Original article

The Role of Total Antioxidant Status in Cerebral Vasoreactivity of Chronic Obstructive Pulmonary Disease Patients

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is associated with an oxidant-antioxidant imbalance. COPD patients have impaired cerebral vasoreactivity (CVR). Impaired CVR could be correlated with total antioxidant status (TAS) in plasma.

Aim: To determine the role of systemic TAS in CVR of COPD patients.

Material and Methods: In this cross-sectional observational study, we included 120 participants (the mean age of 67±7.9, 87 males), 90 COPD patients categorized according to the severity of airway obstruction in mild, moderate, severe/very severe and 30 age- and sex-matched controls. We analyzed baseline mean flow velocities (MFV) of the middle cerebral artery (MCA) and the basilar artery (BA), the mean breath-holding index (BHIm) of those arteries (BHImMCA and BHImBA) by transcranial Doppler ultrasound and TAS in plasma. The level of significance was set to α=0.05.

Results: A significant negative correlation between TAS and BHImBA (Rho=−0.445, P=0.01) was found only in the mild COPD group. In COPD groups, baseline MFV BA is in a significant positive correlation with BHImMCA and BHImBA (Rho=0.336, P=0.001 and Rho=0.647, P<0.001). According to the severity of airway obstruction in COPD groups, a significant positive correlation between baseline MFV BA and BHImBA was found: in mild (Rho=0.731, P<0.001), moderate (Rho=0.574, P=0.001) and severe/very severe (Rho=0.398, P=0.03).

Conclusion: Systemic TAS was not correlated with CVR in all COPD groups. However, perfusion in the BA of COPD groups was in a significant positive correlation with the anterior and posterior CVR. The analysis of perfusion in the basal cerebral arteries should be part of a future study of CVR in COPD patients.

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Introduction

The coexistence of chronic obstructive pulmonary disease (COPD) and vascular disease is known, but the pathophysiology of this association has not been fully elucidated. The regulation of the cerebral blood flow (CBF) could be altered by some extrapulmonary consequences, such as arterial blood gas levels, acid-base imbalance, oxidative burden, endothelial dysfunction and autonomic disorders (1,2). The strongest regulator of the CBF is the arterial partial pressure of carbon dioxide ($PaCO_2$). Large changes in the flow could result even from its small fluctuations. The change in the CBF for a given increase in arterial carbon dioxide (CO_2) is greater than the change in the CBF observed for the same magnitude reduction in arterial CO_2 (3).

COPD patients have high oxidative stress associated with the severity of airway obstruction (4). Oxidative stress occurs when the resident antioxidants are insufficient or fail to upregulate sufficiently to neutralize an increased oxidant burden (5). In COPD, increased oxidative stress causes pulmonary inflammation. There is experimental and clinical evidence that pro-inflammatory mediators from the lungs overflow into the systemic circulation and cause the alteration of blood vessel structure. The vascular modelling and arterial stiffness lead to endothelial dysfunction. Those dysfunctional cells reduce the bioavailability of the vasoactive substance they secrete, such as nitric oxide (NO), which otherwise causes relaxation of smooth muscle cells lining in arterioles (6). The sensitivity of cerebral blood vessels to changes in $PaCO_2$ is termed cerebral vasoreactivity (CVR), where arterial hypercapnia induces vessel dilatation and causes an increase in cerebral perfusion (7). Damaged endothelial function is associated with impaired CO_2 reactivity, causing impairment of CVR. Therefore, CVR could be a surrogate of cerebrovascular endothelial function (8).

COPD patients have impaired CVR in the anterior and posterior cerebral circulation and the impairment increases with the airway obstruction severity (9). A study that analyzed moderate smoking-related COPD postmenopausal women showed altered cerebrovascular responses in the anterior cerebral circulation to hypercapnia. They exhibited increased oxidative stress, suggesting that it may be significant in the dysfunction of CVR observed during hypercapnic challenge in COPD (10). Different methods defining total antioxidant status (TAS) of different biological samples have been developed and some of them can determine TAS in the plasma of COPD patients (11). The aim of this study was to determine the role of systemic TAS in CVR of COPD patients.

Material and Methods

Study subjects

A cross-sectional observational study was conducted at the general hospital between March and August 2018. One hundred twenty participants were included in the study – 90 COPD patients (mild, moderate and severe/very severe group, each consisting of 30 participants) and 30 healthy volunteers with a mean age 67 ± 7.9, from which 87 males (Student's T test, P = 0.27). In terms of gender, there were no significant differences between the proportion of men and women in COPD patients and the control group (74.4 vs. 66.7 and 25.6 vs. 33.3% respectively; P = 0.41; Fisher's exact test). The baseline characteristics of COPD patients and controls were summarized in Table 1. The exclusion criteria were as follows: previous cerebrovascular disease, stenosis of internal carotid arteries (>50%), inadequate trans-temporal acoustic window/s, atrial fibrillation, active malignity, exacerbation of COPD in previous two months, specific therapies (nonsteroidal anti-inflammatory and antioxidant therapy, vitamins A, C and/or E supplementation). COPD patients with arterial hypertension, hyperlipidemia, ischemic heart disease and diabetes mellitus that represent vascular disease risk factors were included if they were under good therapy control.

Table 1. Baseline characteristics, lung functions, laboratory values and pack-years in COPD patients and controls

	Median (interquartile range)		Difference	95% confidence interval	P*
	Controls	COPD			
Age (years)	64 (4)	67 (9)	1.8‡	-5.1 do 1.5	0.27
FEV_1/FVC ratio [Median (interquartile range)]	0.80 (0.75 – 0.83)	0.61 (0.46 – 0.68)	0.20‡	0.15 do 0.25	<0.001†
FEV_1 (%)	106 (12.7)	65 (25.5)	40.7	33.6 do 47.7	<0.001
BMI (kg/m²)	27.2 (3.2)	28.7 (6.7)	1.5	-4.03 do 0.97	0.23
Body temperature (°C)	35.3 (5.7)	35.9 (3.3)	0.64	-2.3 do 1.04	0.45
Systolic blood pressure (mmHg)	130 (13)	135 (16)	5,2	-11.6 do 1.2	0.13
Diastolic blood pressure (mmHg)	80 (5)	81 (6)	0,8	-3.5 do 1.8	0.53
Hemoglobin (g/l)	137 (13)	143 (14)	5,7	-11.6 do 0.08	0.05
CRP (mg/l) [Median (interquartile range)]	2.2 (0.97 – 3.15)	2.6 (1.4 – 6.2)	0.6‡	-0.1 do 1.6	0.08†
Pack-years [Median (interquartile range)]	41 (17.3 - 48)	43 (33 - 55)	9‡	-6.8 do 30	0.27†

*Student T test; †Mann-Whitney U test; ‡Hodges-Lehmann median difference

FEV1/FVC – forced expiratory volume in one second / forced vital capacity; FEV1 – forced expiratory volume in one second; BMI – body mass index; CRP – C-reactive protein; pack-years = number of cigarettes per day x number of smoking years / 20

Study design

The participants were divided into four groups. The first three groups were COPD patients, divided according to spirometry findings and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow obstruction severity based on forced expiratory volume in one second (FEV1) as mild; moderate; and severe to very severe (12). According to GOLD, COPD was defined by post-bronchodilator FEV1 to forced vital capacity (FVC) ratio (FEV1/FVC) of <0.70, confirming a persistent airflow obstruction (13). The fourth group were controls – healthy volunteers (FEV1/FVC >70% and FEV1 >80% of the predicted value).

Anthropometric measurements, comprising height and weight, were determined. Body mass index (BMI) was calculated and expressed as weight (kg) / height (m2). Pack-years were calculated as the number of cigarettes per day x the number of years smoked /20 (14). The participants were asked to abstain from caffeine, alcohol and intense physical activity at least 12 hours prior to participation in the study. For all participants, the exclusion of significant internal carotid stenosis was performed by the method of color and power Doppler flow imaging with a linear probe (VF 10-5 Linear Ultrasound Transducer, Siemens Acuson X300, Germany). For all participants, a specialized technician performed spirometry (Spiroscout^R Ganshorn, Germany) according to the American Thoracic Society/European Respiratory Society standardization (15). Baseline cerebral perfusion and CVR were determined by transcranial

Doppler (TCD) ultrasound (DigiLite™, Rimed, Industrial Park Raanana, Israel).

The study was approved by the Ethics Committee of General Hospital Našice (No. 01-497/3-2017) and by the Ethics Committee of the Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia (No. 2158-61-07-17-209). All data were anonymized and the study was conducted in accordance with the amended Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. All participants signed an informed consent form before entering the study.

Biochemical analysis

Venous blood was taken for a biochemical analysis. We analyzed the complete blood count (Sysmax XN-1000 SA-01, Sysmax Europe GmbH), C-reactive protein (CRP) and TAS (Beckman Coulter DXC 700 AU analyzer, USA). The plasma antioxidant status was measured using a commercially available TAS kit (reagents Randox Laboratories Ltd, United Kingdom). The serum separated from blood was frozen at -20 °C. The samples were collected over a period of 14 days and analyzed in one day by using a colorimetric assay. The procedure was repeated until the target number of participants was reached. The reference range was set at 1.30 – 1.77 and the results were expressed as mmol/L (16). Other blood samples were analyzed immediately after being taken.

TCD monitoring

TCD monitoring of the middle cerebral arteries (MCAs) that represent the anterior cerebral circulation was performed by previously described protocol (9,17,18). The breath-holding test was used as a hypercapnic stimulus. Using an original headband device (Rimed, Industrial Park Raanana, Israel), we secured both 2-MHz Doppler probes over the trans-temporal windows. An optimal insonation position during monitoring was maintained and escaped movement artifacts. Baseline mean flow velocity (MFVbaseline) was defined as a continuous mean velocity over 30 seconds during the resting period in supine position and normal breathing of room air. Maximum MFV (MFVmax) was defined as the last 3 seconds of breath-holding after normal inspiration. The minimum breath-holding time was set at 15 seconds and the maximum at 30 seconds. The test was repeated two times, with a resting period of 2 minutes. Breath-holding index (BHI) was calculated as the difference in the increase of MFV (cm/s) occurring during breath-holding divided by the time (seconds) for which the participant held breath ([MFVmax -MFVbaseline] / breath-holding time). We were calculating BHI of each test on the right and left side and then the mean BHI (BHIm) for each participant. The testing of the basilar artery (BA) that represents the posterior cerebral circulation was performed in a seated position, using the same handheld suboccipital insonation probe. Testing was repeated twice to calculate BHIm of the BA. Among the Croatian population, normal BHI values range from 1.03 to 1.65 (17).

Statistical analysis

The sample size was calculated to be 120, effect size 0.35, level of significance 0.05 and power 0.9 (G*power software, version 3.1.9.2, by Franz Faul, University Kiel, Germany). Local ethics committees approved the study.

The category data are represented by absolute and relative frequencies. Differences between categorical variables were tested by the χ 2 test, and, if necessary, by the Fisher exact test. The normality of the distribution of numeric variables was tested by the Shapiro-Wilk test.

Numerical data are described by the arithmetic mean and standard deviation in case of a normal distribution, and by the median and the limits of the interquartile range in cases where the distribution is not normal. The means of the numerical variables of interest were evaluated using a 95% confidence range. The Student's t-test and Mann-Whitney's U test (with Hodges-Lehmann median difference) were used to test the differences in the numerical variables between two independent groups of subjects, depending on the normality of the distribution. The Kruskal-Wallis (post-hoc Conover) test was

used to test for differences in the numerical variables between three or more independent groups. The correlation of the numerical variables was evaluated by the Spearman correlation coefficient ρ (rho). The differences in the numerical variables before and after testing were tested with the Wilcoxon test (19). All P values were two-sided. The level of significance was set at Alpha=0.05. MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019) and SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) were used for all analyses.

Results

The baseline characteristics of COPD patients according to the severity of airflow obstruction were summarized in Table 2.

Table 2. Baseline characteristics, lung functions, laboratory values and pack-years in COPD groups

	Median (interquartile range)				
	Mild	**Moderate**	**Severe/very severe**	**Total**	**P***
Age (years)	66 (59 – 70)	68 (62 – 72)	71 (65 – 78)	68 (62 – 74)	**0.01**
†FEV₁/FVC ratio [Median (interquartile range)]	0.68 (0.67 – 0.69)	0.645 (0.57 – 0.68)	0.41 (0.34 – 0.48)	0.68 (0.67 – 0.69)	**<0.001**
‡ FEV₁ (%)	93.5 (87.8 – 103.5)	64 (56 – 69.3)	37 (31 – 42.8)	64 (42 - 88)	**<0.001**
BMI (kg/m²)	29.4 (26.9 – 31.7)	31.7 (25.9 - 35)	24 (19.9 – 29.2)	28.6 (24.1 - 33)	**<0.001**
Body temperature (ºC)	36.2 (36 – 36.5)	36.1 (36 – 36.3)	36.3 (36 – 36.5)	36.2 (36 – 36.4)	0.26
Systolic blood pressure (mmHg)	140 (130 - 150)	132.5 (120 - 146)	130 (120 – 142.5)	132.5 (120 - 150)	0.21
Diastolic blood pressure (mmHg)	80 (80 - 90)	80 (80 - 85)	78 (70 - 80)	80 (80 - 80)	0.05
Hemoglobin (g/l)	136 (130.5 – 150.3)	147.5 (135 - 154)	144 (131.75 - 158)	144 (133 -151)	0.44
CRP (mg/l) [Median (interquartile range)]	2.6 (1.3 – 4.6)	2.5 (1.6 – 6.2)	2.95 (1.3 – 7.73)	2.6 (1.4 – 6.2)	0.69
Pack-years	40.5 (31.8 – 52.5)	43 (27.6 - 51)	49 (41.5 – 73.88)	43 (33.5 – 54.4)	0.15

*Kruskal-Wallis test (Post-hoc Conover); †on level P < 0.05 significant differences between mild vs. moderate, mild vs. severe/very severe, moderate vs. severe/very severe; ‡on level P < 0.05 significant differences between mild vs. severe/very severe, moderate vs. severe/very severe

FEV1/FVC – forced expiratory volume in one second / forced vital capacity; FEV1 – forced expiratory volume in one second; BMI – body mass index; CRP – C-reactive protein; pack-years = number of cigarettes per day x number of smoking years / 20

Arterial hypertension was significantly less observed in the severe/very severe COPD group ($\chi 2$ test, P = 0.003) and there were no differences in the presence of hyperlipidemia, ischemic heart disease and diabetes mellitus among the COPD groups (data not presented).

Significant differences were found in baseline MFV of MCA. In COPD groups, higher velocity was on the left, and in controls on the right MCA.

Baseline MFV of MCA and BA and also BHImMCA and BHImBA were significantly lower in COPD patients than in controls. TAS was significantly higher in COPD than controls (Table 3).

Table 3. Baseline MFV, cerebral vasoreactivity and TAS in COPD patients and controls

	Median (interquartile range)				
	Controls	P*	COPD	P*	P*
MFV MCA right (cm/s)	37 (29.8 – 44.3)	<0.001	30 (23 - 35)	<0.001	0.004
MFV MCA left (cm/s)	34.5 (30 – 40.5)		31 (23 – 39.25)		0.01
MFV BA (cm/s)	29.5 (26 – 30.3)	-	24.5 (17 – 30.3)	-	0.003
BHI_mMCA	1.24 (1.16 – 1.39)	-	0.8 (0.7 – 0.9)	-	<0.001
BHI_mBA	1.07 (1.02 – 1.15)	-	0.7 (0.6 – 0.9)	-	<0.001
TAS (mmol/L)	1.6 (1.5 – 1.7)	-	1.7 (1.5 – 1.8)	-	0.03

*Mann-Whitney U test; †Wilcoxon test

MFV – mean flow velocity; TAS – total antioxidant status; MCA – middle cerebral artery; BA – basilar artery; BHIm – breath-holding index mean

In COPD groups, based on greater severity of the disease, there were significant reductions in baseline MFV of MCA and BA and we found significant impairment of BHImMCA and BHImBA (Table 4).

Table 4. Baseline MFV, cerebral vasoreactivity and TAS in COPD groups

	Median (interquartile range)						
	Mild	P§	Moderate	P§	Severe/very severe	P§	P*
MFV MCA right (cm/s)	32 (29.8 - 39)	0.78	30.5 (18 – 39.25)	0.53	26 (19 – 32.5)	0.91	0.02†
MFV MCA left (cm/s)	32.5 (25.8 - 41)		31.5 (23 – 37.75)		24.5 (17.8 – 33.3)		0.04†
MFV BA (cm/s)	28 (20.8 – 32.3)	-	22.5 (17 – 30.3)	-	19 (14.5 – 29.3)	-	0.04†
BHI_mACM	0.94 (0.89 – 1.06)	-	0.8 (0.7 – 0.9)	-	0.7 (0.6 – 0.7)	-	<0.001‡
BHI_mAB	0.83 (0.72 – 1.03)	-	0.7 (0.6 – 0.9)	-	0.6 (0.4 – 0.7)	-	<0.001‡
TAS (mmol/L)	1.6 (1.52 – 1.78)	-	1.7 (1.6 – 1.8)	-	1.7 (1.6 – 1.8)	-	0.52

*Kruskal-Wallis test (post-hoc Conover); †on level P < 0.05 significant differences between mild vs. severe/very severe; ‡on level P < 0.05 significant differences between mild vs. moderate, mild vs. severe/very severe, moderate vs. severe/very severe; §Wilcoxon test

MFV – mean flow velocity; TAS – total antioxidant status; MCA – middle cerebral artery; BA – basilar artery; BHIm – breath-holding index mean

Using the Spearman's rank correlation coefficient, a significant negative correlation was found between age and BHImBA in controls. In COPD groups, we found a significant negative correlation between age and BHImMCA and BHImBA. In the COPD group, baseline MFV of BA is in a significant positive correlation with BHImMCA and BHImBA (Table 5).

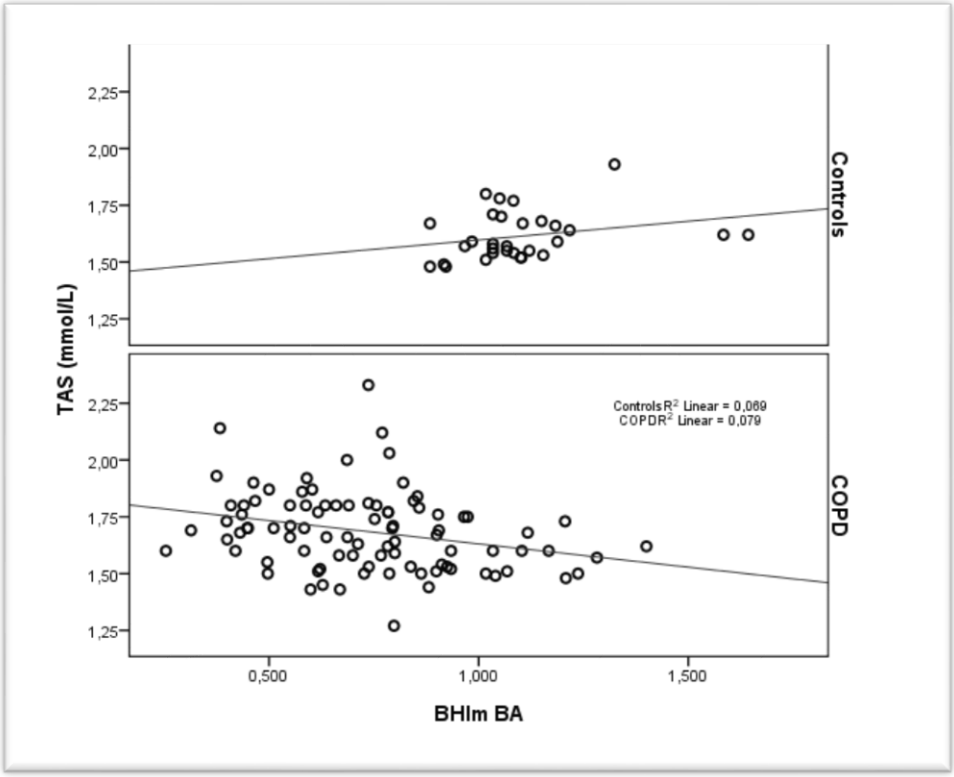
Table 5. Correlation between age and baseline MFV BA with BHIm MCA and BHIm BA in COPD patients and controls

	Spearman's rank correlation coefficient Rho (P value)	
	Age (year)	MFV BA (cm/s)
Controls		
BHI_mMCA	-0.089 (0.64)	0.051 (0.79)
BHI_mBA	-0.376 (0.04)	0.124 (0.51)
COPD		
BHI_mMCA	-0.375 (< 0.001)	0.336 (0.001)
BHI_mBA	-0.376 (< 0.001)	0.647 (<0.001)

MFV – mean flow velocity; BHIm – breath-holding index mean; MCA – middle cerebral artery; BA – basilar artery

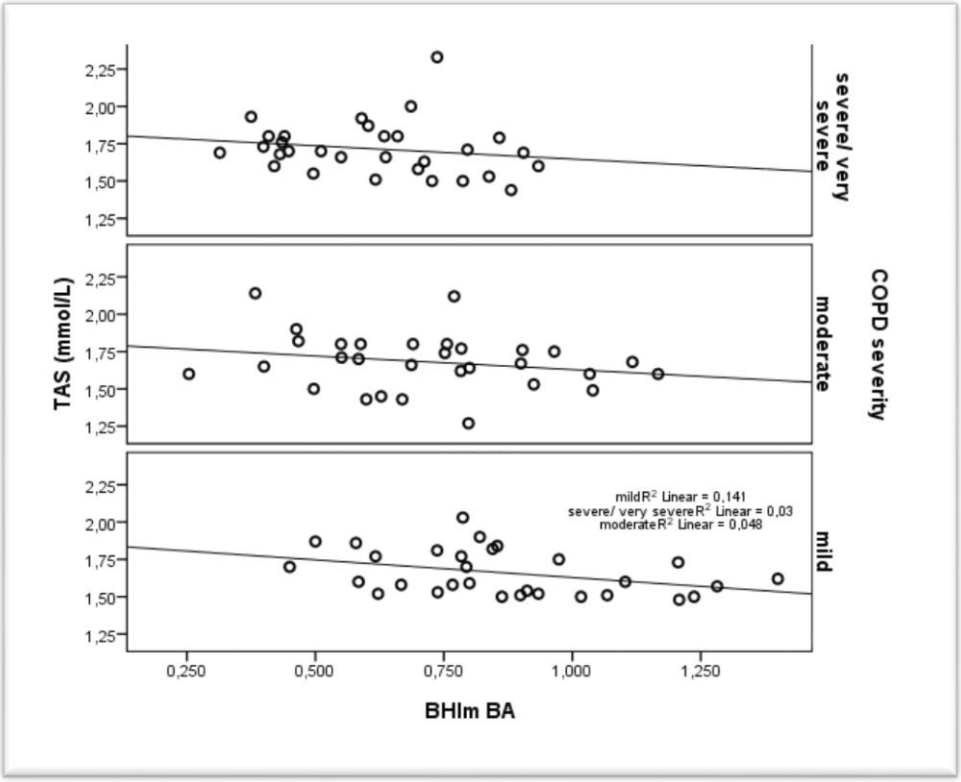
TAS and BHImBA significantly negatively correlated only in COPD groups, but not in the control group (Figure 1).

Figure 1. The correlation between total antioxidant status (TAS) and mean breath-holding index of the basilar artery (BHImBA) in COPD patients and controls



Using the Spearman's rank correlation coefficient Rho, we found a significant negative correlation between TAS and BHImBA (Rho= −0.327, P=0.002) in COPD patients and no significant correlation in controls.

Figure 2. The correlation between total antioxidant status (TAS) and mean breath-holding index of the basilar artery (BHImBA) according to COPD severity

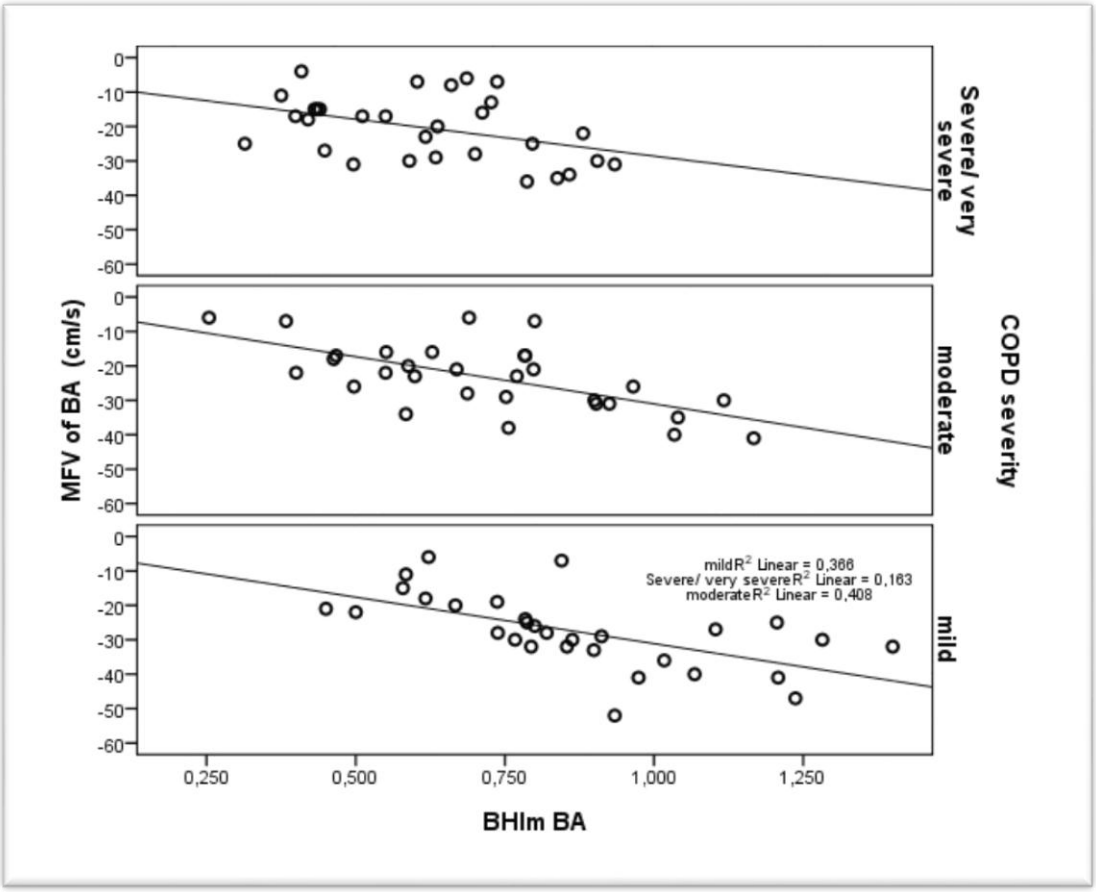


Using the Spearman's rank correlation coefficient Rho, we found a significant negative correlation between TAS and BHIm BA (Rho= −0.445, P=0.01) in the mild COPD groups and no significant correlations in other COPD groups.

There were no significant correlations between TAS and BHImMCA in COPD groups, nor between TAS and BHImMCA in the control group (data not presented). Using the Spearman's rank correlation coefficient Rho, based on the severity of COPD and age, a significant negative correlation was found between age and BHImBA (Rho= −0.398, P=0.03) in the moderate group. In the severe/very severe COPD group, a significant negative correlation was found between age and BHImMCA (Rho= −0.451, P= 0.01), but there were no significant correlations in other COPD groups (data not presented).

In terms of the severity of airway obstruction, we found a significant negative correlation between TAS and BHImBA only in the mild COPD (Figure 2). There was no significant correlation between TAS and BHImMCA in any COPD group (data not presented). In all COPD groups, a significant positive correlation waws found between baseline MFV BA and BHImBA (Figure 3), but there was no correlation between MFV BA and BHImMCA (data not presented).

Figure 3. The correlation between baseline mean flow velocity of the basilar artery (MFV of BA) and mean breath-holding index of the basilar artery (BHIm BA) based on COPD severity



Using the Spearman's rank correlation coefficient Rho, we found a significant positive correlation between baseline MFV BA and BHImBA in all COPD groups: in mild (Rho=0.731, P<0.001), moderate (Rho=0.574, P=0.001) and severe/very severe COPD (Rho=0.398, P=0.03).

Discussion

The aim of the study was to analyze the relationship between CVR and TAS in the plasma of COPD patients and controls. In COPD patients, TAS was in a significant negative correlation with CVR in the posterior cerebral circulation, but in terms of the severity of airflow obstruction, we found a negative correlation between CVR and TAS in the posterior cerebral circulation only in the mild COPD group. There was no significant correlation between CVR and TAS in the anterior cerebral circulation. The results from this study do not allow conclusions that impaired CVR is associated with systemic changes in the antioxidative status of COPD patients.

A significantly higher TAS values were found in COPD patients than in controls, which contrasts the previously published data. The reason for that could be multifactorial, but determining the mechanisms for was in the scope of our recently published manuscript, where we demonstrated that TAS is a predictor of COPD (20).

In terms of gender, there were more men participating in the study than women, but they were equally represented in the COPD groups and in the control group. All women in the study have reached menopause. In that period, oxidative stress was reported to increase because of the decreasing levels of estrogen, which has beneficial vasoactive and antioxidative effects (21). A study of postmenopausal women with moderate COPD

related to smoking analyzed oxidative stress and CVR in the anterior cerebral circulation. The study showed an impaired cerebrovascular response to hypercapnia, significantly higher levels of oxidative stress, a higher level of glutation peroxidase that represents antioxidant enzyme activity and a higher ratio between oxidative stress and antioxidant activity than in controls. There was no significant correlation between the markers of oxidative stress/antioxidants and CVR measured by TCD. The only correlation was found between higher catalase activity, which represents antioxidant enzyme activity associated with higher ventilatory response sensitivity. In the context of higher oxidative stress caused by the decline of estrogen levels in menopause, it was concluded that that could be a possible explanation of the observed differences in cerebrovascular sensitivity to hypercapnia between COPD women and controls (10).

Hypoxemia is part of clinical features of COPD patients. A recent study analyzed how lifetime exposure to hypoxia (3,600 m above sea-level, La Paz, Bolivia) affects oxidative stress, CVR and cognitive function. Chronic mountain sickness is a maladaptation syndrome experienced at high altitudes, characterized by severe hypoxemia. The syndrome is related to a higher risk of stroke, migraine, increased morbidity and mortality. It has been confirmed that individuals with such syndrome have exaggerated oxidative stress and a corresponding decrease in vascular NO bioavailability. Oxidative stress was associated with an impaired cerebrovascular function. The authors of this study concluded that oxidative stress induced by hypoxemia is associated with blunted cerebral perfusion, impaired CVR to hypercapnia, accelerated cognitive decline and depression (18).

In our study, COPD patients had significantly impaired CVR. Impaired CVR, that is impaired CO2 vasoreactivity as a surrogate of endothelial dysfunction, results in a decline of NO (8). A recent study on an animal model showed that an increase in the bioavailability of NO through prostaglandin pathways causes vasodilatation; therefore, it increases oxygenation and produces higher TAS. That way, the antioxidant – TAS eliminates free radicals from biological systems (22). This conclusion is based on a previous study suggesting that as the TAS value increases, free radicals decrease, which protects macromolecules from damage (23). A recent study analyzed age- and apolipoprotein E (APOE) gene-specific hemodynamic changes using the blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (MRI) method. The breath-holding method was used for the examination of CVR and visual stimulation tasks to evoke functional hyperemia. After a three-day intake of nitrate as a NO source, there was no effect on CVR or functional hyperemia, but CVR significantly decreased with age and was dependent on the genotype of participants. That way, the response of vascular functions on breath-holding could be dependent on the APOE-genotype and independent of NO (24). Such an analysis far exceeds our technical capabilities, but it would certainly be useful to include a genetic analysis in the endothelial dysfunction analysis of COPD patients, in order to successfully manage therapeutic decisions, evaluate the response to target interventions and provide prognostic information.

In our study, there were differences between the baseline right and left anterior cerebral perfusion in COPD patients and controls. Determining the mechanism(s) involved in those differences was not the goal of this study. Our opinion is that the differences observed were not significant for the ultimate results of the study.

In COPD, the baseline anterior and posterior cerebral perfusion was significantly lower than in controls. Based on greater severity of COPD, a significant decline of perfusion was recorded in the anterior and posterior cerebral circulation. The baseline posterior cerebral perfusion was in a significant positive correlation with CVR of the anterior and posterior cerebral circulation in COPD, which was not found in controls. In terms of the severity of airway obstruction, all COPD groups had a significant positive correlation between the baseline posterior cerebral

perfusion and CVR in the posterior cerebral circulation. Decreased cerebral perfusion means reduced cerebral blood flow velocity. TCD perfusion analysis assumes that there is no change in the width of the artery lumen and that the blood flow is laminar. Despite that interpretation constraint of TCD, in the study, in cases when lower cerebral perfusion was recorded, that decrease correlated with an impaired ability of the cerebral arteries to respond to vasodilator stimuli, such as hypercapnia (18,25,26).

In our study, a significant negative correlation was found between age and CVR in the posterior cerebral circulation only in the moderate COPD group and between age and CVR in the anterior cerebral circulation in the severe/very severe COPD group. Therefore, the results do not allow a final conclusion on the correlation between the age of COPD patients and CVR in our sample. In the study that used functional TCD and hypercapnic stimuli as a marker of CVR, the cerebral perfusion changes were determined as a response to the language and arithmetic task in healthy young, healthy old and old subjects with the risk factors for atherosclerosis. Arterial hypertension was the most frequent risk factor and the breath-holding method was used as a hypercapnic stimulus. The cerebral perfusion changes were significantly lower during the cognitive task and hypercapnia in the group of old subjects with vascular risk factors compared to the healthy young and old subjects. These results showed that the presence of cardio- and cerebrovascular risk factors significantly reduces the ability of cerebral vessels to react to vasoactive stimuli. Aging alone, without such risk factors, could not be responsible for hemodynamic changes caused by neuronal activation (27). In the study that used the pulsed arterial spin labelling perfusion MRI and BOLD methods, the regional cerebral blood flow and CVR were assessed in young and elderly participants. Age-related decreases in the baseline CBF and CVR were observed in the cerebral cortex, which may be related to the vulnerability to neurological disorders in aging (28). Detection of early dysfunction of the cerebral vascular system in older people with the risk of developing cognitive impairment ensure the possibility of vascular-specific procedures which help maintain cognitive function through a lifetime (29). The TCD ultrasound technique does not allow clear spatial resolution of CVR.

The main limitation of the study was that TAS was the only biomarker that we performed because of technical and financial reasons. In comparison with clinical characteristics and individual biomarker, the combination of COPD markers increases the prognostic value for proper outcome monitoring (30,31). We did not analyze any of the oxidants, so the increased oxidative burden in our population is not clearly proven. On the other side, the choice of peripheral blood among other biological samples is more appropriate because of the non-invasiveness of blood sampling and its property of easily allowing repeated measurements (11).

The main limitations of TCD, and therefore of our study, are its dependency on the operator, the presence of adequate insonation windows and the possibility of recording inaccurate mean flow velocities due to wrong insonation angles (32). The strength of TCD is that the estimation of cerebral blood flow velocity, that is cerebral perfusion and CVR by that method, using CO2 as a hypercapnic stimulus, has acceptable levels of reproducibility. It is an appropriate method for determining the effect of hypercapnia on cerebral haemodynamics (33).

The correlations between CVR and systemic levels of the antioxidant marker of TAS in the plasma have not been demonstrated in all COPD groups. However, based on the decrease in perfusion in the anterior and posterior cerebral circulation detected in COPD patients compared to controls, there is a possible correlation between CVR and the initial perfusion in the basal cerebral arteries.

According to previous studies, there are correlations between impaired CVR and the risk of stroke, progressive global vascular damage, an increased risk of mortality, especially

cardiovascular mortality, cognitive decline and dementia (34–38). Early detection of COPD patients at risk of these comorbidities presents an opportunity for early therapeutic interventions aimed at reducing such risks.

Conclusion

In our study, a negative correlation between CVR and the plasma level of TAS was found only in the posterior cerebral circulation in the mild COPD group. The results do not allow the conclusion that impaired CVR is associated with systemic changes in the antioxidative status of COPD patients. However, we found that decreased perfusion in the basilar artery of COPD patients was in a significant positive correlation with CVR of the anterior and posterior cerebral circulation, which was not observed in controls. The analysis of perfusion in the basal cerebral arteries should be part of a future study of CVR in COPD patients.

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ii

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[ii] **Abbreviations.** COPD – chronic obstructive pulmonary disease; CBF – cerebral blood flow; $PaCO_2$ – arterial partial pressure of carbon dioxide; CO_2 – carbon dioxide; NO – nitric oxide; CVR – cerebral vasoreactivity; TAS – total antioxidant status; GOLD – Global Initiative for Chronic Obstructive Lung Disease; FEV1 – forced expiratory volume in one second; FEV1/FVC – forced expiratory volume in one second FEV1 to forced vital capacity FVC ratio; BMI – body mass index; TCD – transcranial Doppler ultrasound; CRP – C-reactive protein; MCA – middle cerebral artery; MFV – mean flow velocity; BHIm – breath-holding index mean; BA – basilar artery; APOE – apolipoprotein E; BOLD MRI – blood-oxygen-level-dependent magnetic resonance imaging method.