$, $p<0.01$) and tended to be related to IL-6 ($r=0.40$, $p=0.051$).

4.3. Discussion

We investigated the plasma suPAR levels, as a novel biomarker of inflammation in COPD patients, who were free from comorbidities. We found elevated suPAR levels in COPD which correlated with lung function and symptom burden.

A significant association was also found between increased suPAR levels and arterial stiffness, suggesting that this molecule may play a role in development of atherosclerosis in COPD. The prevalence of cardiovascular comorbidities, including atherosclerosis is high in COPD, and COPD is also prevalent in patients with known atherosclerosis. The increased arterial stiffness such as elevated PWVao is not only a marker of clinically symptomatic disease, but preclinical atherosclerosis as well. In this COPD population with PWVao could be identify individuals at high risk.

Our study confirmed the presence of endothelial dysfunction, subclinical atherosclerosis, as we found significantly higher PWVao and SBPao values in the COPD group compared to the control group, which can be attributed to the increased vascular wall stiffness in this population. The pathomechanism linking COPD to atherosclerosis is complex and includes common risk factors, such as smoking, pollution, male gender, and aging and systemic inflammation. Pro-inflammatory cytokines, such as IL-6 or TNF-α are elevated in blood samples of COPD patients and can induce the release of CRP and pro-coagulant mediators by

the liver, but have also a direct effect on endothelium. IL-6 and TNF-α can also induce the production of suPAR from monocytes and lymphocytes, which has a well-established role in the development of atherosclerosis. It induces cellular adhesion, leukocyte migration and eventually leads to the formation of an atherosclerotic plaque. Previous studies revealed that increased plasma suPAR levels were associated with risks for subclinical carotid atherosclerosis and increased occurrence of carotid plaque and cardiovascular disease. The prognostic value was independent from traditional risk factors (i.e. age, gender, smoking, hypertension, dyslipidemia, diabetes), and hsCRP. Furthermore, suPAR is more related to endothelial dysfunction and atherosclerosis than hsCRP.

We included patients with a wide range of lung function and found a significant relationship between airflow limitation and higher suPAR levels. The most likely explanation of increased suPAR levels in COPD is the increase in IL-6 which is in line with the literature. IL-6 upregulates suPAR production which was supported by a significant association between these two molecules in our study. Circulating IL-6 induces the endothelium to release chemotactic factors for leukocytes and adhesion molecules, and high blood levels are associated with cardiovascular comorbidities in COPD. SuPAR which is also induced by IL-6, have more direct effect in formation of the atherosclerotic plaques, and therefore may be a more specific biomarker of endothelial dysfunction in COPD than other circulating mediators. The clinical role of using suPAR as a biomarker for cardiovascular disease has already been assessed. The current study implies that this molecule can also be useful in COPD and associated atherosclerosis.

Apart from lung function, suPAR was associated with symptom burden measured by the mMRC in which score reflects breathlessness in relation to physical exercise. It is likely that this association is not independent from lung function, but due to the low number of subjects we did not test this. There was a tendency for higher plasma suPAR levels in patients with frequent exacerbations. This is in line with the findings of the ECLIPSE cohort that persistent systemic inflammation is related to the frequency of exacerbations. The predictive value of suPAR to detect patients with higher risk for exacerbation has to be assessed in independent cohorts. Interestingly, we did not find any association between plasma suPAR levels and blood gases, suggesting that hypoxia may not be a strong signal for suPAR production, however this has to be tested as well.

Previous studies have demonstrated the importance of hypoxemia and oxidative stress in COPD in the development of endothelial dysfunction, as elevated endothelin-1 levels have been detected in hypoxemic COPD patients with cardiovascular comorbidities. Endothelial dysfunction affects not only the tissue of the lung but all the vascular system. In our study, we found increased plasma levels of endothelin-1 as the sign of the early onset of atherosclerosis in COPD patients without comorbidities.

The control groups included smoker and non-smoker participants. Previous studies reported higher circulating suPAR levels in smokers which was confirmed in this study. Smoker controls had significant lower lung function volumes and elevated markers of arterial stiffness, with significant difference in PWVao and RT. The inter-group difference in suPAR may partially result from smoking.

In summary, we reported higher plasma suPAR levels in COPD, which are associated with impaired lung function and increased arterial stiffness. Plasma suPAR may be a potential link between COPD and cardiovascular comorbidities.

5. Conclusion

Hungary is one of the countries where the cardiovascular risk is very high. At the population level, our first and foremost task is to reduce the incidence of CV disease. Another key priority of the cardiovascular prevention strategy is the early detection of individuals at high cardiovascular risk and the initiation of preventive therapy. It is recommended to perform a non-invasive strategy that is easy to perform in a large population, does not require expensive trained stuff, simple, inexpensive, reproducible, and has no side effects. Methods should be chosen that have an easy-to-use tool for testing large populations.

5.1. The Measurement of Aortic Pulse Wave Velocity (PWVao) and Asymptomatic Carotid Atherosclerosis in a Middle-Aged, Healthy Population

We found that increased PWVao was very strongly associated with the presence of asymptomatic carotid atherosclerosis in apparently healthy individuals, which is of great importance to identify vulnerable individuals with high CV risk, among asymptomatic healthy individuals.

The increased arterial stiffness - oscillometricalliy, non-invasively measured -, due to the simplicity, user independency, and fast performed methodology for measuring PWVao, our study suggests that it helps to improve cardiovascular risk assessment even in a relatively young, middle-aged, apparently healthy population. Non-invasively measured PWVao by Arteriograph, appears to be a suitable functional screening test for the detection of preclinical atherosclerosis.

5.2 The measurement of Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) and Arterial Stiffness parameters in Patients with COPD and healthy individuals.

In our study, we demonstrated elevated plasma suPAR levels, inflammatory parameters and elevated arterial stiffness parameters in the COPD population compared to the control group. Elevated arterial stiffness parameters were measured by non-invasive oscillometric methods in comorbid-free, middle-aged COPD individuals, confirming the presence of early subclinical atherosclerosis, and the higher cardiovascular risk. It is very important to highlight that a significant proportion of mortality in patients with COPD is caused by cardiovascular disease. Thus, the detection of early subclinical atherosclerosis is also important in this group of patients. Persistent inflammation is a breeding ground for early atherosclerosis. Plasma suPAR may be a potential link between COPD and cardiovascular comorbidities.

Our work is not only a novelty in the field of research, but also a help in everyday medical work. Today, when prevention is one of the key priorities of health care, attention is focused on the screening. It is important to find and apply previously unrecognized options that reduce

morbidity and mortality. We need to discover patients who are still asymptomatic, claim to be healthy, but are already in the subclinical stage. Where is this possible? Where a larger number of patients appear, i.e. in general practice, within the framework of medical fitness and occupational medicine examinations. In individuals identified by vascular stiffness screening, there is hope for reversal of the process by lifestyle changes, i.e. changes in eating habits, regular exercise, physical training and early medication, anyway, there is an opportunity to slow the progression.

6. Summary of new results

1. We were the first to determine that asymptomatic carotid atherosclerosis (ACA) in healthy middle-aged individuals was strongly associated with oscillometrically measured increased aortic pulse wave velocity (PWVao). Arteriograph-measured PWVao, smoking habits, SBP, DBP were the independent markers of asymptomatic carotid artery atherosclerosis.

2. We were the first to determine the optimal cut off value of PWVao for the detection of ACA in healthy individuals, which proved to be 8.3 m/s. In addition, we determined the sensitivity, specificity, positive predictive value, negative predictive value of PWVao.

3. We were the first to demonstrate the significantly increased - non-invasively, ocillometricalliy measured - arterial stiffness parameters (PWVao, SBPao and decreased RT) in middle-aged COPD individuals who were free from comorbidity compared with healthy control group. With this, we demonstrated the presence of early subclinical atherosclerosis and a higher cardiovascular risk.

4. We were the first to demonstrate significantly higher plasma suPAR levels in stable COPD patients without CV disease compared to controls.

5. The rate of FEV1 loss was significantly correlated with the increase of suPAR level in comorbid-free COPD individuals. We were the first to demonstrate that with the severity of COPD increase inflammation and increase the risk of atherosclerosis, even in patients with COPD who do not have CV comorbidity.

6. Significant relationship was detected between plasma suPAR levels and COPD symptoms burden (mMRC) in the middle-aged COPD group who did not have comorbidities.

7. List of scientific publications, book chapter and congress summaries related to the thesis

7.1 Original scientific papers related to the thesis

1. **Böcskei RM**, Benczúr B, Müller V, Bikov A, Székely A, Kahan T, Lenkey Zs, Husznai R, Cziráki A and Illyés M. Oscillometrically Measured Aortic Pulse Wave Velocity Reveals Asymptomatic Carotid Atherosclerosis in a Middle-Aged, Apparently Healthy Population. *Biomed Res Int.* 2020;16,2020:8571062. **IF:2.276**
2. **Böcskei RM**, Benczúr B, Losonczy Gy, Illyés M, Cziráki A, Müller V, Bohács A, Bikov A. Soluble Urokinase-Type Plasminogen Activator Receptor and Arterial Stiffness in Patients with COPD. *Lung.* 2019;197(2):189-197. **IF:2.231**
3. **Böcskei RM**, Benczúr B. Az érfali rugalmatlanság jelentősége COPD-ben. *Medicina Thoracalis*. 2017. 70.évf. 3.sz.
4. Illyés M, **Böcskei RM**. Egyszerű, gyors, automatikus, nem-invazív módszer a vérnyomás, az artériás stiffness és más hemodinamikai paraméterek egyidejű mérésére. *Érbetegségek.* 2006/4.13-21.

7.2 The book chapter related to the thesis

1. Jatoi NA, Benczur B, **Bocskei RM**, Sabivic M, Scadale G, Dimitrov G, Al-Baker W, Skrabal F, Catalano M. VAS European Book on Angiology/Vascular Medicine. Reference Book for European Trainig, Courses and UEMS European Exam. Chapter 5. *Arterial stiffness***.** 2018 ISBN 104399/97888255105228, page 81-96

7.3 List of congress posters related to the thesis

1. **Böcskei RM,** Benczúr B, Bikov A, Husznai R, Böcskei Cs, Bohács A, Cziráki A. 24h Arterial stiffness measurement on healthy and COPD patients. *Artery Society 19. Congress.* Budapest. 2019.09.10-12. poster discussion.
2. **Böcskei RM**. Cardiovascular risk detection in COPD. *Erupean Respiratory Socitey Congress* Madrid, Spain. 2019.09.28-10.02
3. **Böcskei RM**, Husznai R, Benczúr B, Böcskei Cs, Müller V, Cziráki A. ABPM és 24h artériás stiffness vizsgálata COPD-s és egészséges egyéneknél. *Magyar Kardilógiai Társaság Kongresszusa.* Balatonfüred. 2019.05.03-05.
4. **Böcskei RM**, Benczúr B, Cziráki A. Determinants of brachial-ankle pulse wave velocity. *Artery Congress.* 2018.10.18. Guimares.
5. **Böcskei RM**. Arterial stiffness and systemic inflammation in COPD patients. *Artery Congress.* 2016 Koppenhága.
6. **Böcskei RM**, Inflammatory markers suPAR and arterial stiffness parameters in COPD patients. *European Respiratory Society Congress.* 2016 London.
7. **Böcskei RM**. Correlation between aortic pulse wave velocity and asymptomatic carotid atherosclerosis in apparently healthy individuals. *Artery.* 2015. Krakkó, poszter előadás
8. **Böcskei** RM. Is Aortic pulse wave velocity a marker of atherosclerosis? *ECCR.* 2015. okt. Lake Garda.
9. Kahan T, **Böcskei RM**, Illyés M, Cziráki A. Aortic pulse wave velocity but not augmentation index is associated with asymptomatic carotid. *Artery Research.* 2013 szept.; 7(s 3–4):112.

10. **Böcskei RM**, Tamási L, Bohács A, Illyés M, Cziráki A, Tünetmentes célszervkárosodások, a carotis atherosclerosis és az artériás stiffness kapcsolata. *Magyar Tüdőgyógyász Társaság 57. Nagygyűlése és 100 éves Centenáriumi Emlékülése*. Budapest. 2012. június 14-16.
11. **Böcskei RM**, Benczúr B, Cziráki A, Molnár F, Illyés M. Correlation Between the Central and Brachial Blood Pressure and Asymptomatic Carotid Atherosclerosis. *Artery Research* 2009 január; 3(4):185-185.
12. Benczúr B, **Böcskei RM**, Illyés M. Reference values for arterial stiffness measured with Arteriograph. *Artery 9 Congress*. 2009. 09. 10–12; Cambridge.
13. **Böcskei RM**, Benczúr B, Molnár F, Illyés M. Correlation between aortic pulse wave velocity and asymptomatic carotid atherosclerosis. *Artery 8 Congress*. 2008.09.25-27; Gent.
14. Benczúr B, **Böcskei RM**, Illyés M. Reference values for arterial stiffness measured with Arteriograph. *Artery 8 Congress*. 2008.09.25-27; Gent.
15. **Böcskei RM**, Benczúr B, Illyés M. Az aorta pulzushullám terjedési sebességének prediktív értéke a tünetmentes carotis atherosclerosis kimutatásában. *Magyar Hypertonia Társaság XV. Kongresszusa és VI. Nemzetközi Továbbképző Kurzusa*. 2007. december 5-8; Budapest.
16. Benczúr B, Baulmann J, Mengden T, Cziráki A, **Böcskei R**, Illyés M. New oscillometric method for assessment of arterial stiffness - comparison with tonometric and piezo-electronic metods. *Magyar Hypertonia Társaság XV. Kongresszusa és VI. Nemzetközi Továbbképző Kurzusa*. 2007.december 5-8; Budapest.

7.4 List of congress oral presentations related to the thesis

1. **Böcskei RM.** Cardiovascular risk detection in COPD. *Eurpean Respiratory Socitey Congress*. 2019.09.28-10.02; Madrid.
2. **Böcskei RM**. Endothel dysfunkció, artériás stiffness vizsgálata krónikus obstruktív tüdőbetegségben a gyulladásos markerek tükrében. *MKT-MTT Kardiopulmonális Szekció 23. ülése és MTT Légzésrehabilitációs szekcióülése*. 2019.09.18-19; Tapolca.
3. **Böcskei RM.** Gyulladásos markerek (hsCRP, IL-6, suPAR), endothel dysfunctio, artériás stiffness vizsgálata krónikus obstruktív tüdőbetegségbe. *MTT 2016 Naggyűlés MPA beszámoló.* 2016, Budapest.
4. **Böcskei RM.** Az aorta oszcillometriásan meghatározott pulzushullám terjedési sebességének szerepe a szív és érrendszeri kockázatbecslésben. *MKT*. 2016 június; Pécs.
5. **Böcskei RM.** PWVao és carotis atherosclerosis kapcsoalta. *Magyar Artériás Stiffnes Társaság Éves Kongresszusa*. 2016. április; Szeged.
6. **Böcskei RM.** Arériás stiffness jelentősége a Pulmonológiában. *Magyar Artériás Stiffnes Társaság Éves Kongresszusa*. 2016. április; Szeged.
7. **Böcskei RM.** Az emelkedett artériás stiffness szenzitív markere a korai, aszimptomatikus carotis ateroszklerózisnak. *Magyar Kardiológiai Társaság Kongresszusa*. 2015.05.08; Balatonfüred.
8. **Böcskei RM.** Az aorta pulzushullám terjedési sebesség és a tünetmentes carotis atherosclerosis kapcsolata. *Magyar Artériás Stiffness Társaság IX. Kongresszusa*. 2014. április 11-12; Győr.
9. **Böcskei RM**, Illyés M. 24 órás monitorozás szerepe az artériás funkció (artériás stiffness) megítélésében. Az artériás funkció napszaki ritmusa. A vérnyomásmonitorozás új aspektusai. *A Magyar Artériás Stiffness Társaság szimpóziuma*. 2012. november 9; Szolnok.

10. **Böcskei RM.** Tünetmentes célszervkárosodások, a carotis atherosclerosis és az artériás stiffness kapcsolata. *7. Nemzetközi Artériás Stiffness Szimpózium és a Magyar Artériá Stiffness Társaság 5. Kongresszusa*. 2011. 04.17-19; Debrecen.
11. **Böcskei RM.** Az aorta pulzushullám terjedési sebességének prediktív értéke a korai aszimptomatikus carotis atherosclerosis kimutatásában. *6. Nemzetközi Arterial Stiffness Szimpózium és a Magyar Artériás Stiffness Társaság 4. Kongresszusa*. 2010. 04.15-17; Pécs.
12. **Böcskei RM.** COPD és az artériás stiffness kapcsolata. *Háziorvosi Továbbképző Tanfolyam.* 2008.03.22; Pásztó.
13. **Böcskei RM.** Összefüggés az aorta PWV és az aszimptomatikus carotis atherosclerosis között. *4. Nemzetközi Arterial Stiffness Szimpózium és a Magyar Artériás Stiffness Társaság 2. Kongresszusa*. 2008.02.22; Budapest.
14. **Böcskei RM.** Új non-invazív módszer az érelmeszesedés korai kimutatására címmel „Az egészséges fejlődés feltételeinek biztosítása a fogamzástól a felnőttkorig". *Nemzeti Népegészségügyi program jegyében*. 2006. okt.19; Budapest Parlament.

8. Other original publications of the author not related to the thesis

8.1. Author's other original scientific papers

1. **Böcskei RM**, Meszaros M, Tarnoki A, et al. Circulating Soluble Urokinase-Type Plasminogen Activator Receptor in Obstructive Sleep Apnoea. *Medicina*. 2020;56(2):77. 14. doi:10.3390/medicina56020077. **IF: 1.467**
2. Molnár V, Nagy A, Tamási L, Gálffy G, **Böcskei RM,** Bikov A, Czaller I, Csoma Zs, Krasznai M, Csáki Cs, Zsigmond Gy et al. From genomes to diaries: a 3-year prospective, real-life study of ragweed-specific sublingual immunotherapy. *Immunotherapy.* 2017;9(15):1279-1294. doi:10.2217/imt-2017-0093 **IF:3.46**
3. Südi A, Bohács A, Bikov A, Czaller I**, Böcskei RM**, Rigó J, Losonczy Gy, Tamási L. A keringő complement 5a és complement factor H szintek és a betegségkontroll kapcsolata asztmás betegekben. *Medicina Thoracalis*. 2017 70. évf.6. sz .339.-345.o
4. Bohács A, Bikov A, Ivancsó I, Czaller I**, Böcskei RM**, Müller V, Rigó J. Relationship of Circulating C5a and Complement Factor H Levels With Disease Control in Pregnant Women With Asthma. *Respir Care.* 2016 Apr;61(4):502-9. doi: 10.4187/respcare.04339. **IF:1.733**
5. Benczúr B, Illyés M, Cziráki A, **Böcskei RM**. Tisztelt Szerkesztőség! *Orv Hetil.* 2015;156(25):1026-8.
6. Lenkey Zs, Illyés M, **Böcskei RM**, Husznai R, Sárszegi Z, Meiszterics Z, Molnár FT, Hild G, Cziráki A, Gaszner B. Comparison of arterial stiffness parameters in patients with coronary artery disease and diabetes mellitus using Arteriograph. *Physiological research / Academia Scientiarum Bohemoslovaca*. 2014; **IF: 1.53**
7. Bikov A, **Böcskei RM**, Eszes N, Bohács A, Losonczy Gy, Rigo J, Horváth I, Tamási L. Circulating survivin levels in healthy and asthmatic pregnancy**.** *Reproductive Biology and Endocrinology*. 2014; 12(1):93. **IF: 2.226**
8. Tamási L, **Böcskei RM.** Asztmás betegek fluticason-salmeterolrol extrafinom részecskés beclometason-formoterolterápiára történő átállításának klinikai- és költséghatékonyság vizsgálata: retrospektív, illesztett, összehasonlító való élet vizsgálat. *Medicina Thoracalis*. 2014; 367-370

9. Hidvégi EV, Illyés M, Molnár FT, **Böcskei RM**, Benczúr B, Lenkey Zs, Cziráki A. Az aorta pulzushullám terjedési sebesség referencia értkei gyermkekben és serdülőkben. *Gyermekgyógyászat*. 2013; 64. évf. 6.sz 287-291
10. Ivancsó I, **Böcskei RM**, Müller V, Tamási L. Extrafine inhaled corticosteroid therapy in the control of asthma. *J Asthma Allergy*. 2013; 6;6:69-80. doi: 10.2147/JAA.S25415. Print 2013.PMID: 23776339 **IF:2.45**
11. **Böcskei RM**, Pápay J, Agócs L, Rényi Vámos F, Losonczy Gy, Tamási L. Endobronchialis eltérés Asztma hátterében. *Medicina Thoracalis*. 2013;.218-224.
12. Ivancsó I, **Böcskei RM**, Müller V, Tamási L. Extrafinom részecskeméretű inhalációs corticosteroid-terápia az asztma kezelésében. *Medicina Thoracalis*. 2013; 235-238.
13. Tamási L, **Böcskei RM**, Ivancsó I. Erlotinib EGFR mutáns és EGFR vad pulmonaris adenocarcinoma kezelésében. *Onkológia*. 2013; 5.sz. 3. évfolyam p. 201-205.
14. Hidvégi EV, Illyés M, Benczúr B, **Böcskei RM**, Rátgéber L, Lenkey Zs, Molnár FT, Cziráki A. Reference values of aortic pulse wave velocity in a large healthy population aged between 3 and 18 years. *Journal of Hypertension*. 2012; **IF: 3.806**
15. Böcskei Cs, Viczián M, **Böcskei RM**, Horváth I. The influence of gastroesophageal reflux disease and its treatment on asthmatic cough. *Beiträge zur Klinik der Tuberkulose*. 2005; 183(1):53-62. **IF: 0.857**
16. Gadó J, Schlick B, Bárány O, Németh J, Müller J, Csóka M, Constantin T, Vonnák E, **Böcskei RM**, Hauser P, Kovács G. The function of the immune system after the treatment of pediatric malignant diseases. *Orv Hetil*. 2006; 10;147(36):1731 8.PMID: 17087017 Hungarian. **IF: 0.25**

8.2. Author's other book detail

1. **Böcskei RM**, Kovalszky I, Pápay J, Süttő Z SrpingMed Orvosi Esettanulmányok Onkopulmonológia. szerkesztette: Szalai ZS, Gálffy GG; II. fejezet / 19. ALK pozitív tüdő adenokarcinómás beteg másodvonalbeli crizotinib kezelésének esetismertetése. *SpringMed kiadó: ISBN:978-615-5166-66-2; ISSN:2498-6305*. 2017;134-138.

8.3. Author’s other congress posters

1. Böcskei RM, Benczúr B, Cziráki A, Müller V, Bohacs A. Arterial stiffness measurement on lung transplanted patients. *Atery Society Congress*. 2017; Pisa, poszter előadás
2. Böcskei RM, Benczúr B, Müller B, Losomczy Gy, Bohacs A, Cziráki A. Endothelial dysfunction measurement on lung transplanted patients. *ERS*. 2017; Milan, poszter előadás
3. Böcskei RM, Artériás stiffness vizsgálata tüdőtranszplantált betegeken. *Magyar Transzplantációs Alapítvány kongresszusa*. 2016; Eger, poszter előadás

8.4 Author’s other congress oral presentations

1. Böcskei RM. Sequencing Approach in Patient Treated with Multiple ALKi. *Case presentation Future Lider Training* 2019; Pozsony.
2. Böcskei RM. Mikor gondoljon a tüdőgyógyász immundeficienciára? *Tüdőgyógyászati, Allergológiai És Immunológiai Megbetegedések” (TAIM) Nemzetközi Alapítvány XIII. Szakmai Továbbképzése*. 2019; DOTE, Debrecen.
3. Böcskei RM. Treatment sequencing in ALK+ NSCLC and the influence on overal survival. *10th Regional Oncology Forum*. 2019; Tallin.
4. Böcskei RM, Eszes N, Pápay J, Balázs Gy, Sax B, Bohács A. Gyógyszer indukálta organizáló pneumónia és pneumomediastinum szívtranszplantált betegnél. *BRONKO. Tata*, 2016.
5. Böcskei RM. Dohányzás és a Tüdőbetegségek Kapcsolata. *Dohányzás Mentes Világnap*. 2015; Tata.

6. Böcskei RM. Autoimmun kórképek pulmonológiai vonatkozásai. *Pulmonológia Szakvizsga Előkészítő Kötelező Tanfolyam*. 2015; Semmelweis Egytetem Pulmonológiai Klinika, Budapest.
7. Böcskei RM. A COPD okozta reggeli és éjszakai tünetek gyakoriságának és súlyosságának felmérése. *LÉT konferencia.* 2015; Velence.
8. Böcskei RM. A Nem kontrollált asztma meglepő okai. *Semmelweis Egyetem Továbbképző tanfolyam*. 2014; Budapest.
9. Böcskei RM.. Légzésfunkciós vizsgálatok. A légzésfunkciós vizsgálatok módszerei, kivitelezése. *GYE.MSZI Szakdolgozói Továbbképző Tanfolyam*. 2014; Budapest.
10. Böcskei RM, Tamási L. Az asztma és szénanátha kezelése a terhesség alatt. *19. Tavaszi Amega Fórum*. 2014; Pécs.
11. Böcskei RM. Az asztma exacerbációk jelentősége. *19. Tavaszi Amega Fórum*. 2014.
12. Böcskei RM. COPD ellátási protokoll. *Semmelweis Egyetem Továbbképző tanfolyam*. 2013; Budapest.
13. Böcskei RM, Bohács A, Markóczy Zs, Balázs Gy, Lang Gy, Losonczy Gy, Müller V. Mi állhat a 21 éves férfi pulmonalis kerekárnyékainak hátterében? *Magyar Tüdőgyógyász Társaság Epidemiológiai és Gondozási Szekciójának rendezvénye*. 2013; Gárdony.
14. Böcskei RM. Dilemmák a COPD-s beteg mindennapi ellátásában. *Amega fórum*. 2013; Budapest.
15. Böcskei RM. Rosszul kooperáló haemoptoés beteg ritka kórképe. *Fiatal pulmonológusok kazuisztikai fóruma*. 2013; Debrecen.
16. Böcskei RM. Asztmás beteg endobronchialis elváltozása. *BRONCO*. 2012; Zalakaros.
17. Böcskei RM. A centrális vérnyomás monitorozásának módszerei és jelentősége a háziorvosi gyakorlatban. (artériás stiffness vizsgálata). *Háziorvosi Kötelező Szintentartó Továbbképzés*. 2011; Tapolca.
18. Böcskei RM. Tarts ki a gyanúd mellett! *MTT. Epidemiológiai és Gondozási Szekció Kazuisztikai Fórum*. 2011; Szarvas.

9. Acknowledgements

I would like to thank Prof. Dr. Lajos Bogár for allowing me to carry out my research within the framework of the Doctoral School he led.

I would like to thank Prof. Dr. István Szokodi for allowing me to carry out my research work under program management and, with his support and encouragement, he helped me move forward.

I am grateful for the help of Prof. Dr. Attila Cziráki, who helped me with his guidance and useful advices during the research, the communication of the results and the writing of the dissertation.

I am grateful to Dr. Miklós Illyés, who introduced me the instrument he invented, the Arteriograph, taught me the basics of scientific research, data processing and communication, and aroused my interest in the physiology of arterial function. He encouraged the international presentation of our scientific work and encouraged me to obtain a Ph.D.

I would like to thank Prof. Dr. Veronika Müller and Prof. Dr. György Losonczy for supporting me to conduct my research on vascular wall stiffness in lung patients at the Department of Pulmonology, Semmelweis University. I am thankful for Associate Professor Dr. Anikó Bohács, who supported and encouraged me for further research, acquainted me with the basics of statistics and became my perpetual role model with her positive attitude and extraordinary willpower.

Thanks to Dr. András Bikov who, despite his young age, helped me to process the data with his vast research experience and statistical knowledge.

My strongest and most persistent supporter was my family during this long road of my research full of bumps. I would like to thank all the members of my family, my husband Tamás Németh, my children Boglárka and Patrik and my parents for encouraging, supporting and giving me all the help during the Ph.D. program.