Endothelial Microparticles in Mild Chronic Obstructive Pulmonary Disease and Emphysema
The Multi-Ethnic Study of Atherosclerosis Chronic Obstructive Pulmonary Disease Study

Michael A. Thomashow[1]*, Daichi Shimbo[1]*, Megha A. Parikh[1], Eric A. Hoffman[2], Jens Vogel-Claussen[3,4], Katja Hueper[3,4], Jessie Fu[1], Chia-Ying Liu[3], David A. Bluemke[3,5], Corey E. Ventetuolo[6], Margaret F. Doyle[7], and R. Graham Barr[1,8]

[1]Department of Medicine, College of Physicians and Surgeons, and [8]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York; [2]Department of Radiology, University of Iowa, Iowa City, Iowa; [3]Department of Radiology, Johns Hopkins University, Baltimore, Maryland; [4]Department of Radiology, Hannover Medical School, Hannover, Germany; [5]Radiology and Imaging Sciences, NIH Clinical Center, Bethesda, Maryland; [6]Department of Medicine, Alpert School of Brown University, Providence, Rhode Island; and [7]Department of Pathology, University of Vermont, Burlington, Vermont

Rationale: Basic research implicates alveolar endothelial cell apoptosis in the pathogenesis of chronic obstructive pulmonary disease (COPD) and emphysema. However, information on endothelial microparticles (EMPs) in mild COPD and emphysema is lacking.

Objectives: We hypothesized that levels of $CD31^+$ EMPs phenotypic for endothelial cell apoptosis would be elevated in COPD and associated with percent emphysema on computed tomography (CT). Associations with pulmonary microvascular blood flow (PMBF), diffusing capacity, and hyperinflation were also examined.

Methods: The Multi-Ethnic Study of Atherosclerosis COPD Study recruited participants with COPD and control subjects age 50–79 years with greater than or equal to 10 pack-years without clinical cardiovascular disease. $CD31^+$ EMPs were measured using flow cytometry in 180 participants who also underwent CTs and spirometry. $CD62E^+$ EMPs phenotypic for endothelial cell activation were also measured. COPD was defined by standard criteria. Percent emphysema was defined as regions less than -950 Hounsfield units on full-lung scans. PMBF was assessed on gadolinium-enhanced magnetic resonance imaging. Hyperinflation was defined as residual volume/total lung capacity. Linear regression was used to adjust for potential confounding factors.

Measurements and Main Results: $CD31^+$ EMPs were elevated in COPD compared with control subjects ($P = 0.03$) and were notably increased in mild COPD ($P = 0.03$). $CD31^+$ EMPs were positively related to percent emphysema ($P = 0.045$) and were inversely associated with PMBF ($P = 0.047$) and diffusing capacity ($P = 0.01$). In contrast, $CD62E^+$ EMPs were elevated in severe COPD ($P = 0.003$) and hyperinflation ($P = 0.001$).

Conclusions: $CD31^+$ EMPs, suggestive of endothelial cell apoptosis, were elevated in mild COPD and emphysema. In contrast, $CD62E^+$ EMPs indicative of endothelial activation were elevated in severe COPD and hyperinflation.

Keywords: chronic obstructive pulmonary disease; emphysema; antigens, CD31; endothelium; pulmonary disease

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Prior research using animal models has implicated the primary destruction of the pulmonary capillary bed in the pathogenesis of chronic obstructive pulmonary disease (COPD) and emphysema. The relevance of these findings to clinical disease in humans is incompletely understood. Endothelial microparticles are microscopic vesicles released into the blood in response to endothelial cell perturbation.

What This Study Adds to the Field

This paper demonstrates that endothelial microparticles suggestive of endothelial cell apoptosis are elevated in COPD and, notably, mild COPD and are positively related to percent emphysema on computed tomography. These cellular markers link endothelial cell apoptosis with COPD and emphysema.

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the United States (1) and is projected to be the third leading cause of death worldwide by 2020 (2). COPD is defined as airflow obstruction that is not fully reversible (3). Many patients with COPD have emphysema, which is characterized by the destruction of alveolar walls with permanent loss of lung architecture and parenchyma (4).

Cigarette smoking, the primary cause of COPD (3), is known to cause endothelial dysfunction (5). Cigarette smoke is delivered directly to pulmonary endothelial cells and contains multiple factors including acrolein that cause endothelial apoptosis (6). Increased endothelial cell apoptosis has been observed in the lung tissue of patients with emphysema compared with control subjects (7, 8). Additionally, reductions in vascular endothelial growth factor (VEGF) and its receptor have been noted

in lung tissue of patients with severe emphysema (8) and COPD (9). In murine models, blockade of VEGF receptor and ceramide up-regulation cause alveolar endothelial apoptosis and emphysema-like changes (10–12); however, the relevance of this work to clinical disease is unclear because the applicability of animal models of COPD to human disease remains controversial (13).

Studies in humans show that endothelial dysfunction, assessed by flow-mediated dilation of the brachial artery, is present in early COPD and is linearly related to decrements in FEV_1 and greater percentage of emphysema-like lung (hereafter referred to as percent emphysema) on computed tomography (CT) among smokers with and without COPD (14, 15). Flow-mediated dilation, however, does not provide information at the cellular level.

Endothelial microparticles (EMPs) (0.1 <1.5 μm in diameter) are vesicles shed from endothelial plasma membranes into the circulation in response to endothelial cell perturbation (16). An EMP contains a number of endothelial cell surface proteins, the composition of which is dependent on the stimulus contributing to its release (17). EMPs expressing CD31 (platelet-endothelial cell adhesion marker 1) are phenotypic for endothelial cell apoptosis (16, 17). In contrast, EMPs expressing CD62E (E-selectin) are phenotypic for endothelial activation (16, 17), and EMPs expressing CD51 (vitronectin receptor) are less specific, reflecting chronic injury (18, 19).

Plasma EMP levels are increased in various vascular-related disorders. $CD31^+$ EMPs are elevated in cardiovascular disease (19), end-stage renal disease (20), pulmonary arterial hypertension (21), sleep apnea (22), severe hypertension (23), and type 2 diabetes (24). $CD62E^+$ EMPs are also elevated in cardiovascular disease (25), pulmonary arterial hypertension (21), and sleep apnea (22). $CD51^+$ EMPs are elevated in type 1 diabetes (26) and multiple sclerosis (18). Plasma EMPs are also elevated in symptomatic and asymptomatic smokers compared with nonsmokers, and among nonsmokers exposed to cigarette smoke (27, 28).

$CD31^+$ EMPs were recently associated with an isolated reduction in the diffusing capacity of carbon monoxide ($D_{L_{CO}}$) (27) and with COPD and its exacerbations (29). The clinical relevance of the former, however, is uncertain and the power of the latter study was not adequate to examine mild COPD or emphysema.

We therefore examined the relationships of circulating levels of EMPs with COPD in a study designed specifically to test the hypothesis that $CD31^+$ EMPs are elevated in mild COPD and emphysema on CT scan. In addition, we examined relationships of EMPs to pulmonary microvascular blood flow (PMBF) assessed on magnetic resonance imaging (MRI), and to $D_{L_{CO}}$ and hyperinflation. Some of the results have previously been reported in abstract form (30, 31).

METHODS

Study Sample

The Multi-Ethnic Study of Atherosclerosis (MESA) COPD Study enrolled cases of COPD and control subjects from two prospective population-based cohort studies, MESA (32) and the Emphysema and Cancer Action Project (EMCAP) (33), who were 50–79 years old with a 10 or more pack-year smoking history and who did not have clinical cardiovascular disease, stage IIIb-V kidney disease, asthma before age 45 years, other lung disease, prior lung resection, cancer, allergy to gadolinium, claustrophobia, metal in the body, pregnancy, or weight greater than 300 lb. We selected all eligible participants in the MESA Lung Study (34) and oversampled participants with COPD or emphysema from the remainder of MESA and EMCAP, in addition to a small number from neither study. The current report includes participants from the one site (Columbia University) where EMPs were measured.

Protocols were approved by the institutional review boards of the participating institutions and the NHLBI. Written informed consent was obtained from all participants.

Endothelial Microparticles

Preparation of EMP samples and measurement using flow cytometry were performed as previously described (17, 19) and as detailed in the online supplement. To exclude the possibility of the unintended measurement of platelet microparticles, EMPs were defined as microparticles positively labeled by CD31 and negatively labeled by CD42, which is expressed only on platelets (19) ($CD31^+$ EMPs); positively labeled by CD51 and negatively labeled by CD42 ($CD51^+$ EMPs); and positively labeled by CD62E ($CD62E^+$ EMPs).

Spirometry

Spirometry was conducted in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (35) on a dry-rolling-sealed spirometer (Occupational Marketing, Inc., Houston, TX). COPD was defined as a post-bronchodilator ratio of FEV_1 to FVC less than 0.70 (2, 3). COPD severity was classified as follows: mild, FEV_1 greater than or equal to 80% predicted; moderate, 50–80% predicted; and severe, FEV_1 less than 50% predicted (3).

Percent of Emphysema-like Lung

All participants underwent full-lung CTs on General Electric 64-slice helical scanners following the MESA-Lung/SPIROMICS full-inspiration protocol (*see* online supplement) (36). Image attenuation was assessed using APOLLO software (VIDA Diagnostics, Coralville, IA) at a single reading center by trained readers without knowledge of other participant information. Percent emphysema was defined as the percentage of total voxels within the lung field below −950 Hounsfield units (HU).

Magnetic Resonance Imaging

Images were obtained using a 1.5-T whole-body MR (Signa LX; GE Healthcare, Waukesha, WI) with phased-array coil for signal reception. Participants underwent dynamic first-pass contrast-enhanced MR of the thorax at functional residual capacity using a coronal three-dimensional gradient echo time resolved imaging of contrast kinetics sequence with a temporal resolution of 1.2–1.8 seconds per frame. After a nonenhanced mask scan, a bolus of 0.1 mmol/kg bodyweight gadolinium–diethylenetriamine pentaacetic acid (Magnevist; Berlex; Wayne, NJ) was injected at 5 ml per second, followed by a saline flush of 20 ml at the same injection rate. Regional PMBF was assessed from a γ-variate function fitted to the signal intensity-time curve of the lung parenchyma (37). Slope increase was defined as the maximum signal increase per time interval.

$D_{L_{CO}}$ and Plethysmography

Single-breath $D_{L_{CO}}$ was measured with a Sensormedics Autobox 220 Series instrument (Viasys Healthcare, Yorba Linda, CA) following ATS/ERS guidelines (38). Body plethysmography was performed using a V6200 Series Autobox (Sensormedics, Yorba Linda, CA) following ATS/ERS recommendations (39).

Covariates

Age, sex, race and ethnicity, educational attainment, smoking status, pack-years, and medical history were self-reported. Height, weight, blood pressure, oxygen saturation, high-density lipoprotein, low-density lipoprotein, and fasting plasma glucose were measured using standardized approaches, and smoking status was confirmed by cotinine (*see* online supplement).

Statistical Analysis

Because EMP counts were skewed in distribution, values were log-transformed to improve normality. Associations between EMPs and COPD severity were initially tested with a linear contrast assuming

the ranked categories of COPD severity were equally spaced, in analysis of variance. Linear regression models were then used to adjust for potential confounders, which were selected based on biologic plausibility and examination of correlations with covariates (*see* Table E1 in the online supplement). The base model was adjusted for age, sex, race and ethnicity, and cohort of selection. We then additionally adjusted for smoking status and pack-years. The full model was additionally adjusted for potential confounders of educational attainment, diabetes, hypertension, oxygen saturation, physician-diagnosed sleep apnea, height, weight, and body mass index in addition to statin use (which may raise EMP levels [40]), high-density lipoprotein (which may affect endothelial health and is related to percent emphysema [41]), and white blood cell count (which, if fragmented, could theoretically be included in $CD31^+$ counts [42]). Models for percent emphysema were additionally adjusted for milliamperes. Models for pulmonary perfusion were additionally adjusted for cardiac output. Additional details on the statistical methods and sensitivity analyses are included in the online supplement.

RESULTS

The study included 180 participants with spirometry, CT, and EMP measures (Figure 1). The mean age of the participants was 68 (SD, 7) years and 58% had COPD (22% mild, 25% moderate, and 11% severe). Thirty-two percent smoked currently and the median pack-years was 38 (interquartile range, 23.3–52.3). The race-ethnic distribution was 57% white, 25% African-American, 16% Hispanic, and 2% Chinese-American. Participants with more severe COPD were more likely to be male, white, and have greater pack-years (Table 1). Of this population, 149 participants completed the gadolinium-enhanced MRI for the perfusion analysis, whereas 118 participants completed $D_{L_{CO}}$ and plethysmography (Figure 1).

EMPs and COPD and Its Severity

$CD31^+$ EMP levels were elevated in COPD compared with control subjects in the fully adjusted model (adjusted mean difference, 0.21 log EMP per microliter; 95% confidence interval [CI], 0.02–0.40; $P = 0.03$). Levels of $CD51^+$ and $CD62E^+$ EMPs were also higher in COPD compared with control subjects, but these differences did not attain statistical significance in the fully

adjusted model (adjusted mean differences of 0.23 log $CD51^+$ EMP per microliter, 95% CI −0.02 to 0.48, $P = 0.07$; and 0.20 log $CD62E^+$ EMP per microliter, 95% CI −0.03 to 0.42, $P = 0.08$).

$CD31^+$ EMPs differed by COPD severity (Table 2; *see* Figure E1) and were significantly elevated not only in severe COPD but also in mild COPD compared with control subjects in adjusted analyses. The magnitude of the association of $CD31^+$ EMPs with mild COPD increased with adjustment particularly for age and race-ethnicity, differences that had attenuated the association in the unadjusted analysis. In contrast, $CD51^+$ EMPs were not significantly elevated and $CD62E^+$ EMPs were only elevated in severe COPD compared with control subjects.

$CD31^+$ EMPs were inversely related to the percent predicted FEV_1 ($P = 0.04$), as were $CD62E^+$ EMPs ($P = 0.02$). However, depiction of these relationships using generalized additive models, which do not force the multivariate relationship to be linear, showed different relationships of EMPs to the percent predicted FEV_1 with an early increase in $CD31^+$ EMPs and a late increase for $CD62E^+$ EMPs (Figure 2).

EMPs and Percent Emphysema

$CD31^+$ EMPs were significantly associated with percent emphysema in adjusted models (Table 3). $CD31^+$ EMPs were increased monotonically across categories of percent emphysema and the relationship of the continuous measures was linear without evidence of a threshold effect (Figure 3). In contrast, there was no significant increase in $CD51^+$ EMPs or $CD62E^+$ EMPs with percent emphysema.

EMPs, PMBF, and Diffusing Capacity

$CD31^+$ EMPs were inversely related to pulmonary microvascular perfusion as assessed by slope increase on contrast-enhanced MR among the 149 participants who completed (Table 4). No significant associations were found between changes in slope increase and the mean number of $CD51^+$ or $CD62E^+$ EMPs.

$CD31^+$ EMPs were inversely associated with $D_{L_{CO}}$ and $D_{L_{CO}}/V_A$, whereas there was no association of $CD51^+$ or $CD62E^+$ EMPs with diffusing capacity in the fully adjusted model (Table 4, Figure 4a).
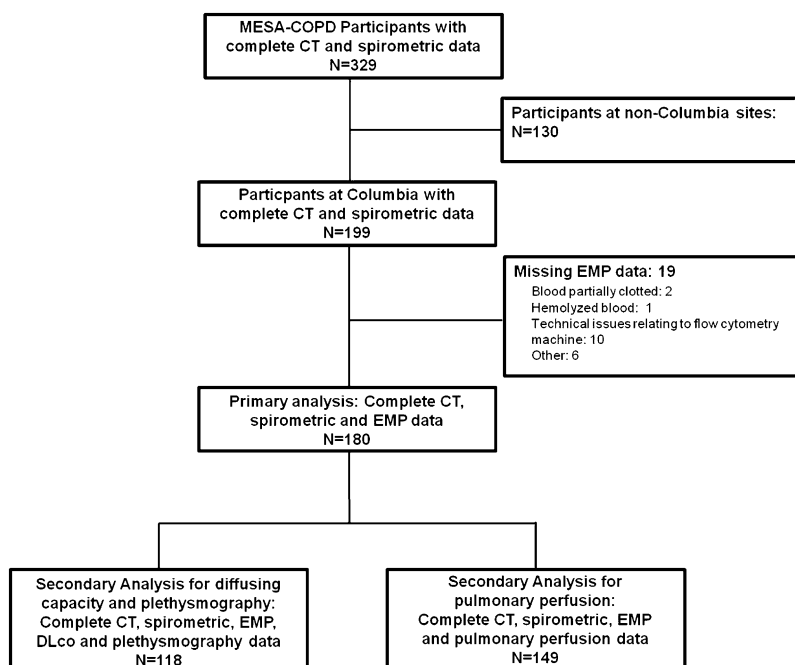

Figure 1. Flowchart of study participants. COPD = chronic obstructive pulmonary disease; CT = computed tomography; EMP = endothelial microparticle; MESA = Multi-Ethnic Study of Atherosclerosis.

TABLE 1. CLINICAL CHARACTERISTICS OF PARTICIPANTS IN THE MESA COPD STUDY WITH MEASURES OF ENDOTHELIAL MICROPARTICLES STRATIFIED BY COPD SEVERITY

	Control Subjects (*n* = 76)	COPD Mild (*n* = 39)	Moderate (*n* = 46)	Severe/Very Severe (*n* = 19)
Age, mean (SD), yr	68.9 (5.6)	69.2 (6.7)	67.3 (8.3)	66.2 (7.3)
Sex, male, No. (%)	39 (51.3)	27 (69.2)	26 (56.5)	14 (73.7)
Race–ethnicity				
White, No. (%)	40 (52.6)	25 (64.10)	25 (55.6)	13 (68.4)
African American, No. (%)	14 (18.4)	10 (25.6)	15 (32.6)	6 (31.6)
Other, No. (%)	22 (29.0)	4 (10.3)	6 (13.0)	0 (0.0)
Educational attainment				
≤High school degree, No. (%)	23 (30.3)	7 (18.4)	12 (26.1)	3 (15.8)
Some college/associate degree/vocational school, No. (%)	22 (29.0)	8 (21.1)	11 (23.9)	8 (42.1)
≥College degree, No. (%)	31 (40.8)	24 (61.5)	23 (50.0)	8 (42.1)
Height, mean (SD), cm	166.43 (9.76)	171.15 (8.80)	169.42 (9.69)	171.78 (10.61)
Weight, mean (SD), kg	79.60 (18.20)	79.05 (14.70)	77.91 (19.89)	81.26 (20.43)
Body mass index, mean (SD), kg/m²	28.63 (5.73)	26.89 (3.89)	26.90 (5.55)	27.31 (5.21)
Cigarette smoking status				
Former, No. (%)	56 (73.7)	29 (74.4)	23 (50.0)	14 (68.42)
Current, No. (%)	20 (26.3)	10 (25.6)	23 (50.0)	5 (26.32)
Pack-years of smoking, median (IQR)	32.0 (20.7–47.5)	40.4 (25.0–63.0)	40.0 (36.0–54.7)	40.0 (20.0, 67.5)
Low-density lipoprotein, mean (SD), mg/dl	110.99 (33.57)	108.05 (31.03)	95.54 (29.25)	102.37 (30.95)
High-density lipoprotein, mean (SD), mg/dl	56.69 (16.69)	59.54 (18.15)	59.87 (21.58)	56.74 (18.64)
Triglycerides, mean (SD), mg/dl	103.92 (42.37)	99.77 (40.04)	108.43 (47.53)	125.11 (70.37)
Cholesterol, mean (SD), mg/dl	188.51 (41.24)	187.54 (37.54)	177.09 (32.43)	184.05 (40.98)
Systolic blood pressure, mean (SD), mm Hg	121.86 (17.28)	120.80 (14.33)	125.45 (15.11)	126.89 (12.19)
Diastolic blood pressure, mean (SD), mm Hg	69.66 (9.93)	71.59 (9.64)	72.83 (9.02)	76.50 (9.72)
Hypertension, No. (%)	25 (32.89)	14 (35.90)	19 (41.30)	8 (42.11)
Fasting plasma glucose, median (IQR),mg/dl	98.5 (92.0–110.0)	100.0 (89.0–108.0)	102.0 (97.0–113.0)	100.0 (87.0–115.0)
Diabetes mellitus, No. (%)	13 (17.1)	6 (15.4)	6 (15.4)	5 (26.3)
Medication use				
Statin, No. (%)	32 (42.1)	18 (46.2)	22 (47.8)	6 (31.6)
ACE inhibitors or angiotensin antagonists, No. (%)	17 (22.4)	13 (33.3)	16 (34.8)	5 (26.3)
Calcium channel blockers, No. (%)	9 (11.8)	2 (5.1)	10 (21.7)	6 (31.6)
β-Blockers, No. (%)	9 (11.8)	5 (12.8)	9 (19.6)	2 (10.5)
Omega-3, No. (%)	12 (15.8)	3 (7.7)	6 (13.0)	0 (0.0)
Inhaled or systemic corticosteroids, No. (%)	3 (4.0)	3 (7.7)	6 (13.0)	17 (89.5)
Aspirin, No. (%)	39 (51.3)	23 (59.0)	22 (47.8)	6 (31.6)
Short-acting β agonists, No. (%)	0 (0.0)	2 (5.1)	9 (19.6)	15 (79.0)
Long-acting β agonists, No. (%)	2 (2.6)	1 (2.6)	2 (4.4)	4 (21.1)
Short-acting anticholinergics, No. (%)	0 (0.0)	0 (0.0)	3 (6.5)	2 (10.5)
Long-acting anticholinergics, No. (%)	1 (1.3)	2 (5.1)	6 (13.0)	15 (79.0)
White blood cell count, mean (SD), billion/L	6.42 (1.67)	6.31 (1.37)	7.17 (1.98)	7.71 (2.43)
Neutrophils, mean (SD), %	58.45 (8.63)	58.39 (8.65)	57.09 (11.76)	62.42 (10.40)
Monocytes, mean (SD), %	7.63 (2.34)	8.77 (2.55)	8.14 (2.40)	8.84 (1.89)
Lymphocytes, mean (SD), %	30.09 (6.87)	29.51 (7.87)	31.70 (10.68)	25.53 (8.35)
Hemoglobin, mean (SD), g/L	13.74 (1.38)	14.14 (0.89)	13.80 (1.24)	14.23 (1.03)
Platelet count, mean (SD), billion/L	223.81 (59.14)	222.56 (48.45)	235.87 (57.77)	231.84 (53.91)
FEV$_1$ percent of predicted, mean (SD)	99.05 (17.77)	91.42 (10.50)	68.50 (7.87)	38.86 (7.16)
FVC percent of predicted, mean (SD)	98.01 (17.19)	108.68 (13.52)	91.66 (13.35)	74.48 (22.17)
FEV$_1$/FVC ratio, mean (SD), %	0.77 (0.04)	0.63 (0.06)	0.57 (0.09)	0.38 (0.07)
D$_{LCO}$ % predicted, mean (SD), %, n = 118	67.52 (10.98)	64.31 (11.93)	56.15 (14.37)	40.07 (13.91)
D$_{LCO}$ VA % predicted, mean (SD), %, n = 118	80.21 (13.03)	70.31 (14.91)	72.50 (20.94)	59.02 (19.70)
RV % predicted, mean (SD), %, n = 118	69.22 (19.41)	84.15 (19.35)	96.32 (29.06)	136.81 (28.57)
TLC % predicted, mean (SD), %, n = 118	88.72 (12.55)	100.15 (11.47)	92.81 (13.33)	99.49 (12.73)
RV/TLC ratio, mean (SD), %, n = 118	0.31 (0.08)	0.31 (0.06)	0.39 (0.08)	0.49 (0.08)
Percent emphysema$_{-910}$, median (IQR)	10.83 (5.02–18.59)	22.87 (12.78–34.02)	18.26 (10.31–30.06)	37.59 (28.07–39.95)
Percent emphysema$_{-950}$, median (IQR)	0.74 (0.40–1.43)	2.74 (1.05–5.20)	2.44 (0.76–6.31)	14.27 (6.16–26.68)
Oxygenation saturation, mean (SD), %	93.30 (6.78)	96.70 (2.28)	94.97 (7.11)	95.17 (3.01)
Home oxygen therapy, No. (%)	1 (1.32)	0 (0.00)	1 (2.17)	8 (42.11)
Sleep apnea, self-reported, No. (%)	5 (6.58)	3 (7.69)	5 (10.87)	3 (15.79)

Definition of abbreviations: ACE = angiotensin-converting enzyme; COPD = chronic obstructive pulmonary disease; D$_{LCO}$ = diffusing capacity of the lung for carbon monoxide; IQR = interquartile range; MESA = Multi-Ethnic Study of Atherosclerosis; RV = residual volume; V$_A$ = alveolar volume.

EMPs and Hyperinflation

As Table 4 shows, in contrast to findings for percent emphysema and pulmonary perfusion, CD62E$^+$ EMPs were highly significantly related to hyperinflation characterized by both higher RV and RV/TLC ratio (Figure 4b), whereas CD31$^+$ EMPs displayed no association with RV or RV/TLC ratio (Table 4).

Sensitivity Analyses

Sensitivity analyses demonstrated similar associations for CD31$^+$ EMPs and COPD with additional adjustment for use of long-acting β agonists, inhaled corticosteroids, long-acting anticholinergics, and omega-3 polyunsaturated fatty acids, and an interaction term between cohort and case status. The results also did not change after restriction to MESA and EMCAP cohorts; former

TABLE 2. ENDOTHELIAL MICROPARTICLE COUNTS BY COPD SEVERITY

	Control Subjects ($n = 76$)	Mild ($n = 39$)	Moderate ($n = 45$)	Severe ($n = 19$)	P Value for Linear Trend Across COPD Severity
CD31$^+$ endothelial microparticles per microliter, log-transformed					
Mean (SD)	7.11 (0.58)	7.12 (0.49)	7.26 (0.71)	7.52 (0.51)	0.01
Predicted mean, model 1*	7.10	7.30	7.27	7.49†	0.02
Predicted mean, model 2‡	7.10	7.32†	7.30	7.51†	0.01
Predicted mean, model 3§	7.07	7.31†	7.27	7.40†	0.03
CD51$^+$ endothelial microparticles per microliter, log-transformed					
Mean (SD)	6.80 (0.79)	6.91 (0.68)	6.94 (0.78)	7.17 (0.83)	0.07
Predicted mean, model 1	6.80	7.06	6.89	7.00	0.43
Predicted mean, model 2	6.80	7.08	6.94	7.02	0.80
Predicted mean, model 3	6.71	7.12†	6.85	6.83	0.47
CD62E$^+$ endothelial microparticles per microliter, log-transformed					
Mean (SD)	5.98 (0.62)	6.09 (0.68)	6.02 (0.71)	6.35 (0.69†)	0.047
Predicted mean, model 1	5.98	6.15	6.11	6.53†	0.05
Predicted mean, model 2	5.98	6.16	6.12	6.54†	0.05
Predicted mean, model 3	5.98	6.22	6.11	6.51†	0.03

Definition of abbreviations: COPD = chronic obstructive pulmonary disease.
* Model 1 adjusted for age, sex, race and ethnicity, and cohort.
† P value less than 0.05, compared with control subjects.
‡ Model 2 adjusted for variables in model 1 in addition to smoking status, and pack-years.
§ Model 3 adjusted for variables in model 2 in addition to educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, and statin use.

smokers; white participants; and those without hypertension, diabetes, asthma after the age of 45, or sleep apnea (*see* Figure E2). The relationship of CD31$^+$ EMPs to mild COPD and percent emphysema were also consistent across these sensitivity analyses (*see* Figures E3 and E4, respectively), as was the relationship of CD62E$^+$ EMPs to RV/TLC ratio (*see* Figure E5). Restriction to participants without subclinical cardiovascular disease strengthened the association of CD31$^+$ EMPs and percent emphysema (*see* Figure E4) and slightly attenuated the association of CD31$^+$ EMPs with COPD status and attenuated the association with mild COPD (*see* Figures E2 and E3, respectively). Such restriction yielded similar associations between CD62E$^+$ EMPs and the RV/TLC ratio (*see* Figure E5).

To better define the apoptotic phenotypes, we additionally adjusted analyses of CD31$^+$ EMPs with COPD and percent emphysema for CD62E$^+$ EMPs. Such further adjustment yielded similar although slightly attenuated associations for COPD status ($P = 0.11$) and percent emphysema ($P = 0.06$).

DISCUSSION

CD31$^+$ EMPs, which are suggestive of endothelial cell apoptosis, were elevated in COPD compared with control subjects and this elevation was observed not only in severe COPD but also in mild COPD. Higher levels of CD31$^+$ EMPs were also associated with the percent emphysema on CT scan, reduced PMBF, and lower $D_{L_{CO}}$. In contrast, elevations in CD62E$^+$ EMPs were observed only in severe COPD and with hyperinflation. These findings suggest endothelial cell apoptosis early in the pathogenesis of COPD and emphysema, and endothelial activation in severe, hyperinflated COPD.

This is the first study of which we are aware to demonstrate that EMPs are increased in mild COPD and are related to a measure of emphysema. The findings, obtained using precise cellular measures linked to state-of-the-art structural and functional imaging in a general-population sample, are consistent with prior work in murine models that suggests a mechanistic role of VEGFR blockade and ceramide up-regulation as a cause of alveolar endothelial apoptosis to epithelial apoptosis and emphysema-like changes (10, 12). Together, these findings suggest a role of endothelial damage and potentially apoptosis in the pathogenesis of emphysema-predominant COPD.

Most prior work on endothelial cells in COPD has been limited to small studies using specimens collected at autopsy or surgery. Reductions in the level of VEGF, a key cytokine involved
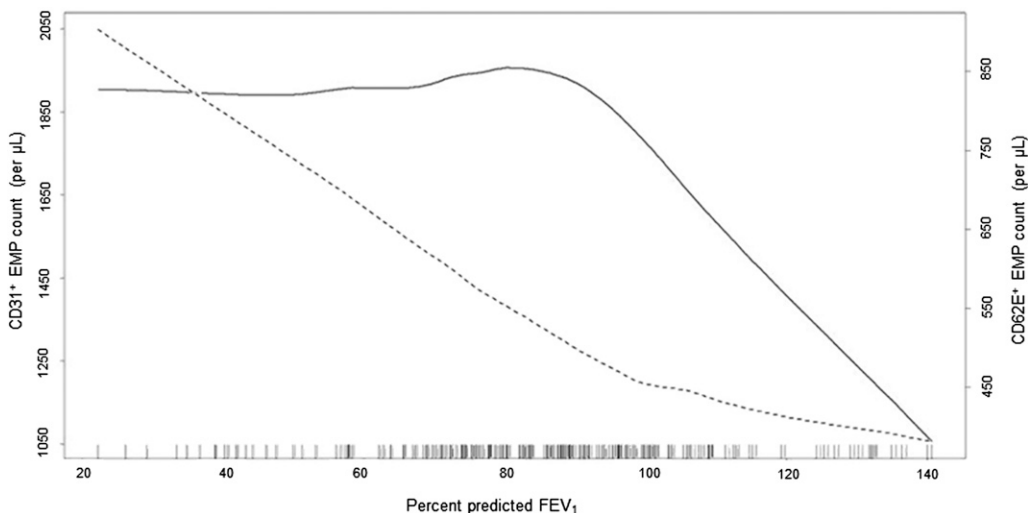

Figure 2. Endothelial microparticles and severity of chronic obstructive pulmonary disease. Smoothed regression plots of the relationship of counts of CD31$^+$ (*dark line*) and CD62E$^+$ (*dashed line*) endothelial microparticles to the percent predicted FEV$_1$. The plots were obtained from regression models adjusted for age, sex, race and ethnicity, cohort, smoking status, pack-years, educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, and statin use. The *hash marks* denote data points. EMP = endothelial microparticle.

TABLE 3. PREDICTED MEAN COUNTS OF ENDOTHELIAL MICROPARTICLES BY QUINTILE OF PERCENTAGE OF EMPHYSEMATOUS LUNG ON COMPUTED TOMOGRAPHY

N = 180	Computed Tomography Percent Emphysema					Difference Per Log Increase in Percent Emphysema (95% CI)	P Value
	Quintile 1 (n = 36)	Quintile 2 (n = 36)	Quintile 3 (n = 36)	Quintile 4 (n = 36)	Quintile 5 (n = 36)		
CD31$^+$ endothelial microparticles per microliter, log-transformed							
Mean (SD)	7.09 (0.50)	7.27 (0.49)	7.07 (0.59)	7.13 (0.55)	7.41 (0.79)		0.13
Predicted mean, model 1*	7.09	7.14	7.22	7.31	7.57	0.082 (0.019 to 0.145)	0.01
Predicted mean, model 2†	7.07	7.13	7.21	7.32	7.60	0.089 (0.026 to 0.152)	0.006
Predicted mean, model 3‡	7.01	7.06	7.11	7.18	7.27	0.075 (0.002 to 0.149)	0.045
CD51$^+$ endothelial microparticles per microliter, log-transformed							
Mean (SD)	6.86 (0.69)	7.13 (0.68)	6.75 (0.77)	6.74 (0.67)	7.02 (0.98)		0.85
Predicted mean, model 1	6.81	6.85	6.90	6.98	7.17	0.059 (−0.032 to 0.150)	0.20
Predicted mean, model 2	6.80	6.84	6.91	6.99	7.20	0.068 (−0.023 to 0.159)	0.14
Predicted mean, model 3	6.88	6.91	6.93	6.96	7.00	0.032 (−0.088 to 0.152)	0.60
CD62E$^+$ endothelial microparticles per microliter, log-transformed							
Mean (SD)	5.94 (0.60)	5.92 (0.68)	6.05 (0.73)	6.11 (0.56)	6.22 (0.71)		0.03
Predicted mean, model 1	5.91	6.00	6.05	6.12	6.24	0.078 (−0.001 to 0.156)	0.05
Predicted mean, model 2	5.94	6.03	6.08	6.15	6.25	0.079 (0.00 to 0.158)	0.05
Predicted mean, model 3	5.98	6.03	6.07	6.12	6.20	0.058 (−0.039 to 0.155)	0.24

Definition of abbreviation: CI = confidence interval.

* Model 1 adjusted for age, sex, race and ethnicity, and cohort.

† Model 2 adjusted for variables in model 1 in addition to smoking status, and pack-years.

‡ Model 3 adjusted for variables in model 2 in addition to educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, statin use, and high milliamperes.

in endothelial cell survival, reductions in VEGFR, and increased endothelial apoptosis have been observed in the lung tissue of patients with emphysema or COPD compared with those without (8, 43). Peinado and coworkers (44) demonstrated increased endothelial progenitor cells (EPCs) in the pulmonary arteries of patients with COPD, suggesting endothelial injury and repair in early COPD. Reductions in circulating EPCs (45), however, may reflect reduced reparative capacity caused by smoking-related suppression of EPC generation in the bone marrow or increased margination of EPCs with increased repair.

EMPs, by contrast, directly reflect endothelial perturbation unrelated to the bone marrow. Consistent with our findings for COPD, Takahashi and coworkers (29) recently showed that CD31$^+$ EMPs were elevated in COPD compared with control subjects and during COPD exacerbations. The current study expands on their findings and demonstrates both that CD31$^+$ EMPs are elevated in mild COPD and that there is a strong, graded, and specific relationship of CD31$^+$ EMPs to percent emphysema, findings that are consistent with animal models and that suggest that EMPs are not merely a biomarker in COPD but that endothelial apoptosis may be involved in the pathogenesis of emphysema and COPD.

Unlike CD31$^+$ EMPs, CD62E$^+$ EMPs in the current study were elevated predominantly in severe COPD and related to functional measures of pulmonary hyperinflation rather than structural measures of pulmonary emphysema. Elevations in CD62E$^+$ EMPs are suggestive of endothelial activation (17), particularly in response to inflammatory cytokines and specifically in response to tumor necrosis factor-α (17). Elevated tumor necrosis factor-α is well-described in severe COPD (46)
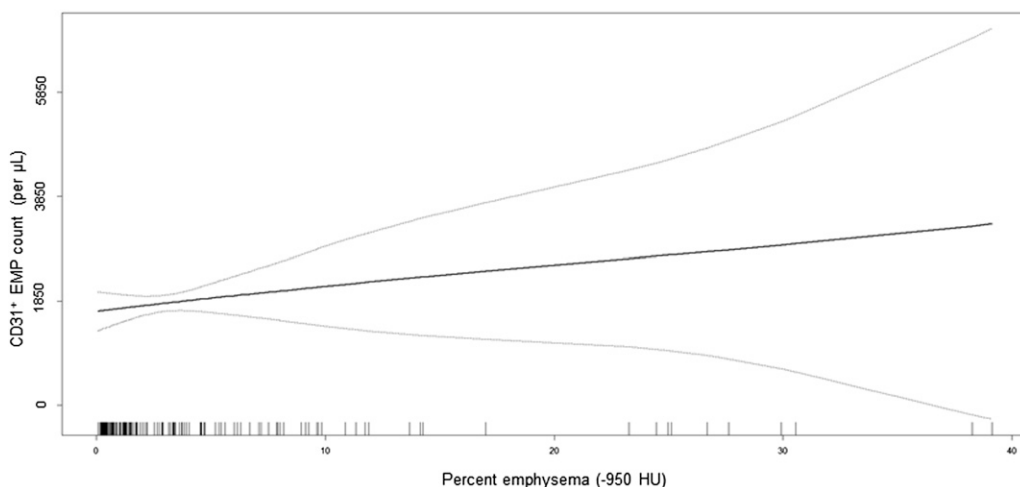

Figure 3. CD31$^+$ endothelial microparticle count and percent emphysema on computed tomography. Smoothed regression plot of the relationship of counts of CD31$^+$ endothelial microparticles to the percentage of emphysema-like lung on computed tomography (*dark line*). The *lighter lines* are 95% confidence intervals. The plot was obtained from a regression model adjusted for age, sex, race and ethnicity, cohort, smoking status, pack-years, educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, statin use, and high milliamperes. The *hash marks* denote data points. EMP = endothelial microparticle; HU = Hounsfield unit.

TABLE 4. THE ASSOCIATION OF ENDOTHELIAL MICROPARTICLES WITH PULMONARY MICROVASCULAR BLOOD FLOW, DIFFUSING CAPACITY, AND HYPERINFLATION

	Slope Increase* ($n = 149$) (per AU/s increase)	P Value	$D_{L_{CO}}$ ($n = 118$) (per ml CO/min/mm Hg increase)	P Value	RV ($n = 118$) (per milliliter increase)	P Value	RV/TLC ratio ($n = 118$) (per unit increase)	P Value
CD31$^+$ endothelial microparticles per microliter, log-transformed								
Mean difference, model 1[†]	−0.014 (−0.027 to −0.001)	0.04	−0.034 (−0.056 to −0.012)	0.003	0.065 (−0.097 to 0.228)	0.43	0.653 (−0.495 to 1.802)	0.26
Mean difference, model 2[‡]	−0.015 (−0.028 to −0.002)	0.02	−0.038 (−0.061 to −0.015)	0.001	0.106 (−0.057 to 0.270)	0.23	0.910 (−0.237 to 2.057)	0.12
Mean difference, model 3[§]	−0.015 (−0.029 to −0.001)	0.047	−0.030 (−0.053 to −0.007)	0.01	0.141 (−0.052 to 0.333)	0.15	1.089 (−0.212 to 2.390)	0.10
CD51$^+$ endothelial microparticles per microliter, log-transformed								
Mean difference, model 1	−0.007 (−0.025 to 0.010)	0.42	−0.030 (−0.063 to −0.002)	0.07	0.113 (−0.121 to 0.347)	0.34	0.519 (−1.143 to 2.182)	0.54
Mean difference, model 2	−0.009 (−0.027 to 0.009)	0.33	−0.033 (−0.066 to −0.001)	0.046	0.161 (−.076 to 0.397)	0.35	0.808 (−0.864 to 2.481)	0.34
Mean difference, model 3	−0.011 (−0.031 to 0.001)	0.26	−0.029 (−0.062 to 0.004)	0.08	0.074 (−0.020 to 0.356)	0.58	0.738 (−1.267 to 2.742)	0.47
CD62E$^+$ endothelial microparticles per microliter, log-transformed								
Mean difference, model 1	0.011 (−0.004 to 0.027)	0.15	−0.025 (−0.055 to 0.006)	0.10	0.202 (−0.009 to 0.414)	0.06	1.821 (0.335 to 3.306)	0.016
Mean difference, model 2	0.011 (−0.005 to 0.027)	0.17	−0.029 (−0.059 to 0.001)	0.06	0.240 (0.026 to 0.455)	0.03	2.043 (0.545 to 3.540)	0.008
Mean difference, model 3	0.012 (−0.005 to 0.028)	0.17	−0.022 (−0.053 to 0.009)	0.17	0.390 (0.180 to 0.601)	<0.001	2.853 (1.147 to 4.558)	0.001

Definition of abbreviations: AU = arbitrary units; $D_{L_{CO}}$ = diffusing capacity of carbon monoxide; RV = residual volume.
* Adjusted for variables in model 3 in addition to cardiac output.
[†] Model 1 adjusted for age, sex, race and ethnicity, and cohort.
[‡] Model 2 adjusted for variables in model 1 in addition to smoking status, and pack-years.
[§] Model 3 adjusted for variables in model 2 in addition to educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, and statin use.

and we speculate that in contrast to CD31$^+$ EMPs, CD62E$^+$ EMPs were elevated as a secondary, late response caused by inflammation in severe COPD.

The present study has several strengths including precisely measured EMPs by flow cytometry; relatively large, population-based sample size; and state-of-the-art assessment of the major phenotypes by spirometry, CT scan, gadolinium-enhanced MRI, diffusing capacity, and plethysmography. Still, there are several reasons why the present results may not support the translation of experimental murine findings on endothelial apoptosis to the human diseases of COPD and emphysema.

First, it is not certain that pulmonary circulation was the origin of the EMP elevation as we sampled EMPs in the peripheral venous circulation. Cell-surface or other markers that definitively label EMPs as pulmonary or systemic are, unfortunately, lacking. Recently, the absence of von Willebrand factor was proposed as a marker for alveolar capillary endothelial cells (29), as has the presence of angiotensin-converting enzyme (CD143) (27). Although we did not use these markers, three lines of reasoning suggest that the origin of the excess EMPs is pulmonary. First, CD31$^+$ EMPs were specifically associated with novel measures of PMBF on contrast-enhanced MRI in addition to $D_{L_{CO}}$, the latter association being previously observed in smokers without COPD (27). Second, patients in this study were specifically selected for COPD and we excluded patients with diseases likely to increase EMPs of systemic origin, such as clinical cardiovascular disease and significant renal disease. Third, the findings were similar in secondary analyses restricted to patients free of hypertension, diabetes, and sleep apnea, which may increase EMPs of systemic origin. Furthermore, restriction to patients free of subclinical cardiovascular disease slightly attenuated the association with COPD status, attenuated that of mild COPD, and strengthened the relationship with percent emphysema.

For obvious reasons, unlike in animal studies, human studies of COPD pathology are limited to observation and cannot include experimentation (i.e., inducing COPD). Therefore, the results may be potentially biased by unmeasured explanatory factors that elevate EMPs and also cause COPD. We adjusted, however, for precise measures of multiple potential confounders and, if anything, the results of the fully adjusted models were of greater significance than the unadjusted results. Furthermore, this potential limitation is offset by the fact that the results apply directly to patients with clinical disease from the general population.

Elevated CD31$^+$ EMP levels in mild COPD is not necessarily synonymous with CD31$^+$ EMP elevations in early COPD, because not all patients with mild COPD progress to severe COPD (47). However, low lung function is the major determinant of accelerated decline in lung function characteristic of COPD (48, 49), and percent emphysema predicts decline in lung function (48). Longitudinal studies are needed to definitively confirm or refute whether elevated CD31$^+$ EMPs contributes to lung function decline and progression of emphysema.

Annexin V on CD31$^+$ EMPs has been used to confirm the apoptotic nature of endothelial cells of origin (50). We did not measure CD31$^+$/annexin V$^+$ EMPs, which limits a definitive statement on the apoptotic nature of the CD31$^+$ EMPs. However, Jimenez and coworkers (17) showed a distinct elevation of CD31$^+$ EMPs in response to the presence of apoptotic agents which, along with our observed findings for CD31$^+$ EMPs that were relatively independent of CD62E$^+$ EMPs, implies that the elevations of CD31$^+$ EMPs discussed herein are suggestive of apoptosis.

Finally, case-control studies can be subject to selection bias; however, the nested design of the current study, in which the sampling probabilities within MESA and EMCAP were known, minimized the possibility of this bias. A small number of participants were recruited from outside the two cohorts and exclusion of these participants yielded consistent results.
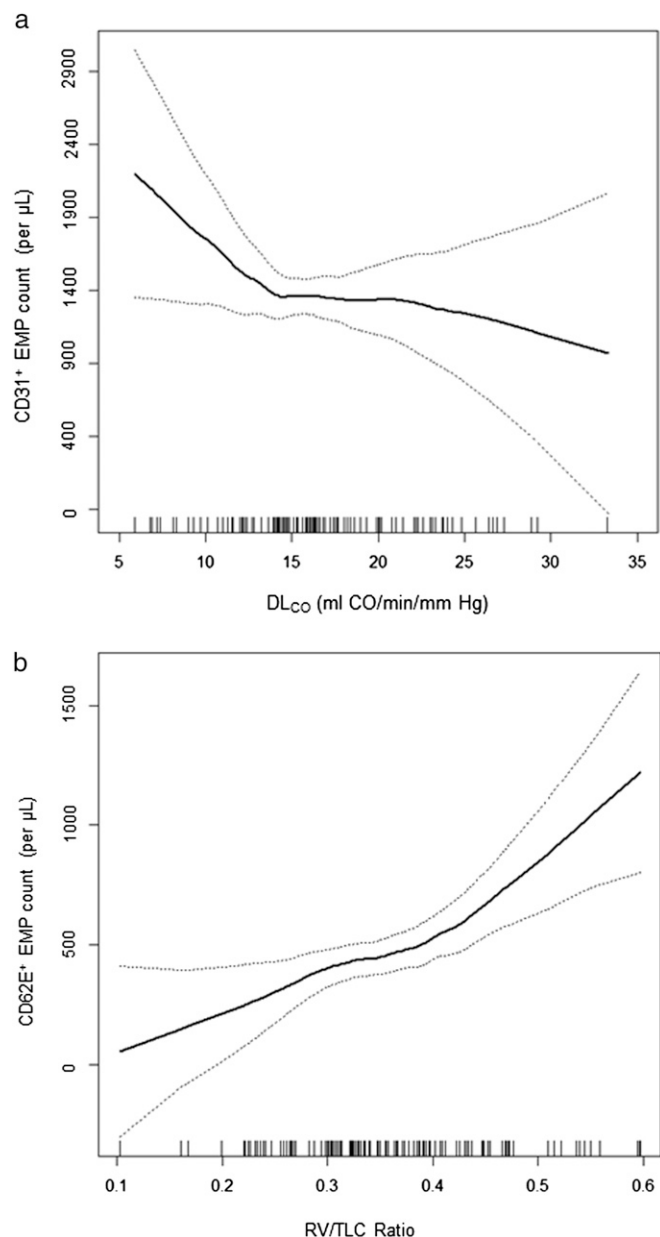
a

b

Figure 4. CD31$^+$ and CD62$^+$ endothelial microparticles and chronic obstructive pulmonary disease (COPD) subphenotypes of pulmonary diffusing capacity and hyperinflation. (*a*) Smoothed regression plot of the relationship of counts of CD31$^+$ endothelial microparticles to diffusing capacity. (*b*) Smoothed regression plot of the relationship of counts of CD62E$^+$ endothelial microparticles to the ratio of residual volume to total lung capacity. The plots were obtained from regression models adjusted for age, sex, race and ethnicity, cohort, smoking status, pack-years, educational attainment, body mass index, height, weight, diabetes mellitus, hypertension, oxygen saturation, white blood cell count, sleep apnea, high-density lipoprotein, and statin use. The *dark lines* are the regression lines; the *lighter lines* are 95% confidence intervals. The *hash marks* denote data points. D$_{LCO}$ = diffusing capacity of the lung for carbon monoxide; EMP = endothelial microparticle; RV/TLC = residual volume/total lung capacity.

In conclusion, CD31$^+$ EMPs were elevated in COPD in a pattern consistent with endothelial apoptosis in mild COPD. CD31$^+$ EMPs were also positively related to percent emphysema and correlated with reductions in pulmonary microvascular perfusion assessed by MRI and diffusing capacity. In contrast,

CD62E$^+$ EMPs suggestive of endothelial activation were elevated in severe COPD and with hyperinflation. These cellular markers may implicate endothelial apoptosis in the pathogenesis of COPD and emphysema.

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