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## **Cardiovascular Risk in COPD: Deciphering the Contribution of Tobacco Smoking**

### **Running head: Cardiovascular risk in COPD and tobacco smoking**

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**Summary conflict of interest statements**

Prof. Roche reports grants and personal fees from Boehringer Ingelheim, Pfizer and Novartis, personal fees from Teva, GSK, AstraZeneca, Chiesi, Mundipharma, Cipla, Sano, Sandoz, 3M, Zambon, outside the submitted work. Prof. Dalphin reports grants, personal fees and non-financial support from Novartis Pharma, personal fees and non-financial support from GSK, Chiesi, Intermune, AstraZeneca and Boehringer Ingelheim, and non-financial support from Stallergenes, outside the submitted work.

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## **ABBREVIATIONS**

AIx = central augmentation index; aPWV = aortic pulse wave velocity; BRS = baroreflex sensitivity; COPD = chronic obstructive pulmonary disease; CVD = cardiovascular disease; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; GOLD = Global lung initiative for Obstructive Lung Disease; HRCT = high-resolution computed tomography; hsCRP = high-sensitivity C-reactive protein; IL = interleukin; LLN = lower limit of normal; OSA = obstructive sleep apnea; PaO<sub>2</sub> = arterial oxygen partial pressure; ST2 = suppression of tumorigenicity 2; TLCO = lung transfer for carbon monoxide; TNFR1 = tumor necrosis factor receptor 1

## **ABSTRACT**

**Background:** The observation that chronic obstructive pulmonary disease (COPD) is an independent risk factor for cardiovascular disease (CVD) comes from comparisons between smokers with COPD and smokers without COPD. The mechanisms that explain increased risk of CVD in patients with COPD are still unclear.

**Objectives:** To assess systemic arterial stiffness (a predictor of CVD mortality) and to evaluate its determinants in a group of patients with mild-to-moderate COPD secondary to organic dust exposure, tobacco smoking or both.

**Methods:** Systemic arterial stiffness was assessed by aortic pulse wave velocity (aPWV). Measurements were made in 142 COPD patients and 155 healthy controls matched for age, sex, body mass index and tobacco smoking, exposed to tobacco smoking (n=56/70 for COPD/controls, respectively), organic dusts (n=44/48), or both (n=42/37).

**Main Results:** aPWV was higher in COPD than in healthy controls in subjects exposed to tobacco smoking and to both dusts and tobacco smoking. By contrast, among never-smokers exposed to organic dusts, COPD patients and matched controls had similar aPWV. Multivariate analysis of the 142 COPD patients (exposed to tobacco smoking and/or to organic dusts) demonstrated that tobacco smoking was associated with high aPWV. Moreover, soluble suppression of tumorigenicity 2 (ST2), a marker of major cardiovascular events, was correlated with aPWV in these patients.

**Conclusions:** Analysis of an unselected group of COPD patients with different causes suggests that 1) COPD by itself is not sufficient to explain increased aPWV, and 2) tobacco smoking is a risk factor for elevated aPWV in COPD.

**KEY WORDS:** cardiovascular diseases; chronic obstructive pulmonary disease; vascular stiffness

Chronic obstructive pulmonary disease (COPD) is a common disease with high global morbidity and mortality.<sup>1</sup> Tobacco smoking is the most frequent cause of COPD in developed countries.<sup>1</sup> Nevertheless, up to 50% of COPD cases worldwide can involve other factors, including indoor and outdoor air pollutants as well as occupational hazards.<sup>2</sup> Among occupational causes, exposure to organic dusts has been associated with accelerated impairment in lung function and with increased risk of COPD.<sup>3-5</sup>

The prevalence of cardiovascular diseases (CVD) is high in patients with smoking-related COPD, and most of these patients die from CVD rather than from respiratory causes.<sup>6-8</sup> Abnormalities of systemic vessels are centrally involved in the genesis of CVD, and measurements of systemic vascular function are therefore used to estimate the risk of CVD.<sup>9</sup> In individuals who are free of clinically apparent heart disease, arterial stiffness, measured non-invasively by aortic pulse wave velocity (aPWV), is an independent predictor of cardiovascular events and mortality.<sup>10</sup> aPWV has been positively associated with aging, chronic elevated blood pressure and tobacco smoking.<sup>9-11</sup> A pathophysiological link between tobacco smoking and arterial stiffness (in COPD as well as in subjects without pulmonary disease) could involve persistent low-grade systemic inflammation.<sup>9,12-14</sup> In this regard, novel biomarkers of CVD have emerged including soluble suppression of tumorigenicity 2 (ST2) which is believed to reflect cardiovascular stress and to have a prognostic value.<sup>15</sup> In addition, sympathetic-vagal imbalance,<sup>16,17</sup> reflected by impaired baroreflex sensitivity (BRS) and hypoxia,<sup>18</sup> may also contribute to the development of increased arterial stiffness.

COPD itself is also believed to be an independent cardiovascular risk factor, as aPWV is higher in patients with COPD than in disease-free controls.<sup>9,19</sup> However, a large majority of studies in this field have been based on comparisons between patients with smoking-related COPD and

matched smokers without COPD.<sup>19</sup> It is therefore impossible to determine whether tobacco smoking remains an independent factor associated with abnormal systemic vascular function in this specific population of patients.

We wondered whether COPD by itself increases cardiovascular risk independent of its underlying risk factors. We therefore measured systemic arterial stiffness (a predictor of cardiovascular events) non-invasively by aortic pulse wave velocity (aPWV) in patients with mild-to-moderate COPD secondary to different exposures (organic dust, tobacco smoking or both). We also aimed to assess potential mechanisms associated with cardiovascular risk in these patients. Therefore we measured systemic inflammation, hypoxia and sympathetic-vagal imbalance.

## **Materials and Methods**

### *Study Design and Subjects*

The current study was a secondary outcome of the BalistiC study (ClinicalTrials.gov Identifier: NCT02540408) that aimed to assess the prevalence of COPD in dairy farmers and in unexposed subjects.<sup>20</sup> Patients and control subjects were consecutively recruited between September 2012 and November 2015 through a regional COPD screening program involving general practitioners and occupational physicians and set up by two national health insurance organizations, as described elsewhere.<sup>20</sup> All patients had a medical history compatible with COPD, a history of risk factors (tobacco smoking and/or daily exposure to organic dusts) and evidence of persistent mild-to-moderate airflow limitation (defined either as a post-bronchodilator forced expiratory volume in 1 second ( $FEV_1$ ) on forced vital capacity (FVC) ratio  $< 0.70$  or as  $FEV_1/FVC < 5^{\text{th}}$  centile

lower limit of normal)<sup>21</sup> together with FEV<sub>1</sub> > 50% of predicted value according to the GLI-2012 equations.<sup>1,22</sup> Patients were in stable condition (no exacerbation of COPD during the previous 6 weeks) and were not taking any oral or inhaled anti-inflammatory drugs, including corticosteroids. COPD subjects were asked to interrupt short and long-acting bronchodilators 72 hours prior to the visit, if required. Exclusion criteria were the following: long-term oxygen therapy; a history of asthma, pulmonary fibrosis, tuberculosis, bronchiectasis, lung cancer, or lung resection; conditions known to affect vascular function, including obstructive sleep apnea, cardiovascular, cerebrovascular, and peripheral vascular disease; uncontrolled hypertension; diabetes; inflammatory conditions such as rheumatoid arthritis or psoriasis.

Healthy subjects participating in the screening program (subjects without airflow limitation or respiratory symptoms) with otherwise identical selection criteria as the COPD patients were also included in the current analysis. These subjects were frequency matched with the COPD patients in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex.

Ethical approval was received from the local ethics committee (CPP Est; P-2011-119) and written consent was obtained from all subjects.

### *Procedures*

*Lung function.* Routine spirometry, constant-volume body plethysmography and single breath lung transfer for carbon monoxide (TLCO) were performed in accordance with recommended techniques (Platinum Elite; MGC Diagnostics Corporation, Saint Paul, Minnesota, USA), as described elsewhere.<sup>23</sup>

*Vascular function.* Studies were performed in the morning, after 30 minutes of supine rest in a quiet, dimly lit, temperature-controlled room (22-25°C). Subjects had fasted overnight and

abstained from coffee, tea, and alcohol for the 24 hours before the study. All medications were withheld on the morning of the study.

Aortic pulse wave velocity (aPWV) was estimated by the noninvasive measurement of carotid-femoral pulse wave velocity using the SphygmoCor™ system (AtCorMedical, Sydney, Australia). Briefly, ECG-gated carotid and femoral artery waveforms were recorded and the distance between the two recording sites was divided by the wave transit time, as described in detail elsewhere.<sup>24,25</sup> A measurement was accepted when it was reproducible three times with minimal variation, and the retained aPWV measurement was the mean of the three measurements.

Baroreflex sensitivity (BRS), a critical physiological feedback mechanism that regulates short-term (beat-to-beat) arterial blood pressure and that demonstrates a negative relationship with aPWV, was assessed as described elsewhere.<sup>26</sup> Briefly, beat-to-beat finger blood pressure and heart rate were measured and recorded over 5 min in the supine position (Finometer-Midi; Finapres Medical Systems, Amsterdam, the Netherlands). Sequences of three or more beats in which progressive increases/decreases in systolic blood pressure were followed by progressive lengthening/shortening in R-R interval were considered as spontaneous baroreflex sequences. A linear regression was calculated for each chosen recording epoch; this slope is considered to depict cardiac BRS.

#### *Laboratory Analysis*

For each patient, blood gases were measured from samples drawn from the arterialized earlobe, and measurements were corrected in order to estimate arterial oxygen partial pressure (PaO<sub>2</sub>).<sup>27</sup> Alveolar oxygen pressure was calculated using the alveolar gas equation.<sup>28</sup> In addition, a venous blood sample was collected in the fasting state, and serum samples were stored at -80°C for

further analyses. Measurement of several inflammatory markers was carried out using a bead-based cytometric immunoassay (R&D Systems Europe, Abingdon, UK). Serum high-sensitivity C-reactive protein (hsCRP) concentrations were measured using a highly sensitive immunonephelometric assay.

### *Statistical Analysis*

Data are presented as number (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively. Quantitative variables were compared with the Student t test or the Wilcoxon test, as appropriate. Qualitative variables were compared with the chi-square ( $\chi^2$ ) test. Bonferroni's correction for multiple comparisons was used as appropriate.

Comparisons were made between six groups defined by their exposures: non-smoking dairy farmers, smokers with no occupational exposure and smoking dairy farmers (with smokers defined as those having smoked on average more than one cigarette, one cigar, or one pipe a day for a year), and by the presence or absence of COPD. To identify factors associated with aPWV among COPD patients, bivariate linear regressions for all candidate factors were performed. The same analyses were repeated twice, once with persistent airflow limitation defined according to the Global lung initiative for Obstructive Lung Disease (GOLD; FEV<sub>1</sub>/FVC <0.70) and once with persistent airflow limitation defined according to the Global Lung function Initiative (GLI; FEV<sub>1</sub>/FVC <LLN). In addition, each variable with  $P < .20$  in the bivariate analysis was then individually analyzed in a model including known confounders, namely, age, BMI and mean blood pressure (multivariate linear regression). Finally, a multivariate backward linear regression model, including all variables associated with aPWV with  $P < .20$  in the bivariate analysis, was performed.

A *P* value < .05 was considered statistically significant. Analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## **Results**

### *Subject Characteristics*

The screening program identified 186 patients with COPD according to the GOLD criterion. Thirty-five patients (19%) were ineligible for the current analysis because of the presence of at least one exclusion criterion (Fig 1). Of the 151 eligible patients, 9 were excluded because of unsuccessful aPWV measurements. The characteristics of these 9 patients were similar to those of the 142 remaining patients (data not shown).

The main characteristics of the 3 subgroups of COPD patients and their healthy counterparts distributed according to exposure (tobacco smoking, organic dusts and both exposures) are given in Table 1. Demographic characteristics of all subgroups were similar. Smoking habits were not significantly different between the subgroups exposed to tobacco smoking. All subgroups exposed to organic dusts also had a similar exposure type and duration (Table 1 and e-Fig 1). The 3 subgroups of COPD patients had similar airflow limitation and similar arterial blood gases. Conversely, the subgroup of patients with COPD due only to exposure to tobacco smoking had a significantly lower TLCO than the other 2 subgroups. Very few COPD patients received long-acting bronchodilators, and the proportion of patients receiving this medication was similar in the 3 subgroups (e-Table 1). About one third had at least one self-reported cardiovascular comorbidity, that is, either high blood pressure, diabetes mellitus and/or dyslipidemia (e-Table 1).

### *Vascular Function*

The group of 142 patients with COPD had a significantly higher aPWV than their healthy matched controls ( $8.7 \pm 2.7$  vs.  $8.0 \pm 1.9$  m/s, respectively,  $P < .01$ ). When analyzing subgroups, aPWV was higher in COPD than in healthy controls only in the 2 subgroups exposed to tobacco smoking: in subjects exposed to tobacco smoking only, aPWV was  $9.0 \pm 2.8$  in patients with COPD vs.  $8.0 \pm 1.9$  in healthy controls ( $P < .02$ ); in subjects exposed to both tobacco and organic dusts, aPWV was  $8.8 \pm 3.0$  (COPD patients) vs.  $7.7 \pm 1.6$  (healthy controls) ( $P < .04$ ). Conversely, in subjects exposed only to organic dusts, aPWV was similar in those with COPD and their matched controls ( $8.4 \pm 2.0$  vs.  $8.3 \pm 2.1$ ) (Table 2 and Fig 2). The central augmentation index (AIx) was similar in all subgroups (Table 2). Among COPD patients, BRS was significantly lower in the subgroup exposed only to tobacco smoking ( $7.3 \pm 4.2$ ) than in the other 2 subgroups ( $9.4 \pm 4.5$  and  $9.8 \pm 4.5$  for subjects exposed to both tobacco and organic dusts and to organic dusts alone, respectively ( $P < .05$  for both)) (Table 2). Of note, COPD patients exposed only to tobacco smoking also had lower diastolic blood pressure than the other 2 subgroups of COPD patients.

### *Factors Associated with aPWV in COPD*

The bivariate analysis performed in the group of 142 COPD patients demonstrated that factors classically associated with poorer systemic vascular function (higher age, higher mean systemic artery pressure and higher body mass index) were significantly correlated with a higher aPWV (Fig 3 and Table 3). In these 142 patients, there was also a significant association between aPWV and the magnitude of tobacco consumption (Fig 3 and Table 3). In addition, the presence of at least one cardiovascular comorbidity, greater gas exchange impairment (lower estimated PaO<sub>2</sub> and higher alveolar-arterial oxygen difference), lower baroreflex sensitivity and several markers

of systemic inflammation and/or of vascular dysfunction were also associated with increased aPWV (Fig 3 and Table 3). In addition, higher alveolar-arterial oxygen difference was associated with higher levels of hsCRP ( $r = .23$ ,  $P = .008$ ) By contrast, in these patients with mild-to-moderate COPD, the magnitude of organic dust exposure, the severity of COPD (assessed by a lower FEV<sub>1</sub>) and the severity of emphysema (assessed by a lower TLCO) did not correlate with aPWV.

After adjustment for age, mean blood pressure and BMI, there was still a significant association between aPWV and the magnitude of tobacco consumption ( $P = .001$ ) (Table 3). The presence of at least one cardiovascular comorbidity ( $P = .002$ ), a higher alveolar-arterial oxygen difference ( $P = .03$ ) and higher sST2 ( $P = .04$ ), a biomarker that provides prognostic information on cardiovascular risk, were also significantly correlated with aPWV.

Moreover, in a multivariate backward linear regression model including variables associated with aPWV in the bivariate analysis, tobacco smoking ( $\beta = 0.027$ ;  $P = .003$ ), alveolar-arterial oxygen difference ( $\beta = 0.052$ ;  $P = .01$ ), mean blood pressure ( $\beta = 0.057$ ;  $P = .001$ ) and age ( $\beta = 0.102$ ;  $P < .0001$ ) were associated with aPWV.

Similar results were found when persistent airflow limitation was defined by an FEV<sub>1</sub>/FVC ratio below the lower limit of normal (e-Table 2).<sup>21</sup>

## **Discussion**

The attempt to dissect the intrinsic contribution of COPD to augmented risk of CVD is complicated by the fact that COPD and CVD share common risk factors, notably smoking.<sup>29,30</sup> To answer that question, a large majority of studies have compared patients with smoking-related

COPD with controls without COPD, matched for tobacco consumption.<sup>19</sup> With such designs, it is impossible to determine whether smoking is 1) solely a risk factor shared by COPD and cardiovascular disease, or 2) a factor that remains associated with increased cardiovascular risk in patients with COPD even after adjustment for known confounders.<sup>30</sup> Using an innovative approach that consisted in analyzing a group of patients with COPD of different causes, that is, exposure to tobacco smoking and/or to organic dusts, we provide here for the first time evidence that tobacco smoking remains associated with a marker of cardiovascular risk in COPD even after adjustment for known confounders. In addition, we find that the magnitude of tobacco smoking, rather than current smoking status, is the main determinant of higher aPWV in patients with mild-to-moderate COPD.

Increased arterial stiffness has been widely reported in patients with COPD, mostly in those with severe forms of the disease.<sup>9,19,25,31,32</sup> Although patients with mild COPD are currently considered at low risk for poor cardiovascular outcomes, systemic vascular dysfunction has been very recently reported in such patients.<sup>18,33</sup> Arterial stiffness is a sign of early cardiovascular damage.<sup>9</sup> By measuring carotid-femoral pulse wave velocity, a surrogate for aPWV that is considered the gold standard for assessment of arterial stiffness, our study corroborates in a large number of unselected consecutive patients the finding of impaired systemic vascular function in the less severe forms of COPD.<sup>33</sup>

The pathophysiological links between cardiovascular risk and COPD remain debated.<sup>9</sup> A link between persistent low-grade systemic inflammation and cardiovascular diseases in COPD has been suggested, but clear evidence for this relationship is still lacking.<sup>12,13</sup> One study has shown a weak positive correlation between arterial stiffness and two inflammatory biomarkers, (interleukin (IL)-6 and soluble tumor necrosis factor receptor 1 (sTNFR1)), in COPD,<sup>32</sup> but this

finding has not been confirmed by others.<sup>25</sup> We found an association between aPWV and sTNFR1 only on bivariate analysis, and we were unable to find any association between aPWV and IL-6. We were also unable to find any correlation between aPWV and hsCRP, an inflammatory marker that was found to be elevated only in a small subset of our patients. Our findings therefore go against the hypothesis of a low-grade systemic inflammation that results from lung-to-plasma spillover of inflammatory mediators and that may determine vascular dysfunction, at least in the mild stages of COPD.

Excess activation of the sympathetic nervous system may explain how COPD leads to the development of increased arterial stiffness.<sup>34</sup> In line with this hypothesis, we found that decreased baroreflex sensitivity, indicating sympathetic/parasympathetic imbalance in favor of the sympathetic system, was associated (in bivariate analysis) with aPWV in our series of patients. Nevertheless, this association was no longer found after adjustment for age, systemic blood pressure and BMI, suggesting that one or more of these factors contributed significantly to BRS impairment.

Chronic hypoxia is thought to alter the structure and function of the vessels and is associated with systemic inflammation.<sup>35,36</sup> In patients with mild to severe COPD, hypoxia has been reported to affect arterial stiffness.<sup>18</sup> In agreement with these results, we found that increased alveolar-arterial difference was associated with both aPWV and hsCRP in mild to moderate COPD. However, the cascade of events linking increased alveolar-arterial difference and systemic inflammation and leading to arterial stiffness could not be clarified.

In recent years, novel biomarkers of CVD have emerged including soluble ST2 (sST2), a member of the interleukin-1 receptor family that is believed to reflect cardiovascular stress and to have a prognostic value.<sup>15</sup> In population-based studies, higher sST2 levels have been associated with

increased risk of heart failure, death and major cardiovascular events.<sup>15</sup> Here, we report for the first time an association between aPWV and sST2 in COPD patients with no overt cardiovascular disease. Further studies are needed to determine whether sST2 has some value in routine clinical practice for the estimation of cardiovascular risk in COPD patients.

In patients with severe COPD, arterial stiffness is independently associated with the severity of emphysema<sup>31</sup> and of airflow limitation.<sup>32</sup> Systemic elastin degradation (elastin fragmentation and collagen replacement in the extracellular matrix) has been suggested as a common mechanism for arterial stiffness and pulmonary emphysema.<sup>37</sup> Even in mild COPD, a positive correlation between arterial stiffness and emphysema burden, as quantified by high-resolution computed tomography (HRCT), has recently been reported in 12 patients with a history of tobacco smoking.<sup>33</sup> Low single breath lung transfer for carbon monoxide (TLCO) has been clearly linked to structural markers of early emphysema.<sup>38</sup> HRCT was not available for emphysema quantification, but we were unable to find any correlation between TLCO and aPWV in our large group of unselected COPD patients. The systemic consequences of COPD caused by exposures other than tobacco smoking have not been fully investigated. In particular, the cardiovascular consequences of COPD secondary to organic dust exposure are unknown.<sup>2</sup> We provide the first evidence that organic dust exposure is not associated with increased risk of CVD in patients with mild-to-moderate COPD. Only one recent study has assessed cardiovascular variables in patients with COPD related to an exposure other than tobacco smoking: Golpe *et al.* compared a group of patients with COPD caused by biomass smoke exposure with a group of patients with tobacco-related COPD matched for sex, age, and pulmonary function.<sup>39</sup> These authors found similar carotid intima-media thickness, number of carotid plaques and endothelial function variables in both patient groups.<sup>39</sup> There could be several explanations for the discrepancies with our findings.

First, Golpe *et al.* studied COPD patients with a mean post-bronchodilator FEV<sub>1</sub> that was ~ 30% lower than in the current study, and it is plausible that cardiovascular alterations depend at least in part on COPD severity (due to severity of lung hyperinflation, inflammation and hypoxemia).<sup>9</sup> Second, exposures to biomass smoke and to organic dust are not equivalent, as biomass smoke is composed of both gaseous pollutants (including carbon monoxide and nitrogen dioxide) and microparticles (including black carbon),<sup>2</sup> while the organic dusts to which dairy farmers are exposed include mostly bacterial and fungal components.<sup>40</sup>

### *Limitations of the Study*

There are several limitations in our study. Firstly, the number of patients in each group was small, leading to some lack of power. Statistical power had been calculated for the primary endpoint of the BalistiC study.<sup>20</sup> For the present study, 76 subjects had to be excluded (therefore, only 297 subjects of the 373 included in the original BalistiC study were analyzed). The initial power calculation was therefore no longer appropriate for the current study, resulting in a lower effective statistical power that could explain the lack of significance of our results. Secondly, we acknowledge that arterial stiffness is only one among other surrogate markers of cardiovascular morbidity in COPD, and we did not evaluate cardiovascular function with other measures such as flow-mediated vasodilation and/or carotid intima-media thickness. Nevertheless, among these markers, arterial stiffness has the strongest predictive value for cardiovascular events beyond that of classic cardiovascular risk factors and is the most suited for use in routine clinical practice.<sup>9,41</sup> Thirdly, some other factors linked to the severity of airway obstruction that may contribute to arterial stiffness, including low level of physical activity, were not specifically assessed. Fourthly, the cross-sectional nature of this study did not permit any assessment of causality.

## **Conclusions**

In addition to confirming that increased arterial stiffness may be found in patients with COPD even at mild-to-moderate severity stages, our study indicates for the first time that tobacco smoking is associated with increased arterial stiffness in patients with an established diagnosis of COPD even after adjustment for known confounders. We also report that patients with mild-to-moderate COPD secondary to organic dust exposure do not have increased arterial stiffness. This suggests that COPD by itself is not sufficient to explain increased arterial stiffness, and that causal factors of the disease are also of importance. Finally, soluble ST2, a marker associated with increased risk of heart failure, death and major cardiovascular events, is correlated with arterial stiffness in COPD and could be of interest for prospective evaluations of cardiovascular risk in COPD patients.

### **Guarantor statement**

TS is the guarantor of the content of the manuscript, including the data and analysis.

### **Author Contributions**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

- BD and TS made substantial contributions to the conception and design of the work.
- AG, LB, SH, MB, BD and TS performed all analyses.
- JCD and BD obtained study funding.
- All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work.
- TS wrote the first draft of the manuscript. BD, TS, MB, SH, SR, FC, PA, NR and JCD revised the manuscript for important intellectual content.
- All authors approved the manuscript.

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**TABLE 1.** Main Characteristics of Patients with COPD and Control Subjects, Separated Into 3 Subgroups According to Exposure to Tobacco Smoking, to Organic Dusts, or Both

	Exposure to tobacco smoking			Exposure to both tobacco and organic dusts			Exposure to organic dusts		
	COPD (n = 56)	Controls (n = 70)	P Value	COPD (n = 42)	Controls (n = 37)	P Value	COPD (n = 44)	Controls (n = 48)	P Value
<b>Demographics</b>									
Age	60.7 ± 8.0	60.4 ± 6.7	.83	58.2 ± 8.3	57.2 ± 8.9	.58	59.6 ± 9.1	58.8 ± 8.7	.67
Men, %	82	74	.29	86	92	.49	82	89	.29
Body mass index, kg.m <sup>-2</sup>	25.7 ± 3.7	26.8 ± 3.7	.09	25.8 ± 3.3	26.9 ± 4.2	.19	25.9 ± 3.8	26.1 ± 3.0	.74
<b>Exposure</b>									
Former/current smokers, %	43 <sup>a</sup> /57 <sup>a</sup>	51/49	.34	55/45	70/30	.16	-	-	
Tobacco, pack-years	34.1 ± 23.2 <sup>a</sup>	28.8 ± 15.9	.15	24.4 ± 16.9	17.6 ± 11.5	.04	-	-	
Exposure to organic dust, years	-	-		37.7 ± 5.9	36.4 ± 5.8	.35	38.2 ± 6.3	37.7 ± 5.9	.73
<b>Pulmonary function tests</b>									
Post-bronchodilator FVC, % predicted	103 ± 13	107 ± 12	.14	105 ± 17	105 ± 14	.91	107 ± 14	106 ± 10	.70
Post-bronchodilator FEV <sub>1</sub> , % predicted	81 ± 14	110 ± 12	< .001	85 ± 15	105 ± 11	< .001	87 ± 14	107 ± 11	< .001
Post-bronchodilator FEV <sub>1</sub> / FVC, %	61 ± 7	80 ± 4	< .001	63 ± 6	79 ± 4	< .001	63 ± 7	78 ± 4	< .001
TLCO, z-score	-1.43 ± 1.33 <sup>a,b</sup>	0.03 ± 1.19	< .001	0.09 ± 1.36	0.54 ± 1.08	.12	-0.36 ± 1.20	0.61 ± 1.16	< .001
PaCO <sub>2</sub> at rest, mmHg	37.5 ± 3.2	37.7 ± 3.2	.68	37.9 ± 4.1	37.0 ± 3.1	.32	36.8 ± 3.8	37.1 ± 2.9	.68
PaO <sub>2</sub> at rest*, mmHg	79.1 ± 10.4	84.6 ± 10.3	.003	83.6 ± 9.9	85.6 ± 10.2	.38	80.0 ± 7.9	86.7 ± 9.6	< .001
Alveolar-arterial difference, mmHg	23.8 ± 10.1	18.1 ± 10.1	.002	19.0 ± 9.4	17.8 ± 11.6	.62	23.7 ± 10.0	16.7 ± 9.9	.001

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; FEV<sub>1</sub> = forced expiratory volume in 1 s; FVC = forced vital capacity; PaCO<sub>2</sub> = arterial carbon dioxide tension; PaO<sub>2</sub> = estimated arterial oxygen tension according to the correction proposed by Aguilaniu *et al.* <sup>27</sup>; TLCO = lung transfer for carbon monoxide.

Data are presented as mean ± SD or as percentages.

<sup>a</sup>: *P* < .01 vs. organic dust COPD; <sup>b</sup>: *P* < .01 vs. COPD with both exposures (Bonferroni's correction).

**TABLE 2.** Vascular Function and Systemic Inflammation in Subjects with COPD and in Matched Controls

	Exposure to tobacco smoking			Exposure to both tobacco and organic dusts			Exposure to organic dusts		
	COPD (n = 56)	Controls (n = 70)	<i>P</i> Value	COPD (n = 42)	Controls (n = 37)	<i>P</i> Value	COPD (n = 44)	Controls (n = 48)	<i>P</i> Value
<b>Vascular function</b>									
Aortic pulse wave velocity, m.s <sup>-1</sup>	9.0 ± 2.8	8.0 ± 1.9	.02	8.8 ± 3.0	7.7 ± 1.6	.04	8.4 ± 2.1	8.3 ± 2.1	.85
Central augmentation index, %	25.6 ± 9.9	25.2 ± 8.1	.79	23.7 ± 9.9	20.0 ± 10.2	.12	21.0 ± 12.0	19.6 ± 10.7	.56
Systolic blood pressure, mmHg	125 ± 15	132 ± 18	.02	126 ± 18	126 ± 15	.96	131 ± 19	129 ± 15	.55
Diastolic blood pressure, mmHg	70 ± 9 <sup>b</sup>	75 ± 9	.006	75 ± 10	75 ± 9	.93	74 ± 11	75 ± 10	.68
Mean blood pressure, mmHg	89 ± 10	94 ± 12	.02	93 ± 12	93 ± 11	.96	94 ± 14	93 ± 11	.96
Cardiac frequency, beats per min	69 ± 11	64 ± 8	.003	68 ± 9	66 ± 11	.42	65 ± 7	66 ± 7	.43
Baroreflex sensitivity, ms.mmHg <sup>-1</sup>	7.3 ± 4.2 <sup>a</sup>	8.5 ± 3.7	.09	9.4 ± 4.5	9.5 ± 4.3	.97	9.8 ± 4.5	9.7 ± 4.5	.85
<b>Systemic inflammatory markers</b>									
hsCRP, mg.L <sup>-1</sup>	3.27 ± 2.78	2.78 ± 2.68	.32	2.15 ± 1.94	2.99 ± 3.50	.21	3.12 ± 4.05	2.06 ± 1.89	.12
sTNFR1, pg.mL <sup>-1</sup>	4,391 ± 1,278 <sup>b</sup>	4,251 ± 912	.51	3,747 ± 708	4,523 ± 2,463	.07	4,244 ± 955 <sup>b</sup>	4,243 ± 1,165	1.00
Interleukin-8, pg.mL <sup>-1</sup>	15.1 ± 11.0 <sup>a</sup>	13.1 ± 5.7	.22	12.1 ± 5.4	11.7 ± 5.3	.72	10.8 ± 4.1	12.7 ± 5.7	.08
sST2, ng.mL <sup>-1</sup>	13.1 ± 5.5	12.5 ± 5.0	.55	13.9 ± 8.7	14.0 ± 4.9	.98	12.9 ± 5.9	15.2 ± 6.1	.09
sICAM-1, ng.mL <sup>-1</sup>	1,031 ± 425	966 ± 481	.43	922 ± 405	895 ± 304	.74	845 ± 325	1,004 ± 437	.05
sVCAM-1, ng.mL <sup>-1</sup>	409 ± 108	381 ± 136	.22	364 ± 106	398 ± 137	.22	386 ± 135	420 ± 170	.30

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; hsCRP = high-sensitivity C-reactive protein; sICAM-1 = intercellular adhesion molecule-1; sST2 = soluble ST2; sTNFR1 = soluble tumor necrosis factor receptor 1; sVCAM-1 = vascular cell adhesion molecule-1.

Data are presented as mean ± SD or as percentages.

<sup>a</sup>: *P* < .017 vs. organic dust COPD; <sup>b</sup>: *P* < .017 vs. COPD with both exposures (Bonferroni's correction).

**TABLE 3.** Simple and Multivariate Linear Regression with Aortic Pulse Wave Velocity as the Dependent Variable in Patients with COPD (n = 142)

	Simple linear regression		Multiple linear regression <sup>#</sup>	
	$\beta$	<i>P</i> Value	$\beta$	<i>P</i> Value
Age, years	0.11	< .001	-	-
Mean systemic blood pressure, mmHg	0.06	.002	-	-
Body mass index, kg.m <sup>-2</sup>	0.17	.005	-	-
Tobacco smoking, pack-years	0.03	.001	0.03	.001
Smoking status ( <i>vs.</i> never-smoker)				
Former smoker	1.00	.07	0.84	.10
Current smoker	0.04	.95	0.64	.20
Organic dust exposure ( <i>vs.</i> no exposure)	-0.43	.35	-0.47	.26
Duration of exposure to organic dust, years	-0.005	.66	-0.02	.15
Post-bronchodilator FEV <sub>1</sub> , % predicted	0.01	.44	0.003	.86
Post-bronchodilator FEV <sub>1</sub> / FVC, %	0.005	.88	0.001	.97
TLCO, % predicted	0.002	.82	0.002	.85
At least one cardiovascular comorbidity, %	1.84	< .001	1.28	.002
Baroreflex sensitivity, ms/mmHg	-0.14	.005	-0.06	.16
PaO <sub>2</sub> at rest, mmHg	-0.07	.004	-0.04	.09
Alveolar-arterial difference, mmHg	0.08	.0004	0.05	.03
Inflammatory markers				
hsCRP, mg.L <sup>-1</sup>	0.04	.63	-0.06	.34
sTNFR1, pg.mL <sup>-1</sup>	0.05	.07	0.03	.26
Interleukin-8, pg.mL <sup>-1</sup>	0.0005	.01	0	.25
sST2, ng.mL <sup>-1</sup>	0.097	.002	0.06	.04
sICAM-1, ng.mL <sup>-1</sup>	0	.95	0	.72
sVCAM-1, ng.mL <sup>-1</sup>	0.004	.02	0	.21

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; hsCRP = high-sensitivity C-reactive protein; FEV<sub>1</sub> = forced expiratory volume in 1 s; FVC = forced vital capacity; sICAM-1 = intercellular adhesion molecule-1; PaO<sub>2</sub> = estimated arterial oxygen tension according to the correction proposed by Aguilaniu *et al.* <sup>27</sup>; sST2 = soluble ST2; TLCO = lung transfer for carbon monoxide; sTNFR1 = soluble tumor necrosis factor receptor 1; sVCAM-1 = vascular cell adhesion molecule-1.

<sup>#</sup>: after adjustment for age, mean blood pressure and body mass index (BMI)

## Figure Legends

Figure 1 – *Flow chart of participants included in the study.*

Subjects were recruited through a screening program set up by two national health insurance organizations. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma and hypersensitivity pneumonitis.

Abbreviations: aPWV = aortic pulse wave velocity; COPD = chronic obstructive lung disease; OSA = obstructive sleep apnea.

Figure 2 – *Aortic pulse wave velocity in patients with COPD and controls.*

\*  $P < .05$  versus controls.

Figure 3 – *Relationship between aPWV and (A) age, (B) tobacco smoking, (C) baroreflex sensitivity, (D) alveolar-arterial difference, (E) soluble ST2 and (F) soluble VCAM-1 in subjects with mild-to-moderate COPD*





