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Original article

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Take-Home Message
In community-dwelling adults, adhesion molecules sICAM-1, sVCAM-1 and P-selectin are associated with CT measures of early lung injury, lower FVC, ILD hospitalizations and deaths, suggesting they may contribute to the development of pulmonary fibrosis.

ABSTRACT

Adhesion molecules may contribute to the development of interstitial lung disease (ILD) and have been proposed as prognostic biomarkers in idiopathic pulmonary fibrosis. Our objective was to determine whether circulating adhesion molecules sICAM-1, sVCAM-1 and P-selectin are associated with subclinical ILD in community-dwelling adults.

The Multi-Ethnic Study of Atherosclerosis enrolled men and women aged 45-84 from six communities in the United States in 2000-2002. High attenuation areas were defined as the percentage of imaged lung volume with attenuation -600 to -250 Hounsfield Units on cardiac computed tomography. Interstitial lung abnormalities (ILA) were visually assessed on a full lung computed tomography. Spirometry was performed on a subset of individuals. ILD hospitalizations and deaths were adjudicated.

In fully adjusted analyses, higher levels of sICAM-1 , sVCAM-1 and P-selectin were associated with greater high attenuation areas (2.94%, 95%CI 1.80-4.07; 1.24%, 95%CI 0.14-2.35; 1.58%, 95%CI 0.92-2.23, respectively), and greater rate of ILD hospitalizations (HR 1.36, 95%CI 1.03-1.80; 1.40, 95%CI 1.07-1.85, 2.03, 95%CI 1.16-3.5, respectively). sICAM-1 was associated with greater prevalence of interstitial lung abnormalities (OR 1.39, 95%CI 1.13-1.71). sICAM-1 and P-selectin were associated with lower forced vital capacity (44ml, 95%CI 12-76; 29ml, 95%CI 8-49, respectively). sVCAM-1 and P-selectin were associated with increased risk of ILD death (HR 2.15, 95%CI 1.26-3.64; 3.61, 95%CI 1.54-8.46, respectively).

Higher levels of circulating sICAM-1, sVCAM-1, and P-selectin are independently associated with CT and spirometric measures of subclinical ILD, and increased rate of adjudicated ILD events among community-dwelling adults.

Introduction

Interstitial lung disease (ILD) is characterized by the presence of cellular proliferation, cellular infiltration, and/or fibrosis of the lung parenchyma not due to infection or malignancy [1, 2]. Cellular-level changes that lead to ILD likely begin years prior to the onset of symptoms [3]. Fibrotic ILDs generally have a poor prognosis and few treatment options [4]. The investigation of subclinical ILD has gained momentum in recent years as a way to identify novel risk factors and early mechanisms of lung fibrosis in humans [5-9]. These studies may lead to future interventions to prevent lung fibrosis [1].

Adhesion molecules mediate leukocyte recruitment into the lung, and may play a role in the development of ILD [10, 11]. Intracellular adhesion molecule-1 (ICAM-1) is overexpressed in idiopathic pulmonary fibrosis (IPF) lungs. Serum levels of soluble ICAM-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) correlate with the extent of fibrosis and poor survival in IPF, and have been proposed as potential prognostic biomarkers [10, 12, 13]. The selectin family of adhesion molecules, including E-selectin and L-selectin, are an important part of the innate immune response [11, 14]. P-selectin has been implicated in lung injury but has not been extensively studied in ILD [15]. The role of these adhesion molecules in adults with subclinical ILD is not known.

In the current study, we sought to determine the associations of circulating sICAM-1, sVCAM-1, E-selectin, L-selectin, and P-selectin with CT measures of subclinical ILD, forced vital capacity (FVC), and clinical ILD events in a large cohort of community-dwelling adults enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that higher

levels of circulating cell adhesion molecules will be associated with greater high attenuation areas (HAA), higher odds of interstitial lung abnormalities (ILA), lower FVC, and increased risk of ILD events during follow-up.

Methods

Participants

MESA is a multi-center population-based prospective cohort study that enrolled 6814 men and women aged 45-84 without evidence of clinical cardiovascular disease at study entry. MESA participants were sampled from six U.S. communities in years 2000 to 2002, regardless of lung disease, respiratory symptoms, or smoking history, as previously described [16]. Circulating adhesion molecules were measured in a random sample of MESA participants stratified by race/ethnicity. The samples sizes for each biomarker and timeline of measurements are shown in Figure S1. Written informed consent was obtained from all participants and the study was approved by Institutional Review Boards at all collaborating centers.

Circulating adhesion molecules

sICAM-1 and E-selectin were measured in blood samples collected at the MESA baseline exam in years 2000-2002. Samples were stored at -80ºC and analyzed in year 2004 after the first thaw. sICAM-1 was measured by ELISA (Parameter Human sICAM-1 Immunoassay, R&D Systems) in plasma from 2,621 participants. E-selectin was measured by ELISA (Human Soluble E-Selectin kit, R&D Systems) in serum from 999 participants. sVCAM-1, L-Selectin and P-Selectin were measured in blood samples collected at the MESA follow-up

exam in years 2002-2004. Samples were stored at -80ºC, sent for processing after one thaw and analyzed in years 2010-2011 after the second thaw cycle. Serum levels of sVCAM-1 and L-Selectin were measured by ELISA (Quantikine Human Soluble VCAM Immunoassay kit, Human Soluble L-Selectin Immunoassay kit, R&D Systems) in 2,440 participants. Plasma levels of P-selectin were measured in two independent batches from 6,147 participants by ELISA (Human Soluble P-Selectin Immunoassay kit, R&D Systems). Different sets of ELISA reagents and separate human control pool were used for each batch. The inter-assay coefficients of variation were 5.0%, 3.6%, 6.7%, 7.3% and 6.7% for sICAM-1, sVCAM-1, P-selectin, E-selectin and L-selectin, respectively.

Genotyping

The sICAM-1 ELISAs can differ in their ability to recognize two of the less frequent sICAM-1 variants due to poor binding to the immunoassay [17]. Thus, the genotype of the sICAM-1 single nucleotide polymorphism (SNP) rs5491 was attained from all consenting individuals in order to ensure that the results were not affected by inclusion of individuals with the AT and TT variants. Genotyping was performed using the ITMAT-Broad-CARe (IBC) microarray (Illumina, Inc; San Diego, CA).

High Attenuation Areas on CT scan

Lung attenuation was measured on exam 2 or 3 cardiac CT scans in 5,703 MESA participants at the University of Iowa Imaging Lab using a modified version of Pulmonary Analysis Software Suite. HAA was defined as percentage of imaged lung with attenuation

between -600 and -250 Hounsfield units (HU), as previously described [5, 18]. Percent

emphysema was defined as the percentage of lung voxels below −950 HU

Interstitial Lung Abnormalities on CT scan

Full-lung CT scans were performed at follow-up exam 5, in years 2010-2012, using multi-

detector CT-scanners, as previously described [7]. A total of 2,907 participants had CT

scans visually assessed by one of five expert radiologists (inter-reader kappa 0.47) for the

presence of ILA, which was defined as the presence of ground-glass, reticular

abnormalities, diffuse centrilobular nodularities, honeycombing, traction bronchiectasis,

non-emphysematous cysts or architectural distortion in at least 5% of nondependent

portions of the lung, as previously described [7, 19, 20]. Of these scans, 477 were classified

as indeterminate for ILA and were excluded from ILA analyses.

Spirometry

Spirometry was performed at follow-up exams 3 and 4, between years 2004 and 2007, in

accordance with American Thoracic Society/European Respiratory Society guidelines,

using protocol that was previously described [21, 22].

Cause of death and hospitalization

Data on hospitalizations and deaths was obtained through direct contact with MESA participants

or surrogates at 9-12 month intervals, and was supplemented by review of the National Death

Index. Mortality data were complete as of March 2015 and hospitalization data were complete as

of December 2013. A two-member adjudication panel adjudicated all ILD deaths and

hospitalizations, as previously described [9]. Respiratory death was determined based on the ICD-10 diagnosis code for underlying cause of death on the death certificate.

Statistical Analysis

We examined the distributions of each biomarker using box plots and computed pearson correlation coefficients between individual adhesion molecules. We used generalized linear models to examine the associations of circulating adhesion molecules, modeled in a continuous fashion, with HAA and FVC. We report all results per standard deviation (SD) of each adhesion molecules to facilitate interpretation. HAA was expressed as percentage of total imaged lung volume and was log-transformed. We used logistic regression to examine the association of adhesion molecules with ILA. HAA models were adjusted for age, sex, body mass index, waist circumference, height, race/ethnicity, current smoking status and pack-year smoking history, educational attainment, glomerular filtration rate (GFR), study site/scanner, radiation dose, percent emphysema, and total imaged lung volume. ILA and FVC models were adjusted for age, gender, race, smoking status, BMI, and study site, as previously described [23]. We examined models stratified by SNP rs5491, smoking-status, age and BMI, and used the likelihood ratio test to test for effect modification. To account for differences in P-selectin measurement between the two batches, we obtained residuals from a linear regression model using log-transformed raw P-selectin values as a response variable and batch number as a predictor, as previously described, and used the P-selectin residuals in our fully adjusted models [24]. We used Cox regression and proportional means models, which allow for recurrent events, to examine the associations of circulating adhesion molecules with time to ILD death and hospitalization, respectively, as previously

described [9]. Due to low event rates, we controlled for confounding in these models by calculating a generalized propensity score (GPS), as previously described [9]. We used generalized additive models with loess smoothers of continuous variables to examine for non-linear associations between our predictors and outcomes, and to generate graphs. Statistical significance was defined using the Benjamini-Hochberg procedure to control the false discovery rate at 0.05. All analyses were done in Stata (version 14) and R (version 3.5.1).

Results

The baseline characteristics of MESA participants overall and by quartile of each adhesion molecule are shown in Tables 1, and S1-S4. Overall, for participants with measured sICAM-1 and HAA, the mean (SD) age was 59 (9.6) years. 56% were female, 49% were white, 18% were black, 22% were Hispanic and 12% were Chinese. 45% were current or former smokers with a median of 15 pack years smoking history (Table 1). Participants in the highest quartile of sICAM-1 were more likely to be female, white or Hispanic, ever-smokers and have higher BMI. The mean (SD) levels of sICAM-1, sVCAM-1, P-selectin, E-selectin, and L-selectin were 274 ng/mL (78.4), 740 ng/mL (232.0), 36 ng/mL (13.9), 55 ng/mL (25.3), and 891 ng/mL (200.0), respectively (Figure S2). Pearson correlation coefficients demonstrated only weakly positive relationships between the adhesion molecules (Table S5).

Table 1. Baseline characteristics of MESA participants by quartile of sICAM-1

	Overall	Quartile of sICAM-1			
		Q1	Q2	Q3	Q4
Number	2233	559	558	558	558
sICAM-1	273.8 ± 78.39	187.84 ± 35.21	248.43 ± 11.02	288.26 ± 12.03	373.29 ± 70.38
Age, years	59.1 ± 9.6	58.1 ± 9.5	59.8 ± 9.8	60.0± 9.9	60.0 ± 9.7
Women, %	56.2	55	55.2	55.5	59.2
BMI, kg/m^2	28.3	27.1	29.1	29.1	29.9
Waist Circumference, cm	97.6	93.8	96.1	99.8	102
Height, cm	166.8	166.9	167.2	166.4	165.6
eGFR, mL/min/1.73 m^2		82.9	80.4	80.4	81.2
Race					
White, %	49.2	30	55.1	55.7	49.1
Chinese American, %	12.2	24.2	13.2	6	3.3
Black, African American, %	18	30.8	13	13.2	17.3
Hispanic, %	20.8	14.7	18.7	25.1	30.2
Smoking					
Ever Smokers, %	45.7	47.7	45.3	49.6	60.9
Current Smokers, %	15.1	7.5	8.3	12.1	28.8
Site					
Wake Forest, %	16.8	13.4	18.9	14.4	17.3
Columbia, %	16.7	17.9	12.6	16.3	19
John's Hopkins, %	12.7	15.6	11.5	12.9	12.1
UMN, %	15.8	6.6	14.1	14.34	24.8
Northwestern, %	15.5	19.5	18.4	17.2	8.3
UCLA, %	22.5	27	24.5	22.9	18.4

Data presented as mean +/- SD, n (%) or median (interquartile range), unless otherwise stated. All parameters were collected at MESA baseline visit in years 2000-2002. HAA: high attenuation areas; sICAM: soluble intracellular adhesion molecule; BMI: body mass index; eGFR: effective glomerular filtration rate

High Attenuation Areas

In fully adjusted models, higher levels of sICAM-1, sVCAM-1 and P-selectin were associated with greater HAA. Each SD increment of sICAM-1, sVCAM-1, and P-selectin was associated with a 2.94% (95% CI 1.80-4.07, p<0.001,), 1.24% (95% CI 0.14-2.35, p=0.03), and 1.58% (CI 0.92-2.23, p<0.001) increase in HAA, respectively (Table 2, Figure 1A-C). There was no evidence of effect modification by smoking status, age or BMI on the association between

these adhesion molecules and HAA (p for interaction >0.08, Table S6-8). After correction for multiple comparisons, the p-values for sICAM-1, sVCAM-1 and P-selectin remained significant. Findings were consistent in a less adjusted model (Table S9). The effect estimate for sICAM-1 was similar when the analysis was limited to individuals with the AA genotype of SNP rs5491 (Table S10). There were no significant associations of E-selectin or L-selectin with HAA.

Interstitial Lung Abnormalities

In fully adjusted models, higher levels of sICAM-1 were associated with a greater prevalence of ILA on CT scan. Each SD increment of sICAM-1 was associated with 1.39 (95% CI 1.13-1.71, p=0.002) higher odds of ILA, (Table 2, Figure 1D-E). Unadjusted analyses by ILA subtype are shown in Table S11. Each SD increment of sICAM-1 was associated with 1.39 (95% CI 1.14 to 1.70, p=0.001) higher odds of fibrotic ILA subtypes, as defined by the presence of reticular abnormalities, traction bronchiectasis or honeycombing. There was no evidence of effect modification by smoking status, age or BMI on the association between sICAM-1 and ILA (p for interaction >0.05, Table S6-8). When corrected for multiple comparisons, the p-value remained significant. Results for sICAM-1 were similar in analyses restricted to individuals with AA genotype of SNP rs5491 (Table S10). There were no significant associations of sVCAM-1, P-selectin, E-selectin or L-selectin with ILA (Table 2, Figure 2F). Each SD increment of VCAM-1 was associated with 1.34 higher odds of fibrotic ILA (Table S11).

Lung function

In fully adjusted models, higher levels of sICAM-1 and P-selectin were associated with a lower FVC. Each SD increment in sICAM-1 and P-selectin was associated with a 44.1mL (95% CI 12.4-75.9, p=0.006) and 28.5ml (95% CI 8.0-49.1, p=0.007) lower FVC, respectively (Table 2, Figures 1G-I). The association of sICAM-1 with FVC was modified by smoking status, with stronger effect estimates among ever-smokers (p for interaction 0.01, Table S6). There was no evidence of effect modification by age or BMI (p for interaction >0.05, Table S7-8). There were significant associations of sVCAM-1 and E-selectin with FVC among ever-smokers, although the p values for interaction were not significant (0.08 and 0.28, respectively, Table S6). Association between adhesion molecules with percent predicted FVC are shown in Table S12. Results were consistent when participants with an obstructive ventilator defect (FEV1/FVC ratio< 70%) were excluded from the analyses and among participants with the AA genotype of SNP rs5491 (Tables S9 and S13). There was no significant association between L-selectin and FVC.

ILD hospitalizations and death

In fully adjusted models, higher levels of sICAM-1, sVCAM-1 and P-selectin were all associated with greater rate of respiratory hospitalizations among those with ILD, as well as hospitalizations due to ILD. Each SD increment of sICAM-1, sVCAM-1 and P-selectin was associated with a HR of 1.36 (95% CI 1.03-1.80, p=0.03), 1.40 (95% CI 1.07-1.85, p=0.02), and 2.03 (95% CI 1.16-3.55, p=0.01) for ILD hospitalization, respectively (Table 3, Figure 2A-C). In fully adjusted models, both sVCAM-1 and P-selectin were associated with increased risk of death due to respiratory causes and ILD deaths. Each SD increment in

sVCAM-1 and P-selectin was associated with a HR of 2.15 (95% CI 1.26-3.64, p=0.005) and 3.61(95% CI 1.54-8.46, p=0.003) for ILD death, respectively (Table 3, Figure 2E-F). There was a trend toward an increased risk for both respiratory and ILD mortality in participants with higher sICAM-1 levels, but the association did not reach statistical significance (Table 3, Figure 2D). There were no significant associations of E-selectin and L-selectin with ILD events.

Table 2. Associations of circulating adhesion molecules with HAA, ILA and FVC.

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
HAA										
N participants	2,226		2,187		5,498		864		2187	
% change (95% CI)*										
Unadjusted	2.87 (1.38 to 4.36)	<0.001	1.82 (0.34 to 3.31)	0.02	1.79 (0.87 to 2.72)	<0.001	4.87 (2.51 to 7.23)	0.01	-0.96 (-2.45 to 0.53)	0.21
Adjusted	2.94 (1.80 to 4.07)	<0.001	1.24 (0.14 to 2.35)	0.03	1.58 (0.92 to 2.23)	<0.001	0.64 (-1.21 to 2.42)	0.51	0.40 (-0.70 to 1.49)	0.48
ILA										
N participants	1,028		1,008		2,239		507		1,008	
OR (95% CI)†										
Unadjusted	1.41 (1.18 to 1.67)	<0.001	1.33 (1.13 to 1.56)	0.001	1.10 (0.97 to 1.24)	0.14	0.88 (0.67 to 1.17)	0.37	0.91 (0.75 to 0.09)	0.30
Adjusted	1.39 (1.13 to 1.71)	0.002	1.20 (0.99 to 1.45)	0.07	1.08 (0.94 to 1.24)	0.27	0.96 (0.79 to 1.30)	0.78	0.93 (0.75 to 1.15)	0.49
FVC										
N participants	1,549		1,680		3,449		808		1,688	
Change, mL (95% CI)†										
Unadjusted	-47.8 (-94.5 to -0.6)	0.05	-77.8(-120.0 to -35.6)	0.001	60.7 (30.0 to 91.5)	<0.001	-36.81 (-102.6 to 28.9)	0.27	-9.47 (-51.8 to 32.9)	0.66
Adjusted	-44.1 (-75.9 to -12.4)	0.006	-29.1 (-57.7 to -0.4)	0.047§	-28.5 (-49.1 to -8.0)	0.007	-43.9 (-87.6 to -0.1)	0.049§	10.6 (-17.7 to 38.9)	0.46

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas; ILA: interstitial lung abnormalities; FVC: forced vital capacity; OR: odds ratio

Effect estimates are per standard deviation of each adhesion molecule.

* adjusted for age, sex, body mass index, waist circumference, race/ethnicity, eGFR, current smoking status and pack-year smoking history, educational attainment, study site/scanner, mA dose, percent emphysema, and total imaged lung volume.

† adjusted for age, gender, race, smoking status, eGFR, BMI and site

§ p value no longer significant after Benjamini-Hochberg correction for multiple comparisons

Table 3. Associations circulating adhesion molecules with ILD events.

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
Respiratory hospitalization with concomitant ILD										
Number of events	20		18		77		9		18	
Person-years (PY)	30,295		28,014		70,176		11,457		28,014	
Rate per 10,000 PY (95% CI)	6.6 (4.3 to 10.2)		6.4 (4.0 to 10.2)		11.0 (8.8 to 13.7)		7.9 (4.1 to 15.1)		6.4 (4.0 to 10.2)	
Hazard ratio (95% CI)										
Unadjusted	1.75 (1.46 to 2.10)	<0.001	1.56 (1.27 to 1.90)	<0.001	1.73 (1.23 to 2.43)	0.002	1.39 (1.02 to 1.89)	0.04	1.09 (0.67 to 1.71)	0.78
Adjusted: Model 1*	1.36 (1.13 to 1.63)	0.001	1.62 (1.21 to 2.16)	0.001	1.84 (1.24 to 2.72)	0.002	1.29 (0.71 to 2.35)	0.40	1.09 (0.65 to 1.84)	0.75
Adjusted: Model 2†	1.34 (1.09 to 1.66)	0.006	1.68 (1.25 to 2.27)	<0.001	2.07 (1.25 to 3.43)	0.005	1.42 (0.75 to 2.70)	0.29	1.09 (0.66 to 1.80)	0.75
ILD hospitalization										
Number of events	12		11		44		8		11	
Person-years (PY)	30,295		28,014		70,176		11,457		28,014	
Rate per 10,000 PY (95% CI)	4.0 (2.3 to 7.0)		3.9 (2.2 to 7.1)		6.3 (4.7 to 8.4)		7.0 (3.5 to 14.0)		3.9 (2.2 to 7.1)	
Hazard ratio (95% CI)										
Unadjusted	1.70 (1.32 to 2.21)	<0.001	1.57 (1.21 to 2.05)	<0.001	1.95 (1.32 to 2.88)	<0.001	1.34 (0.94 to 1.91)	0.11	1.10 (0.52 to 2.33)	0.80
Adjusted: Model 1*	1.30 (1.00 to 1.70)	0.047§	1.53 (1.24 to 1.89)	<0.001	1.87 (1.19 to 2.92)	0.007	1.25 (0.65 to 2.41)	0.51	1.03 (0.58 to 1.84)	0.91
Adjusted: Model 2†	1.36 (1.03 to 1.80)	0.03	1.40 (1.07 to 1.85)	0.02	2.03 (1.16 to 3.55)	0.01	1.30 (0.72 to 2.36)	0.38	1.05 (0.56 to 1.97)	0.87
Respiratory mortality										
Number of events	28		25		63		7		25	
Person-years (PY)	30,295		28,014		70,176		11,457		28,014	
Rate per 10,000 PY (95% CI)	9.2 (6.4 to 13.4)		8.9 (6.0 to 13.2)		9.0 (7.0 to 11.5)		6.1 (2.9 to 12.8)		8.9 (6.0 to 13.2)	
Hazard ratio (95% CI)										
Unadjusted	1.43 (1.08 to 1.91)	0.01§	1.71 (1.42 to 2.06)	<0.001	1.48 (1.14 to 1.92)	0.003	1.78 (1.13 to 2.81)	0.01§	0.85 (0.56 to 1.29)	0.43
Adjusted: Model 1*	1.25 (0.94 to 1.65)	0.13	1.80 (1.38 to 2.36)	<0.001	1.49 (1.12 to 1.98)	0.006	1.95 (0.97 to 3.94)	0.06	0.85 (0.59 to 1.23)	0.39
Adjusted: Model 2†	1.29 (0.96 to 1.72)	0.09	2.06 (1.56 to 2.72)	<0.001	1.54 (1.15 to 2.08)	0.004	1.95 (1.04 to 3.65)	0.04§	0.84 (0.58 to 1.21)	0.35
ILD mortality										
Number of events	9		7		16		3		7	
Person-years (PY)	30,295		28,014		70,176		11,457		28,014	
Rate per 10,000 PY (95% CI)	3.0 (1.5 to 5.7)		2.5 (1.2 to 5.2)		2.3 (1.4 to 3.7)		2.6 (0.8 to 8.1)		2.5 (1.2 to 5.2)	
Hazard ratio (95% CI)										
Unadjusted	1.83 (1.31 to 2.55)	<0.001	1.71 (1.21 to 2.43)	0.003	2.04 (1.25 to 3.33)	0.004	1.35 (0.56 to 3.26)	0.51	1.47 (0.76 to 2.87)	0.25
Adjusted: Model 1*	1.41 (0.93 to 2.14)	0.11	2.02 (1.19 to 3.41)	0.009	3.30 (1.40 to 7.80)	0.006	1.28 (0.45 to 3.65)	0.64	1.29 (0.67 to 2.50)	0.45
Adjusted: Model 2†	1.39 (0.94 to 2.07)	0.10	2.15 (1.26 to 3.64)	0.005	3.61 (1.54 to 8.46)	0.003	1.36 (0.52 to 3.58)	0.53	1.29 (0.66 to 2.53)	0.45

ILD: interstitial lung disease; HR: hazard ratio;

Effect estimates are per standard deviation of each adhesion molecule.

*Adjusted for generalized propensity score 1, which includes age, gender, race/ethnicity, smoking status, cigarette pack-years, BMI, waist circumference, height, educational attainment, study site, and glomerular filtration rate.

†Adjusted for generalized propensity score 2, which includes age, gender, race/ethnicity, smoking status, cigarette pack-years, BMI, waist circumference, height, educational attainment, study site, glomerular filtration rate, percent emphysema on CT, alcohol use, total intentional exercise (metabolic equivalent minutes/week), coronary artery calcium, diabetes medication use, insulin use, fasting glucose, hypertension, antihypertensive medication use, systolic and diastolic blood pressures, cholesterol medication use, total and high-density lipoprotein cholesterol levels, C-reactive protein, d-dimer, and cancer history.

§ p value no longer significant after correcting for multiple comparisons

Figure 1. Continuous associations of adhesions molecules sICAM-1, sVCAM-1 and P-selectin with HAA (A-C, respectively), ILA (D-F, respectively), and FVC (G-I, respectively). HAA models (A-C) are adusted for age, sex, body mass index, waist circumference, height, race/ethnicity, current smoking status and pack-year smoking history, educational attainment, glomerular filtration rate (GFR), study site/scanner, radiation dose, percent emphysema, and total imaged lung volume. ILA and FVC models (D-I) are adjusted for age, gender, race, smoking status, BMI, and study site. Solid line is the overall effect estimate, and dashed lines are the 95% confidence bands. Each vertical hashmark in the rug plot along the x-axis represent one study participant.
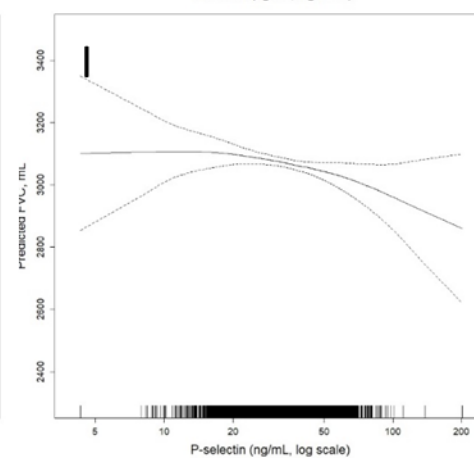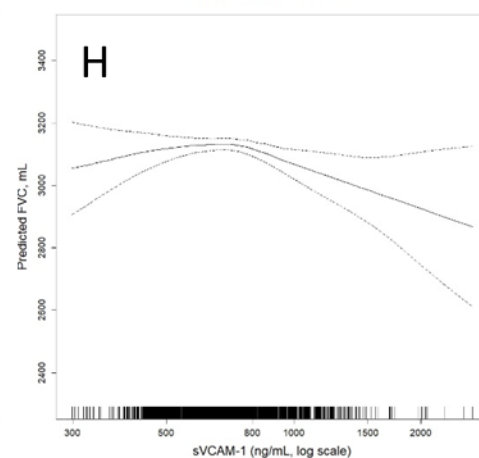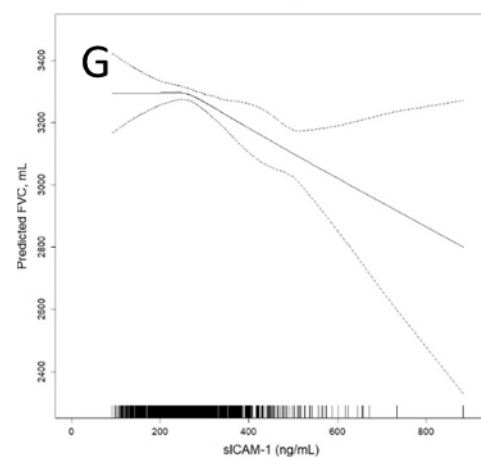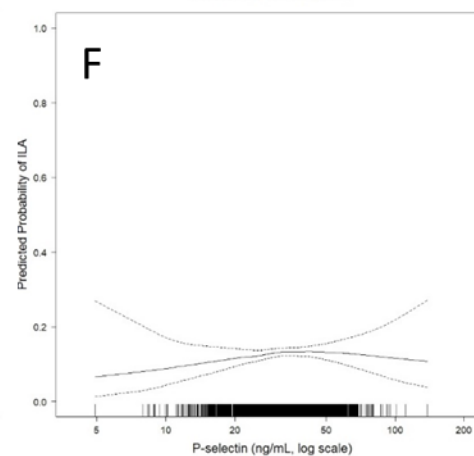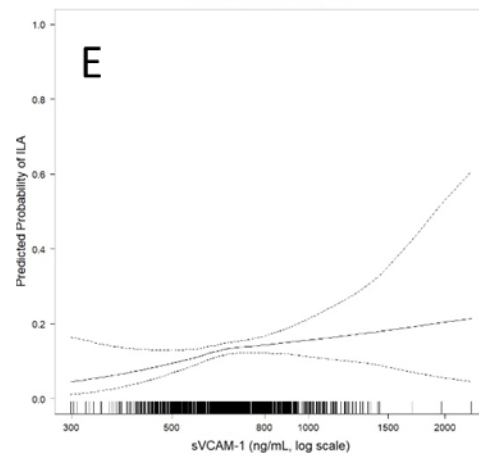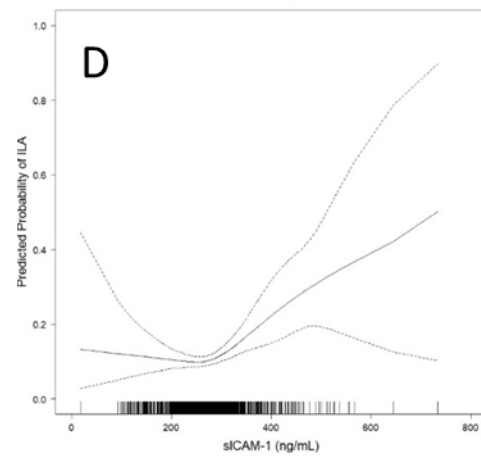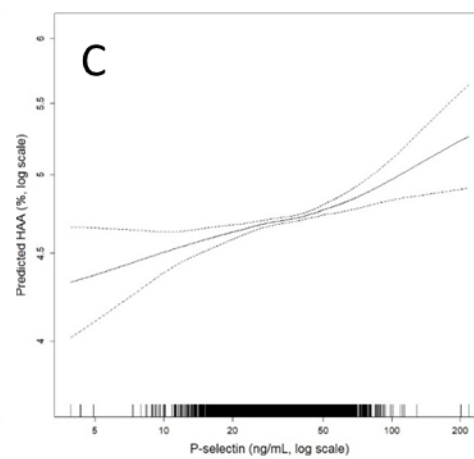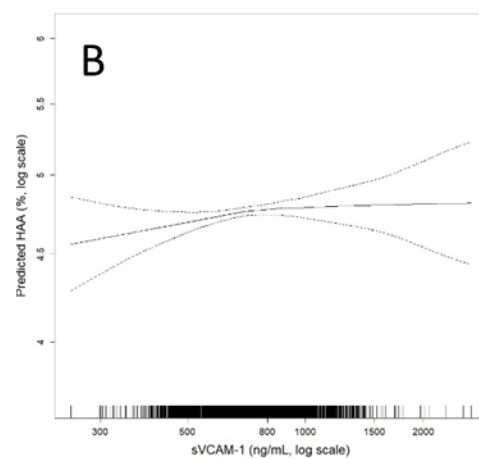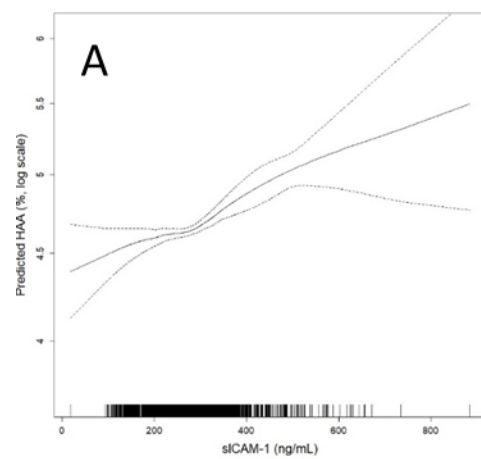
Figure 2. Continuous associations of adhesions molecules sICAM-1, sVCAM-1 and P-selectin with ILD hospitalization (A-C, respectively), and death due to ILD (D-F, respectively). Models adjusted for generalized propensity score 2, which included age, gender, race/ethnicity, smoking status, cigarette pack-years, BMI, waist circumference, height, educational attainment, study site/scanner, glomerular filtration rate, radiation dose, alcohol use, total intentional exercise (metabolic equivalent minutes/week), coronary artery calcium, diabetes medication use, insulin use, fasting glucose, hypertension, antihypertensive medication use, systolic and diastolic blood pressures, cholesterol medication use, total and high-density lipoprotein cholesterol levels, C-reactive protein, d-dimer, and cancer history. Solid line is the overall effect estimate, and dashed lines are the 95% confidence bands. Each vertical hashmark in the rug plot along the x-axis represent one study participant.
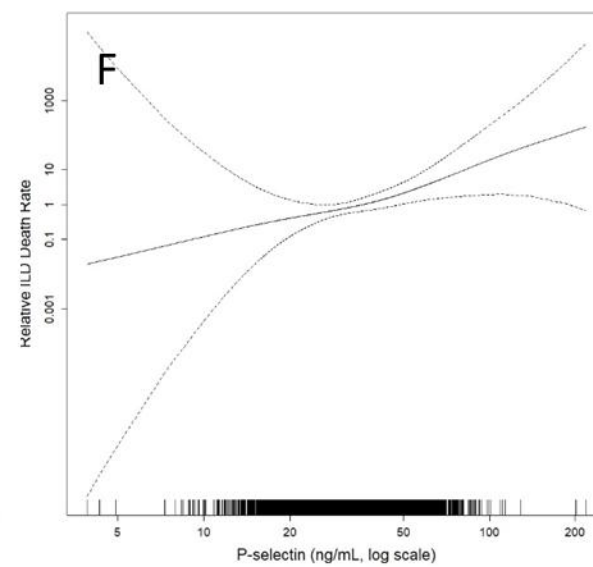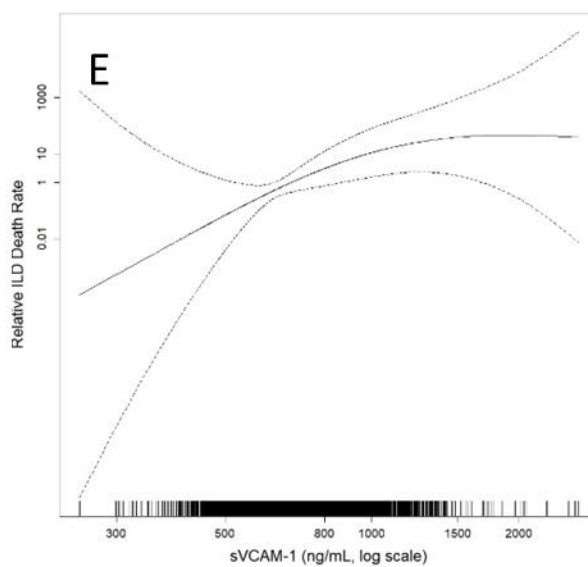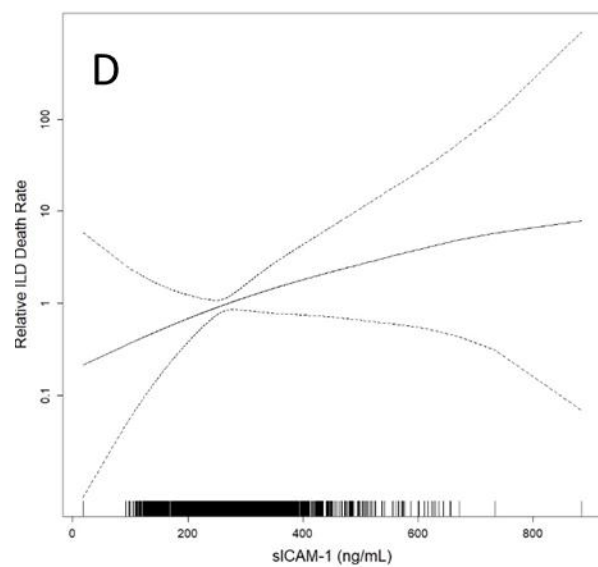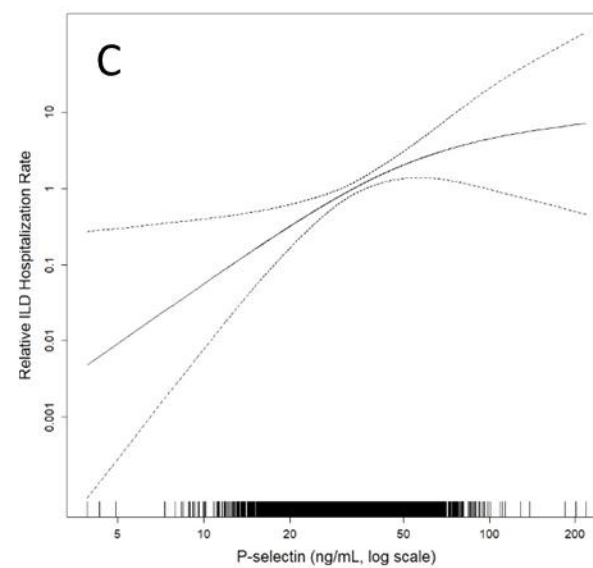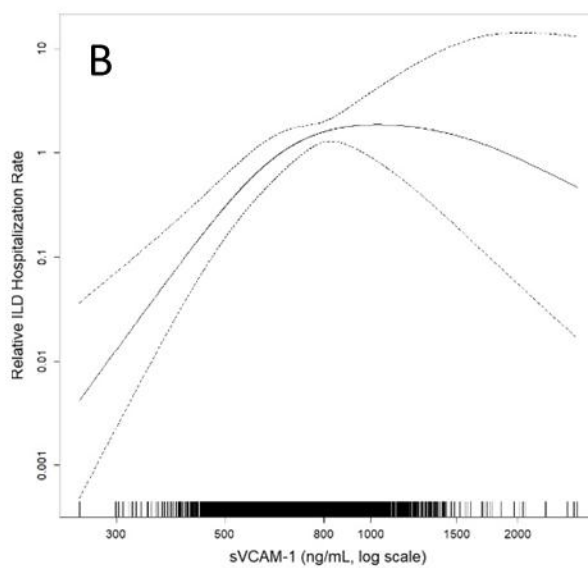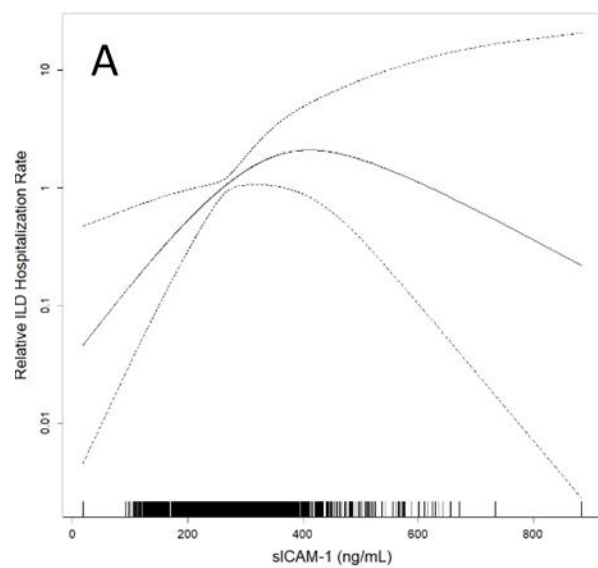
Discussion

We found that higher levels of circulating sICAM-1, sVCAM-1, and P-selectin were each independently associated with CT measures of subclinical ILD, lower FVC, increased rate of adjudicated ILD hospitalizations, and ILD death among community-dwelling adults. E-selectin was associated with lower FVC and respiratory mortality, but not our other outcome measures. These findings strongly support roles for circulating adhesion molecules sICAM-1, sVCAM-1 and P-selectin in the development of ILD and offer important insights into the biologic processes behind previous studies showing association of HAA and ILA with disease progression and mortality[9, 25].

ICAM-1 is essential for leukocyte diapedesis, which may be important in the earliest stages of pulmonary fibrosis [10, 12]. In the healthy lung, ICAM-1 is expressed in alveolar macrophages and, at low levels, in type II alveolar epithelial cells (AEC's) and capillary endothelial cells [26, 27]. In mouse models, administration of bleomycin leads to upregulation of ICAM-1 expression on macrophages and type II AEC's [10]. In humans with IPF, plasma concentrations of sICAM-1 are associated with poor progression-free and overall survival [12]. Ours is the first study to demonstrate associations between circulating levels of sICAM-1 and subclinical ILD. These findings may point to a role for sICAM-1 as a serum marker of type II AEC injury, or perhaps of activated macrophages, both of which have been implicated in the pathobiology of early ILD. Aaron *et al* previously showed that higher sICAM-1 levels are associated with greater progression of emphysema, but not with longitudinal change in lung function, as measured by FEV1 or FEV1/FVC [28].

We demonstrate an association of sICAM-1 with both CT measures of early ILD and lower FVC, even after adjusting for emphysema. Further studies are needed to determine whether sICAM-1 is directly involved in the pathobiology of early ILD, or is a biomarker of the disease process.

VCAM-1 is expressed within the vascular endothelium and functions similarly to ICAM-1 with regard to leukocyte recruitment [27]. Exposure to radiation increases endothelial expression of endothelial VCAM-1 in mouse models of pulmonary fibrosis [29]. In humans with IPF, both lung tissue expression of VCAM-1 and plasma levels of sVCAM-1 are significantly increased compared to controls [30]. Plasma levels of sVCAM-1 have also been shown to be predictive of mortality [12]. VCAM-1 is densely expressed in fibrotic foci of IPF lungs and its protein and mRNA levels significantly increase when human lung fibroblasts are treated with TGF-β [30]. Therefore, VCAM-1 may play a role in fibroblast proliferation in addition to leukocyte recruitment.

Ours is the first study to implicate P-selectin in the development and progression of ILD in humans. P-selectin is expressed on the surface of both activated endothelial cells and platelets [31]. When platelets adhere to endothelium at a site of injury, P-selectin on the surface of platelets binds neutrophils, monocytes and lymphocytes and slows their flow [31]. Activated platelets, bound to the capillary endothelium, also induce fibrin binding and upregulate endothelial expression of ICAM-1 and VCAM-1 [32]. Little is known about the role of P-selectin in the development of fibrosis, but there is substantial data supporting the role of platelets and P-selectin in acute lung injury, which is particularly relevant given that

repetitive injury to alveolar membrane has been implicated in the development of ILD, and subclinical ILD has been linked to increased risk of acute respiratory distress syndrome [33]. In animal models of acute lung injury, plasma levels of P-selectin correlated with the extent of lung inflammation after infusion of lipopolysaccharide, and an increase in platelet-derived P-selectin led to downstream ICAM-1 expression and neutrophil activation [34, 35]. In patients with acute respiratory distress syndrome, plasma P-selectin levels are associated with both higher lung injury scores and increased mortality [2, 15]. Our findings of an association of plasma P-selectin levels with subclinical ILD may therefore reflect increased platelet activity, fibroblast activation and upregulation of other adhesion molecules in early ILD.

We found that E-selectin was associated with lower FVC and respiratory mortality, but not with other measures of subclinical ILD, and that L-selectin was not associated with any of our outcomes. E-selectin is expressed on endothelial cells and has been shown to be upregulated in bleomycin-induced endothelial injury [36]. Given the relatively low sample size of the E-selectin analyses, it is possible that our study was not powered to detect an association between E-selectin and subclinical ILD. Alternatively, it is possible that E-selectin plays a role in more advanced stage of ILD, especially given prior data, which showed that in IPF lungs, E-selectin expression is restricted to areas of honeycombing [37]. L-selectin is constitutively expressed on the surface of leukocytes and its expression is not affected by endothelial perturbation, which may be one explanation for our null findings [38].

The association between sICAM-1and lung function was modified by smoking status. Smoking is a well-known risk factor for development of IPF and tobacco use is associated with both decreased FVC as well as increased HAA in the MESA cohort [5]. Underlying mechanisms may include oxidative stress to alveolar epithelium, and the release of profibrotic factors like TGF-β from pulmonary fibroblasts [39]. Schaberg *et al* demonstrated that smoking upregulates endothelial expression of ICAM-1 within the peripheral pulmonary vasculature, suggesting that smoking may lead to recruitment of leukocytes [40]. Thus, while higher sICAM-1 levels are associated with a decrease in FVC when adjusted for smoking status, smoking and sICAM-1 may interact to jointly cause additional oxidative stress to the epithelium and recruitment of leukocytes.

There were several limitations to our study. First, all adhesion molecules were measured in serum or plasma rather than lung tissue or single lung cells and thus may not fully reflect pathobiological changes in the lung. Moreover, measurements were done in stored samples and were not run in duplicate. Therefore, there is some concern about protein degradation or even estimate inflation. However, we believe that the relative differences are important even if the absolute values are not completely accurate. Second, adhesion molecules were measured at a single time point, limiting our ability to study the associations of longitudinal trends in these adhesion molecules with both subclinical and clinical measures of ILD. Third, analyses of clinical ILD events are limited by low event rates. Fourth, multiple comparisons increase the chances of type I error while varying sample sizes limits our ability to compare results between biomarkers. Finally, while we adjusted for a number of

demographic and clinical variables in our models, there remains the potential that residual confounding may affect some or all of our results.

In summary, we found that circulating adhesion molecules sICAM-1, sVCAM-1 and P-selectin are linked to CT measures of early lung injury, inflammation and fibrosis, lower FVC, and clinical ILD events in community-dwelling adults, supporting their role as a potential peripheral blood biomarkers of subclinical ILD. These findings suggest that sICAM-1, sVCAM-1 and P-Selectin may be important to the pathogenesis to ILD, and warrant future studies of the role these adhesion molecules play in the development and progression of pulmonary fibrosis.

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Circulating Adhesion Molecules and Subclinical Interstitial Lung Disease: The Multi-Ethnic Study of Atherosclerosis

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ONLINE DATA SUPPLEMENT

Figure S1. Study timeline of exams and measurements.
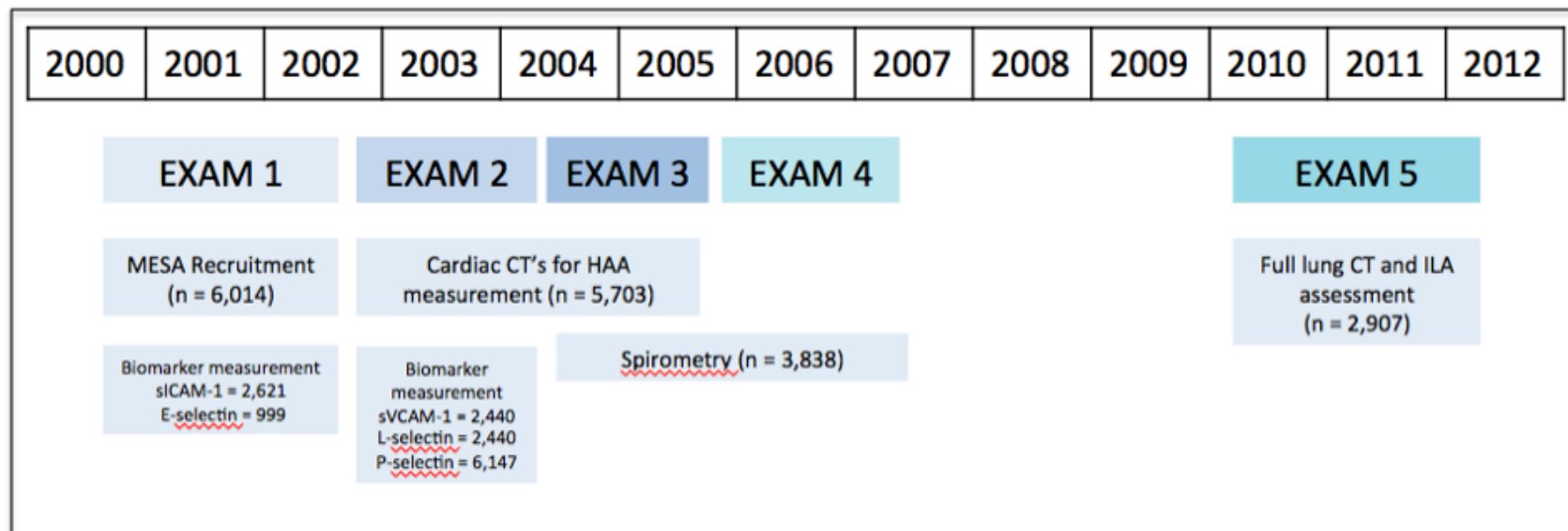
MESA: Multi-Ethnic Study of Atherosclerosis; sICAM: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas; ILA: interstitial lung abnormalities

Figure S2: Boxplots of distribution of adhesion molecule levels measured in MESA. Area within the box represents 25-75th percentiles, or interquartile range. Solid horizontal lines represent the median value. Whiskers extend to 1.5-times the interquartile range. Solid circles represent all individual data points.
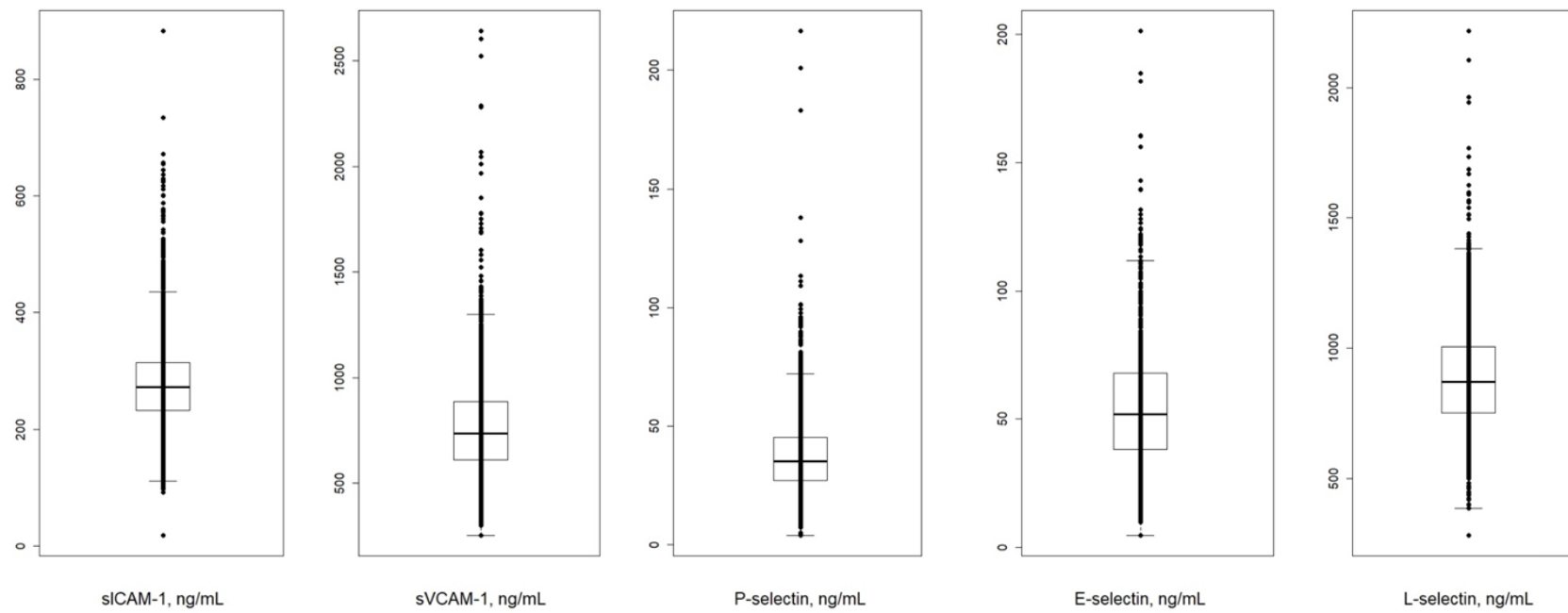
Table S1. Demographics per quartile of sVCAM-1 in patients with HAA measurements

	Overall	Quartile of VCAM-1			
		Q1	Q2	Q3	Q4
Number	2201	551	550	550	550
P-Selectin	733.4 ± 223.0	503.7 ± 62.4	642.1 ± 31.1	763.5 ± 42.0	1024 ± 213.3
Age, years	61.1 ± 10.0	56.5 ± 8.7	59.1 ± 9.1	62.8 ± 9.4	65.8 ± 10.1
Women, %	52.5%	51.4%	57.5%	52.0%	49.3%
BMI, kg/m²	27.9	28.3	27.8	27.5	27.9
Waist Circumference, cm	96.5	96.5	95.8	96	97.7
Height, cm	165.5	166.9	164.9	165.1	165
eGFR, mL/min/1.73 m²	80.1	85.4	80.9	78.6	75.5
Race					
White, %	26.2%	14.2%	28.2%	28.9%	33.5%
Chinese American, %	25.4%	24.9%	25.1%	26.0%	25.8%
Black, African American, %	23.9%	41.0%	20.5%	20.5%	13.5%
Hispanic, %	24.5%	20.0%	26.2%	24.5%	27.3%
Smoking					
Ever Smokers, %	50.6%	52.3%	51.1%	48.7%	50.2%
Current Smokers, %	14.7%	19.1%	15.4%	10.7%	13.4%
Site					
Wake Forest, %	13.1%	14.2%	12.9%	12.9%	12.4%
Columbia, %	16.9%	18.5%	16.9%	15.5%	16.5%
John's Hopkins, %	10.1%	12.9%	8.5%	9.6%	9.3%
UMN, %	13.5%	9.8%	14.9%	13.6%	15.6%
Northwestern, %	17.6%	17.6%	17.5%	19.1%	16.4%
UCLA, %	28.8%	29.2%	29.3%	29.3%	29.8%

Data presented as mean +/- SD, n (%) or median (interquartile range), unless otherwise stated. All parameters were collected at MESA baseline visit in years 2000-2002. HAA: high attenuation areas; sICAM: soluble vascular adhesion molecule; BMI: body mass index; eGFR: effective glomerular filtration rate

Table S2. Demographics per quartile of P-selectin in patients with HAA measurements

	Overall	Quartile of P-selectin			
		Q1	Q2	Q3	Q4
Number	5530	1383	1383	1382	1382
P-Selectin	36.2 ± 13.7	21.2 ± 3.9	30.5 ± 2.3	38.9 ± 2.7	54.4 ± 11.4
Age, years	61.8 ± 10.1	61.1 ± 10.1	61.9 ± 10.1	62.1± 10.2	62.2 ± 10.1
Women, %	52.1%	61.5	54.6	48.4	43.7
BMI, kg/m^2	28.3	27.7	27.9	28.5	29
Waist Circumference, cm	97.9	95.3	96.6	98.9	100.1
Height, cm	166.7	165.3	166.1	167.5	168.1
eGFR, mL/min/1.73 m^2	79.9	84.3	80.7	78.6	77.3
Race					
White, %	40.2%	34.1	37	45.4	44.2
Chinese American, %	11.8%	21.5	14.2	8.6	2.9
Black, African American, %	26.9%	28.1	28.3	24.7	26.3
Hispanic, %	21.2%	16.3	20.5	21.3	26.6
Smoking					
Ever Smokers, %	55.0%	48.4%	60.0%	56.2%	62.7%
Current Smokers, %	13.8%	10.5%	12.8%	14.8%	17.1%
Site					
Wake Forest, %	16.4	15.5%	16.2%	16.9%	17.1%
Columbia, %	15.5	20.0%	18.9%	14.3%	12.9%
John's Hopkins, %	15.0	14.7%	15.0%	15.3%	14.9%
UMN, %	15.8	5.8%	10.9%	18.5%	23.6%
Northwestern, %	17.5	18.7%	18.2%	18.5%	14.5%
UCLA, %	18.8	21.1%	20.7%	16.6%	17.0%

Data presented as mean +/- SD, n (%) or median (interquartile range), unless otherwise stated. All parameters were collected at MESA baseline visit in years 2000-2002. HAA: high attenuation areas; BMI: body mass index; eGFR: effective glomerular filtration rate

Table S3. Demographics per quartile of E-selectin in patients with HAA measurements

	Overall	Quartile of E-Selectin			
		Q1	Q2	Q3	Q4
Number	864	216	216	216	216
P-Selectin	54.5 ± 25.4	27.3 ± 7.3	44.8 ± 3.9	57.7 ± 4.1	88.2 ± 22.8
Age, years	58.9 ± 9.5	59.8 ± 10.2	59.0 ± 9.4	58.5 ± 9.5	58.2 ± 8.8
Women, %	57.8%	68.5%	58.3%	52.3%	52.3%
BMI, kg/m²	28.4	26.9	27.8	28.3	30.6
Waist Circumference, cm	97.6	92.6	96.4	98	103.6
Height, cm	166.5	165.6	167.9	166.7	165.7
eGFR, mL/min/1.73 m²	79.5	77.4	79.7	79.9	81
Race					
White, %	46.5%	54.6%	52.8%	45.4%	33.3%
Chinese American, %	9.9%	13.9%	7.9%	8.8%	8.8%
Black, African American, %	20.8%	15.3%	23.6%	19.9%	24.5%
Hispanic, %	22.8%	16.2%	15.7%	25.9%	33.3%
Smoking					
Ever Smokers, %	54.4%	46.8%	51.9%	55.6%	63.4%
Current Smokers, %	16.9%	14.8%	14.8%	15.3%	25.5%
Site					
Wake Forest, %	17.4%	18.1%	20.4%	13.9%	17.6%
Columbia, %	19.1%	13.0%	20.8%	21.3%	21.3%
John's Hopkins, %	11.5%	11.6%	19.0%	15.3%	9.3%
UMN, %	16.9%	18.1%	11.1%	18.1%	20.4%
Northwestern, %	15.3%	19.4%	18.1%	15.3%	8.3%
UCLA, %	19.8%	19.9%	15.3%	20.8%	23.1%

Data presented as mean +/- SD, n (%) or median (interquartile range), unless otherwise stated. All parameters were collected at MESA baseline visit in years 2000-2002. HAA: high attenuation areas; BMI: body mass index; eGFR: effective glomerular filtration rate

Table S4. Demographics per quartile of L-selectin in patients with HAA measurements

| | Overall | Quartile of L-Selectin | | | |
		Q1	Q2	Q3	Q4
Number	1066	552	549	550	550
P-Selectin	892.1 ± 197.1	661.8 ± 72.5	816.4 ± 34.5	936.9 ± 36.8	1154.0 ± 134.2
Age, years	61.1 ± 10.0	63.8 ± 9.9	61.1 ± 10.0	60.0 ± 9.6	59.4 ± 9.8
Women, %	52.5%	37.0%	48.3%	58.0%	66.9%
BMI, kg/m²	27.9	27.1	27.8	28.1	28.6
Waist Circumference, cm	96.5	95.3	96.7	96.5	97.4
Height, cm	165.5	166.3	165.9	164.9	164.8
eGFR, mL/min/1.73 m²	80.1	80.3	81.4	79.7	79.1
Race					
White, %	26.2%	15.8%	53.4%	27.6%	37.1%
Chinese American, %	25.4%	34.4%	103.4%	24.9%	14.4%
Black, African American, %	23.9%	27.0%	89.9%	22.7%	21.5%
Hispanic, %	24.5%	22.8%	85.9%	24.7%	27.1%
Smoking					
Ever Smokers, %	50.6%	55.3%	54.0%	46.7%	46.4%
Current Smokers, %	14.7%	16.3%	16.9%	12.9%	12.7%
Site					
Wake Forest, %	13.1%	12.3%	14.0%	12.4%	13.6%
Columbia, %	16.9%	14.1%	13.5%	17.3%	22.5%
John's Hopkins, %	10.1%	8.7%	8.0%	12.5%	11.1%
UMN, %	13.5%	10.3%	15.1%	12.2%	16.4%
Northwestern, %	17.6%	21.7%	18.2%	15.6%	14.9%
UCLA, %	28.8%	32.8%	30.1%	30.0%	21.5%

Data presented as mean +/- SD, n (%) or median (interquartile range), unless otherwise stated. All parameters were collected at MESA baseline visit in years 2000-2002. HAA: high attenuation areas; BMI: body mass index; eGFR: effective glomerular filtration rate

Table S5. Pearson correlation coefficients between biomarkers

	sICAM-1	sVCAM-1	E-selectin	L-selectin	P-selectn
sICAM_1	1.000	X	X	X	X
Sig (2-tailed)	<0.001				
sVCAM-1	0.275	1.000	X	X	X
Sig (2-tailed)	<0.001	<0.001			
E-selectin	0.394	0.148	1.000	X	X
Sig (2-tailed)	<0.001	<0.001	<0.001		
L-selectin	0.142	0.315	0.010	1.000	X
Sig (2-tailed)	<0.001	<0.001	0.774	<0.001	
P-selectin	0.223	0.147	0.389	0.031	1.000
Sig (2-tailed)	<0.001	<0.001	<0.001	0.127	<0.001

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule

Table S6. Associations of circulating adhesion molecules with HAA, ILA and FVC, stratified by smoking status.

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
HAA										
N participants	2,226		2,187		5,498		864		2187	
% change (95% CI)*										
Never Smokers	3.31 (1.55 to 5.08)	<0.001	1.10 (-0.40 to 2.60)	0.15	1.21 (0.25 to 2.19)	0.01	0.96 (-1.66 to 3.57)	0.94	-0.05 (-1.55 to 1.46)	0.95
Ever Smokers	3.32 (1.96 to 4.68)	<0.001	0.99 (-0.55 to 2.48)	0.21	2.26 (1.41 to 3.13)	<0.001	0.55 (-1.77 to 2.88)	0.64	3.00 (-0.96 to 2.02)	0.49
ILA										
N participants	1,028		1,008		2,239		507		1,008	
OR (95% CI)†										
Never Smokers	1.29 (0.93 to 1.80)	0.12	1.21 (0.91 to 1.58)	0.20	1.02 (0.83 to 1.25)	0.87	0.65 (0.38 to 1.12)	0.12	1.02 (0.77 to 1.34)	0.91
Ever Smokers	1.47 (1.14 to 1.90)	0.003	1.16 (0.90 to 1.50)	0.25	1.15 (0.96 to 1.38)	0.12	1.20 (0.82 to 1.76)	0.35	0.80 (0.60 to 1.07)	0.14
FVC										
N participants	1,549		1,680		3,449		808		1,688	
Change, mL (95% CI)†										
Never Smokers	-13.6 (-63.4 to 36.1)	0.59	-11.8 (-50.5 to 26.9)	0.55	-14.0 (-44.1 to 16.0)	0.36	-16.1 (-78.3 to 46.1)	0.87	-3.2 (-41.6 to 35.2)	0.87
Ever Smokers	-89.7 (-127.9 to -51.4)	<0.001	-46.5 (-86.9 to -6.1)	0.02	-49.8 (-77.1 to -22.5)	<0.001	-85.2 (-143.2 to -27.2)	0.004	25.9 (-13.3 to 65.1)	0.20

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas; ILA: interstitial lung abnormalities; FVC: forced vital capacity; OR: odds ratio

Effect estimates are per standard deviation of each adhesion molecule.

P for interaction by smoking status >0.05 for all analyses except for the association of sICAM-1 with FVC (p=0.01)

* adjusted for age, sex, body mass index, waist circumference, race/ethnicity, eGFR, current smoking status and pack-year smoking history, educational attainment, study site/scanner, mA dose, percent emphysema, and total imaged lung volume.

† adjusted for age, gender, race, smoking status, eGFR, BMI and site

Table S7. Associations of circulating adhesion molecules with HAA, ILA and FVC, stratified by age

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
HAA % change (95% CI)*										
45-54	2.32 (0.08 to 4.56)	0.04	1.37 (-1.08 to 4.30)	0.241	1.01 (-0.49 to 2.66)	0.17	3.12 (-0.32 to 6.58)	0.76	0.29 (-2.26 to 2.85)	0.82
55-64	3.44 (0.74 to 6.13)	0.013	2.94 (-0.29 to 6.17)	0.08	2.06 (0.30 to 3.82)	0.02	2.84 (-1.59 to 7.27)	0.21	0.57 (-2.59 to 3.73)	0.72
65+	3.93 (0.52 to 7.35)	0.02	2.56 (0.25 to 4.87)	0.30	2.21 (0.75 to 2.67)	0.003	-1.38 (-7.41 to 3.73)	0.51	-3.11(-5.51 to -0.71)	0.01
ILA OR (95% CI)†										
45-54	1.48 (1.05 to 2.09)	0.03	1.21 (0.85 to 1.74)	0.28	1.04 (0.76 to 1.41)	0.81	0.93 (0.56 to 1.55)	0.79	0.99 (0.65 to 1.49)	0.94
55-64	1.96 (1.27 to 3.03)	0.002	1.11 (0.69 to 1.75)	0.67	1.37 (1.03 to 1.83)	0.03	1.18 (0.62 to 2.24)	0.60	1.17 (0.74 to 1.85)	0.51
65+	1.08 (0.74 to 1.61)	0.66	1.48 (1.09 to 2.00)	0.012	1.02 (0.85 to 1.24)	0.81	0.87 (0.44 to 1.72)	0.69	0.85 (0.62 to 1.16)	0.31
FVC Change, mL (95%CI)†										
45-54	-38.6 (-89.3 to 12.1)	0.14	-50.3 (-107.6 to 7.08)	0.09	-43.0 (-81.8 to -4.3)	0.03	-36.9 (-106.9 to 33.0)	0.30	-0.75 (-52.8 to 51.3)	0.98
55-64	-69.2 (-129.3 to -9.12)	0.02	-0.37 (-55.9 to 55.2)	0.98	-51.2 (-90.2 to -12.1)	0.01	-92.3 (-172.3 to -12.2)	0.02	41.7 (-14.1 to 97.6)	0.14
65+	-40.59 (-102.6 to 21.4)	0.20	-69.1 (-112.6 to -25.7)	0.002	-19.1 (-51.7 to 12.4)	0.25	-51.1 (-144.2 to 42.0)	0.28	14.4 (-31.3 to 60.0)	0.53

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas; ILA: interstitial lung abnormalities; FVC: forced vital capacity; OR: odds ratio

Effect estimates are per standard deviation of each adhesion molecule.

P for interaction by age >0.05 for all analyses except for the associations of L-selectin with HAA (p=0.02)

* adjusted for age, sex, body mass index, waist circumference, race/ethnicity, eGFR, current smoking status and pack-year smoking history, educational attainment, study site/scanner, mA dose, percent emphysema, and total imaged lung volume.

† adjusted for age, gender, race, smoking status, eGFR, BMI and site

Table S8. Associations of circulating adhesion molecules with HAA, ILA and FVC, stratified by BMI

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
HAA % change (95% CI)*										
Underweight & normal	1.40 (-1.54 to 4.34)	0.35	-1.31 (-4.07 to 1.44)	0.34	2.91 (1.29 to 4.52)	<0.001	1.12 (-3.34 to 5.73)	0.60	-2.51 (-5.23 to 0.19)	0.07
Overweight	3.90 (1.35 to 6.46)	0.003	3.54 (1.06 to 6.01)	0.005	1.07 (-0.44 to 2.59)	0.17	0.61 (-3.69 to 4.91)	0.78	0.30 (-2.16 to 2.77)	0.81
Obese	3.18 (0.48 to 5.87)	0.02	2.64 (-0.34 to 5.63)	0.08	1.74 (0.11 to 2.28)	0.04	0.91 (-3.27 to 5.09)	0.67	-0.60 (-3.51 to 2.32)	0.67
ILA OR (95% CI)†										
Underweight & normal	1.66 (1.07 to 2.58)	0.023	1.115 (0.77 to 1.71)	0.49	0.93 (0.75 to 1.29)	0.91	0.65 (0.26 to 1.58)	0.34	1.29 (0.87 to 1.91)	0.21
Overweight	1.22 (0.93 to 1.59)	0.14	1.22 (0.93 to 1.61)	0.15	1.12 (0.94 to 1.33)	0.19	1.53 (0.87 to 2.68)	0.14	0.77 (0.58 to 1.03)	0.07
Obese	2.81 (1.15 to 6.85)	0.024	1.26 (0.79 to 2.02)	0.39	1.11 (0.69 to 1.81)	0.69	0.72 (0.44 to 1.18)	0.20	1.11 (0.62 to 1.95)	0.72
FVC Change, mL (95%CI)†										
Underweight & normal	-39.4 (-106.3 to 27.8)	0.25	-38.7 (-91.2 to 13.8)	0.15	-58.4 (-96.3 to -20.5)	0.003	-171.2 (-273.9 to -68.6)	0.001	-15.6 (-66.5 to 35.3)	0.55
Overweight	-38.4 (-94.4 to 17.5)	0.18	7.8 (-38.7 to 54.4)	0.74	-11.2 (-45.9 to 23.5)	0.53	-20.5 (-96.8 to 55.7)	0.58	23.1 (-23.6 to 70.0)	0.33
Obese	-60.7 (-109.4 to -12.0)	0.015	-84.9 (-140.0 to -29.8)	0.003	-21.1 (-56.3 to 14.2)	0.24	-30.1 (-98.7 to 36.5)	0.37	14.9 (-37.6 to 67.5)	0.58

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas; ILA: interstitial lung abnormalities; FVC: forced vital capacity; OR: odds ratio

Effect estimates are per standard deviation of each adhesion molecule

P for interaction by BMI >0.05 for all analyses

* adjusted for age, sex, body mass index, waist circumference, race/ethnicity, eGFR, current smoking status and pack-year smoking history, educational attainment, study site/scanner, mA dose, percent emphysema, and total imaged lung volume.

† adjusted for age, gender, race, smoking status, eGFR, BMI and site

Table S9. Associations of circulating adhesion molecules with HAA in a less adjusted model

	sICAM-1	P value	sVCAM-1	P value	P-selectin	P value	E-selectin	P value	L-selectin	P value
HAA										
N participants	2,226		2,187		5,487		864		2187	
% change (95% CI)*	3.10 (1.55 to 4.65)	<0.001	2.31 (0.77 to 3.85)	0.003	1.52 (0.61 to 2.42)	<0.001	1.78 (-0.64 to 4.20)	0.15	-0.77 (-2.17 to 0.88)	0.40

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; HAA: high attenuation areas

Effect estimates are per standard deviation of each adhesion molecule.

*adjusted for age, gender, race, smoking status, eGFR, BMI and site

Table S10. Association between sICAM-1 and HAA, ILA and FVC by genotypes at SNP rs5491

Change %HAA	sICAM1	p-value
AA genotype, n = 2,198	3.56% (2.28-4.84)	<0.001
TA genotype, n = 310	5.96% (1.20-10.72)	0.01
Odds of ILA		
AA genotype, n = 968	1.45 (1.02-2.05)	0.04
TA genotype, n = 133	0.33 (0.05-2.40)	0.27
Dec in FVC, mL		
AA genotype, n = 1,616	-56.2 (-103.9 to -8.6)	0.02
TA genotype, n = 224	-2.9 (685.2-679.4)	0.99

* P for interaction of all associations is > 0.10

Table S11. Associations between sICAM-1, sVCAM-1, P-selectin with ILA patterns

ILA abnormality	#	ICAM-1 OR(95% CI)	p-value	#	VCAM-1 OR (95% CI)	p-value	#	P-selectin OR (95% CI)	p-value
Ground glass opacities	71	1.37 (1.10 to 1.71)	0.003	79	1.30 (1.07 to 1.58)	0.007	162	1.05 (0.89 to 1.23)	0.54
Reticular abnormalities	76	1.36 (1.10 to 1.68)	0.005	84	1.41 (1.17 to 1.69)	<0.001	193	1.06 (0.92 to 1.23)	0.42
Centrilobular nodules	8	1.37 (0.75 to 2.51)	0.30	13	1.09 (0.65 to 1.81)	0.75	24	1.36 (.89 to 2.07)	0.15
Honeycombing	1	2.23 (0.76 to 6.47)	0.14	0	N/A		6	1.25 (0.55 to 2.87)	0.56
Traction Bronchiectasis	14	1.49 (0.96 to 2.29)	0.07	15	1.23 (0.81 to 1.87)	0.32	41	0.86 (0.63 to 1.16)	0.32
Non-emphysematous cysts	7	1.06 (0.49 to 2.25)	0.88	3	1.03 (0.34 to 3.11)	0.95	11	0.97 (0.53 to 1.75)	0.92
Architectural Distortion	6	1.74 (0.99 to 3.07)	0.05	8	1.63 (1.11 to 2.39)	<0.001	14	1.31 (0.76 to 2.26)	0.32
Fibrotic ILA*	84	1.39 (1.14 to 1.70)	0.001	93	1.34 (1.12 to 1.61)	0.002	226	1.09 (0.95 to 1.25)	0.25

sICAM-1: soluble intracellular adhesion molecule ILA; sVCAM-1: soluble vascular adhesion molecule; Interstitial lung abnormalities
*Fibrotic ILA was defined as the presence of reticular changes, traction bronchietasis and/or honeycombing

Table S12. Associations of circulating adhesion molecules and percent predicted FVC, mL

	sICAM1	p-value	sVCAM1	p-value	P-selectin	p-value	E-selectin	p-value	L-selectin	p-value
N for FVC	1,549		1,680		3,449		808		1,688	
Change in % pred. FVC, mL										
Unadjusted	-1.87 (-2.64 to -1.11)	<0.001	0.18 (-0.92 to 0.57)	0.64	-0.69 (-1.22 to -0.17)	0.01	-1.60 (-2.67 to -0.53)	0.003	-0.63 (-1.37 to 0.11)	0.10
Adjusted*	-0.74 (-1.53 to 0.06)	0.07	-0.40 (-1.15 to 0.34)	0.30	-0.24 (-0.77 to 0.30)	0.38	-0.54 (-1.42 to 0.14)	0.11	-0.46 (-1.20 to 0.28)	0.23
Never Smokers	0.14 (-1.11 to 1.38)	0.83	0.04 (-1.04 to 0.96)	0.93	0.42 (-0.35 to 1.18)	0.29	0.14 (-1.11 to 1.38)	0.83	-0.83 (-1.87 to 0.19)	0.11
Ever Smokers	-1.90 (-2.87 to -0.93)	<0.001	-0.56 (-1.62 to 0.50)	0.30	-1.17 (-1.86 to -0.47)	0.001	-1.90 (-2.87 to 0.93)	<0.001	0.16 (-0.88 to 1.20)	0.76

sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; FVC: forced vital capacity

p for interaction by smoking is > 0.10 in all models

* adjusted for smoking status and BMI

Table S13. Associations of circulating adhesion molecules and FVC when excluding participants with obstructive disease
sICAM-1: soluble intracellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; FVC: forced vital capacity
*adjusted for age, gender, race, smoking status, eGFR, BMI and site

	sICAM1	p-value	sVCAM1	p-value	P-selectin	p-value	E-selectin	p-value	L-selectin	p-value
N for FVC*	1,549		1,680		3,449		808		1,688	
Change in FVC, mL, when exluding obstructive disease	-41.5 (-74.6 to -8.5)	0.02	-19.0 (-49.1 to 11.1)	0.21	-17.5 (-38.8 to 3.9)	0.11	-46.6 (-93.1 to 0.10)	0.05	15.7 (-14.9 to 46.3)	0.31