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Matrix metalloproteinase-9 and plasminogen activator inhibitor-1 are associated with right ventricular structure and function: The MESA-RV Study

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Abstract

Elevated resistance and reduced compliance of the pulmonary vasculature increase right ventricular (RV) afterload. Local and systemic inflammation and haemostatic abnormalities are prominent in pulmonary vascular diseases. We hypothesized that plasma biomarker levels indicating greater inflammation and coagulability associated with pulmonary vascular disease would be associated with RV structure and function measured by cardiac magnetic resonance imaging (MRI). The Multi-Ethnic Study of Atherosclerosis (MESA) performed cardiac MRI among participants aged 45–84 years without clinical cardiovascular disease. We assessed the associations of RV mass, RV end-diastolic volume (RVEDV), RV stroke volume (RVSV) and RV ejection fraction (RVEF) with plasma measures of inflammation (matrix metalloproteinase (MMP)-3 and -9, intercellular adhesion molecule (ICAM)-1, tumour necrosis factor receptor (TNF-R1), and E-selectin) and thrombosis (plasminogen activator inhibitor (PAI)-1, tissue factor, tissue factor pathway inhibitor and CD40 ligand).The study sample included 731 subjects. Higher MMP-9 levels were associated with lower RV mass before and after adjustment for left ventricular (LV) mass ($p = 0.008$ and $p = 0.044$, respectively). Higher levels of MMP-9 and PAI-1 were also associated with smaller RVEDV ($p<0.05$). Higher PAI-1 levels were associated with lower RVEF even after adjustment for LV ejection fraction ($p = 0.017$). In conclusion, MMP-9 and PAI-1 are associated with changes in RV structure and function which could be potentially related to a subclinical increase in pulmonary vascular resistance.

Keywords: *Inflammation; thrombosis; hypertension; pulmonary*

Introduction

Pulmonary hypertension with subsequent right ventricular (RV) failure is an increasingly important cause of morbidity and mortality. Recent evidence suggests that clinically significant pulmonary vascular disease may be caused by both local and systemic inflammation. Elevated plasma levels of inflammatory cytokines (including chemokine (C-C motif) ligand 2) are found in patients with pulmonary arterial hypertension (PAH)(Humbert et al. 1995, Sanchez et al. 2007). There is also extensive evidence for a hypercoagulable state in pulmonary hypertension. *In situ*

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thrombosis is commonly seen in the small pulmonary arteries in PAH, and plasminogen activator inhibitor (PAI)-1, tissue factor (TF) and CD40 ligand (CD40L) are all dysregulated in the plasma or lungs in PAH (White et al. 2007, Martin et al. 2002, Christ et al. 2001, Bakouboula et al. 2008, Hoeper et al. 1998). Parenchymal lung disease and left-sided cardiac disease are also characterized by inflammation and hypercoagulability, potentially leading to subclinical pulmonary vascular disease and changes in RV morphology.

Systemic inflammation and thrombosis have important effects on the left ventricle (LV) (Bahrami et al. 2008). For example, increased matrix metalloproteinase (MMP)-9 and PAI-1 levels are associated with altered LV geometry (Ahmed et al. 2006, Velagaleti et al. 2008). Unfortunately, there are no large multiethnic studies of the association between biomarkers reflecting inflammation and thrombosis associated with subclinical pulmonary vascular disease and RV parameters. Studies of individuals without clinical pulmonary or cardiovascular disease might enlighten the early pathogenic processes which result in clinical RV dysfunction.

We examined the relationships of a variety of biomarkers with magnetic resonance imaging (MRI) measures of RV structure and function in a large, population-based cohort free of clinical cardiovascular disease. We hypothesized that plasma levels of biomarkers indicating greater inflammation (MMP-3 and MMP-9, soluble intercellular adhesion molecule (ICAM)-1, soluble tumour necrosis factor-α receptor 1 (TNF-R1) and soluble E-selectin) and coagulability (PAI-1, soluble TF, tissue factor pathway inhibitor (TFPI) and soluble CD40L) in the pulmonary vasculature would be associated with increased RV mass, larger RV end-diastolic volume (RVEDV) and lower RV stroke volume (RVSV) and RV ejection fraction (RVEF).

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicentre prospective cohort study to investigate the prevalence, correlates and progression of subclinical cardiovascular disease in whites, African-Americans, Hispanics and Chinese (Bild et al. 2002). In 2000–2002, MESA recruited 6814 men and women aged 45–84 years old from six US communities: Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; St Paul, MN; Chicago, IL; and Los Angeles, CA. Exclusion criteria included clinical cardiovascular disease (physician diagnosis of heart attack, stroke, transient ischemic attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure), weight >136 kg (300 lbs), pregnancy, or impediment to long-term participation. The protocols of MESA and all studies described herein were approved by the Institutional Review Boards of all collaborating institutions and the National Heart Lung and Blood Institute.

The MESA-Right Ventricle Study is an ancillary study focused on measuring RV morphology in MESA participants eligible for MRI (without metal implants, device or fragment) with interpretable studies at the baseline examination.

Cardiac magnetic resonance imaging measures

The cardiac MRI protocol has been previously described (Natori et al. 2006). All imaging was performed on 1.5 T magnets with a four-element phased-array surface coil positioned anteriorly and posteriorly and electrocardiographic gating. Imaging consisted of fast-gradient echo cine images of the LV with temporal resolution ≤50 ms.

Methods for interpretation of LV and RV parameters have been previously reported (Bluemke et al. 2008, Chahal et al. 2010). Briefly, RV image analysis was performed by two independent analysts on Windows workstations using QMASS software (Medis, Leiden, the Netherlands). The endocardial and epicardial borders of the RV were traced manually on the short axis cine images at the end-systolic and end-diastolic phase. RV end-systolic volume (RVESV) and RVEDV were calculated using Simpson's rule by summation of areas on each slice multiplied by the sum of slice thickness and image gap. RV mass was determined at the end-diastole phase as the difference between end-diastolic epicardial and endocardial volumes multiplied by the specific gravity of the heart (1.05gcm^{-3}). RVSV was calculated by subtracting RVESV from the RVEDV. RVEF was calculated by dividing RVSV by RVEDV.

Biomarkers

Fasting blood samples were drawn, processed and stored using standardized procedures (Cushman et al. 1995). All plasma biomarkers were measured in the Laboratory for Clinical Biochemistry Research at University of Vermont (Burlington, VT, USA). Plasma biomarkers of inflammation and thrombosis were assayed in 999 MESA participants.

Inflammation

MMP-3 was measured by an ultrasensitive, solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using a polyclonal antibody specific for both the pro- and active forms of MMP-3 (Quantikine Human MMP-3 (total) Immunoassay; R&D Systems, Minneapolis, MN, USA)(coefficient of variation (CV) ~7.0–8.6%). MMP-9 was measured by a high-sensitivity quantitative sandwich enzyme immunoassay (Quantikine Human MMP-9 (total)

Immunoassay; R&D Systems)(CV ~6.9–7.9%). Soluble ICAM-1 was measured by ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems,) (CV 5.0%). Soluble TNF-R1 was measured using an ultrasensitive ELISA assay (Quantikine Human sTNF RI Immunoassay; R&D Systems) (CV 5%). Soluble E-selectin was measured using a high-sensitivity quantitative sandwich enzyme assay (Parameter Human sE-Selectin Immunoassay; R&D Systems) (CV ~5.7–8.8%).

Thrombosis

The PAI-1 assay was a two-site ELISA sensitive to free PAI-1 (both latent and active) but not PAI-1 in complex with tissue plasminogen activator (CV 3.5%) (Declerck et al. 1988) Soluble TF was measured by an enzyme-linked immunoassay that employs an anti-TF monoclonal capture antibody (Imubind Tissue Factor ELISA Kit, American Diagnostica, Inc., Stamford, CT, USA) (CV 14.6%). TFPI was measured by enzyme-linked sandwich ELISA using a polyclonal anti-TFPI antibody as the capture antibody (Imubind Total TFPI ELISA Kit, American Diagnostica, Inc.) (CV ~5.5–7.3%). CD40L was measured by an ultrasensitive quantitative sandwich enzyme immunoassay technique (Quantikine Human soluble CD40 Ligand Immunoassay; R&D Systems) (CV ~6.0–6.4%).

Other covariates

Race/ethnicity was self-reported during the baseline MESA examination according to the 2000 US Census criteria. Height was measured to the nearest 0.1 cm with the subject in stocking feet and weight was measured to the nearest pound with the subject in light clothing using a balanced scale. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or current use of anti hypertension medication. Presence of diabetes mellitus was based on self-reported physician diagnosis, use of medication for hyperglycaemia, or a fasting glucose value ≥126 mg dl^{-1}, the latter measured by rate reflectance spectrophotometry (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY, USA). Fasting glucose of 100–125 $mgdl^{-1}$ was considered impaired fasting glucose. Smoking was classified as current, past or never. Spirometry, urine cotinine and computed tomographic (CT) lung density measures were available for a subset of 607 participants(Hankinson et al. 2009, Hoffman et al. 2009).

Statistical analysis

Continuous variables are expressed as means and standard deviations or ranges. Categorical variables are expressed as percentage. Multivariate linear regression was used to assess the relationship of each biomarker (in quintiles) with RV mass, RVEDV, RVSV and RVEF. Results are expressed as least-square means. Initial models were adjusted for age, gender, race/ethnicity, height and weight. Adjustment for height and weight in all analyses avoided the assumptions made in indexing the RV measures to certain parameters of body size (e.g. body surface area), while achieving the same end of accounting for differences in body size between participants. Biomarkers which were possibly associated with RV parameters in this analysis ($p<0.20$) were then further adjusted for smoking, hypertension, diabetes mellitus, impaired glucose tolerance, hypertension, and systolic and diastolic blood pressure, as well as the respective LV parameter. Adjustment for LV parameters was performed both to account for the contribution of LV abnormalities to RV changes (e.g. increased LV mass causing pulmonary venous hypertension leading to increased RV mass), to better account for differences in body size, and to examine RV-specific associations (rather than more general associations with bi-ventricular morphology). RVSV was not adjusted for LV stroke volume considering the significant interdependence of these measures. The subset of the study sample with available spirometry, urine cotinine and CT lung density measurements was included in further adjusted analyses. Statistical significance was defined as $p<0.05$. Analyses were performed using STATA 10.0 (StataCorp, College Station, TX, USA).

Results

MESA included 6814 participants. Of these, 1000 were randomly selected for biomarker sampling, which 999 completed. Of these 999, 785 were eligible for cardiac MRI and had MRI performed, which was interpretable in 734. Three participants had missing data for clinical variables, leaving 731 in the final study sample (Table 1). The mean age was 59.0 years, 41.9% were male, 45.6% were white, 21.1% were African-American, 22.9% were Hispanic and 10.4% were Chinese. The mean body mass index (BMI) was 27.7 kgm^{-2}. The study sample was similar to those excluded in terms of age, gender, race/ethnicity, educational achievement, hypertension and diabetes mellitus. There were differences between included and excluded participants in terms of weight, BMI and smoking status with the study sample being somewhat leaner and more likely to be non-smokers. Biomarker values were similar between those in the study sample and those excluded.

The mean RV mass in the study sample was 22.3 ± 4.8 g, the mean RVEDV was 127.3 ± 32.4 ml and the mean RVSV was 86.9 ± 21.4 ml. The mean RVEF was 68.7 ± 6.9%. The significant associations of quintiles of biomarkers with RV parameters after adjustment for age, sex, race/ethnicity, height and weight are shown in Table 2. Higher MMP-9 levels were associated with lower RV mass and

Table 1. Characteristics of the study sample compared with those excluded.

	Study sample (n=731)	Excluded (n=268)
Age (years)	59.0±9.6	60.2±10.0
Male (%)	41.9	45.9
Race/ethnicity (%)		
White	45.6	47.0
African-American	21.1	20.5
Hispanic	22.9	24.3
Chinese	10.5	8.2
Educational attainment (%)		(n=266)
No high school degree	16.6	16.9
High school degree	17.2	19.2
Some college	27.5	32.0
College degree	18.5	15.0
Higher than bachelor's degree	20.3	16.9
Height (cm)	166.1±9.6	167.4±10.5
Weight (kg)	76.6±16.2	85.5±19.5
Body mass index (kgm^{-2})	27.7±5.1	30.5±6.5
Cigarette smoking status (%)		
Never	52.5	43.6
Former	32.6	39.5
Current	14.9	16.9
Hypertension (%)	39.3	45.2
Systolic blood pressure (mmHg)	123.2±21.0	126.0±21.0
Diastolic blood pressure (mmHg)	71.2±10.0	71.7±10.8
Diabetes mellitus (treated or untreated) (%)	11.2	12.0 (N=267)
Biomarkers		
Inflammation		
MMP-3 (ngml^{-1})	16.1±15.5	14.3±11.5
MMP-9 (ngml^{-1})	241.4±143.2	256.0±140.2
ICAM (ngml^{-1})	280.0±79.1 (n=717)	292.0±86.4 (n=265)
TNF-R1 (pgml^{-1})	1.3±0.4	1.3±0.4
E-selectin (ngml^{-1})	53.8±24.7	57.1±26.8
Thrombosis		
PAI-1 (ngml^{1})	25.8±27.1 (n=711)	30.5±29.2 (n=259)
TF (pgml^{-1})	126.2±87.5 (n=727)	116.5±81.0 (n=266)
TFPI (ngml^{-1})	48.2±14.3 (n=730)	49.5±14.1 (n=265)
CD40L (pgml^{-1})	4.0±2.7	4.2±2.7

Data shown as mean ± standard deviation or %. CD40L, CD 40 ligand; ICAM, intercellular adhesion molecule; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor 1; TF, tissue factor; TFPI, tissue factor pathway inhibitor;TNF-R1,tumour necrosis factor-α receptor 1.

smaller RVEDV (both p<0.05). Higher TNF-R1 levels had a borderline association with lower RV mass (p=0.08) and were significantly associated with smaller RVEDV and RVSV (p<0.02). In addition, higher E-selectin levels were associated with smaller RVEDV and RVSV. Higher PAI-1 levels were significantly associated with lower RV mass (p=0.046), smaller RVEDV and RVSV (both p<0.01), and possibly lower RVEF (p=0.06). All results are shown in supplementary Tables 1 and 2 (see online version of this article).

Multivariable models with further adjustment for other covariates confirmed the association between higher MMP-9 levels and lower RV mass, which was attenuated (but still significant) after adjustment for LV mass (Table 3). MMP-9 levels were inversely proportional to RVEDV with adjustment, but this relationship did not persist after further adjustment for LV end-diastolic volume (LVEDV), indicating a biventricular effect. Higher TNF-R1 levels were associated with smaller RVEDV (p=0.01) and RVSV (p=0.02). The former association diminished after controlling for LVEDV. Higher PAI-1 levels were associated with lower RVEDV (p=0.02), RVSV (p=0.004), and was borderline significant for RVEF (p=0.08) in adjusted models. The association between PAI-1 levels and RVEF was statistically significant after adjustment for LVEF (p=0.02). Multivariate analyses other than those in the Table did not reveal significant relationships (data not shown). Other multivariate analyses are shown in supplementary Table 3 (see online version of this article).

Analyses of the smaller subset of participants with available lung measures (n=604) showed reductions in the associations between TNF-R1 and RV volumes after adjustment for forced expiratory volume in 1 s (FEV_1), FEV_1/forced vital capacity, urine cotinine and CT lung density. Similarly, the associations between PAI-1 levels and RVEDV and RVSV were no longer present after adjustment for these measures (p>0.30); however the association with RVEF persisted.

Discussion

Several biomarkers of inflammation and thrombosis were associated with RV structure and function in an adult, multiethnic cardiovascular disease-free cohort. We have shown that higher plasma levels of MMP-9 and TNF-R1 were associated with lower RVEDV, but not significantly so after adjusting for LVEDV, suggesting a biventricular relationship. Levels of MMP-9 were inversely associated with RV mass, which remained significant after adjustment for LV mass, consistent with an independent effect on the RV out of proportion to that on the LV. Higher plasma PAI-1 levels were associated with lower RVSV and lower RVEF even after consideration of LVEF. RV parameters differed ~3–8% between the lowest and highest quintiles of the biomarkers. The magnitudes of these effects were equivalent to the impact of traditional cardiovascular risk factors (e.g. current smoking or diabetes mellitus) on LV mass and LVEF in MESA (Heckbert et al.2006) suggesting physiological and clinical importance of our findings in

Table 2. Associations between quintiles of each biomarker and right ventricular (RV) mass, RV end-diastolic volume (RVEDV), RV stroke volume (RVSV) and RV ejection fraction (RVEF) after adjustment for age, sex, race/ethnicity, height, and weight.

	Quintile of biomarker					
	1	2	3	4	5	*p* for trend
MMP-9						
Mean (range) (ng ml^{-1})	99.4 (34.2–132.9)	157.7 (133.3–186.2)	208.1 (186.6–236.7)	275.3 (238.0–326.3)	465.1 (328.7–1351.6)	
n	146	146	146	146	147	
RV mass (g)	22.1	22.2	21.7	21.2	21.2	0.005
RVEDV (ml)	122.1	124.6	121.1	118.0	118.4	0.02
RVSV (ml)	84.6	87.6	84.1	83.2	83.3	0.12
RVEF (%)	70.2	71.1	70.2	71.0	70.8	0.57
TNF-R1						
Mean (range) (pgml^{-1})	0.9 (0.6–1.0)	1.1 (1.0–1.1)	1.2 (1.1–1.3)	1.4 (1.3–1.5)	1.9 (1.5–5.5)	
n	146	146	145	146	148	
RV mass (g)	21.9	22.1	21.7	21.4	21.3	0.08
RVEDV (ml)	124.2	123.1	122.1	118.2	118.0	0.01
RVSV (ml)	86.7	86.5	86.0	82.7	82.6	0.02
RVEF (%)	70.2	70.9	71.1	70.6	70.6	0.82
PAI-1						
Mean (range) (ng ml^{-1})	4.9 (2.0–7.0)	10.3 (8.0–13.0)	17.9 (14.0–22.0)	29.1 (23.0–38.0)	64.3 (39.0–357.0)	
n	141	135	143	144	148	
RV mass (g)	22.2	21.6	21.5	22.0	21.0	0.046
RVEDV (ml)	125.6	120.6	118.0	122.0	115.9	0.006
RVSV (ml)	88.4	85.3	82.7	83.9	80.9	0.001
RVEF (%)	71.2	71.4	70.5	69.7	70.2	0.06
E-selectin						
Mean (range), (ng ml^{-1})	24.6 (4.7–33.4)	39.8 (33.6–45.6)	51.0 (45.7–55.6)	62.1 (55.6–70.5)	91.4 (71.0–201.4)	
n	146	146	147	146	146	
RV mass (g)	21.8	21.8	21.4	21.7	21.1	0.13
RVEDV (ml)	121.3	122.6	120.0	119.0	117.2	0.06
RVSV (ml)	85.4	85.7	83.7	83.6	81.9	0.04
RVEF (%)	71.0	70.7	70.3	70.9	70.5	0.69

Right ventricular data shown as least-squares means. MMP, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor 1;TNF-R1,tumour necrosis factor-α receptor 1.

the RV. This is the largest study ever performed assessing the association of plasma biomarkers of inflammation with RV morphology.

Circulating MMP-9 is produced by atherosclerotic plaques, circulating neutrophils and monocytes, the pulmonary and systemic vascular beds, and the myocardium itself. MMP-9 leads to collagen degradation and remodelling and may therefore be beneficial or harmful, depending on the organ, study and pathological model of disease. For example, the monocrotaline animal model of pulmonary hypertension demonstrates increased RV myocardial MMP-9, suggesting a pathogenic role in cardiac remodelling (Umar et al. 2007) However, MMP inhibition in the chronic hypoxia animal model of pulmonary hypertension actually caused more severe pulmonary vascular remodelling, suggesting a protective effect of MMP-9 in the lung (Vieillard-Baron et al. 2000).

The multiple effects of MMP-9 extend beyond animal models. The Framingham Heart Study showed that detectable circulating MMP-9 was associated with increased LV end-diastolic diameter and LV hypertrophy in men, but not in women (Sundstrom et al. 2004). The MMP-9-associated increase in LV mass was attributed to maladaptive cardiac remodelling, enabled by matrix breakdown. However, in patients with clinical pulmonary hypertension, higher MMP-9 content in circulating monocytes was significantly associated with higher cardiac index, suggesting either a potential protective effect on RV function or adaptive remodelling (Cantini-Salignac et al. 2006). Our findings suggest that MMP-9 is associated with a lower RV mass, potentially by preventing collagen accumulation in cardiovascular disease-free individuals.

TNF-R1 is shed from the cell surface in response to inflammation, and higher soluble TNF-R1 levels are

Table 3. Adjusted regression models.

	Quintile of biomarker					
	1	2	3	4	5	*p* for trend
MMP-9						
n	146	146	146	146	147	
RV mass (g)						
Adjusted[a]	21.9	22.1	21.6	21.1	21.1	0.008
Adjusted + LV mass	22.5	22.9	22.4	22.2	21.9	0.04
RVEDV (ml)						
Adjusted	122.4	125.0	121.4	118.3	118.9	0.02
Adjusted + LVEDV	122.4	124.3	123.8	121.5	122.2	0.44
RVSV (ml)						
Adjusted	85.6	88.9	85.2	84.4	85.1	0.20
TNF-R1						
n	146	146	145	146	148	
RV mass (g)						
Adjusted	21.8	22.0	21.6	21.3	21.2	0.07
Adjusted + LV mass	22.4	22.7	22.2	22.4	22.1	0.34
RVEDV(ml)						
Adjusted	124.3	123.6	122.6	118.2	118.3	0.01
Adjusted + LVEDV	124.3	123.3	123.2	123.3	120.8	0.15
RVSV (ml)	87.9	87.8	87.4	83.9	83.8	0.016
PAI-1						
n	141	135	143	144		
RV mass (g)						
Adjusted	21.9	21.4	21.3	21.8	20.8	0.10
Adjusted + LV mass	22.4	22.4	22.3	22.5	21.9	0.37
RVEDV(ml)						
Adjusted	125.2	120.6	118.0	122.4	116.4	0.02
Adjusted + LVEDV	122.2	122.7	121.6	124.2	123.8	0.35
RVSV(ml)						
Adjusted	89.0	86.2	83.6	83.2	82.3	0.004
RVEF (%)						
Adjusted	71.6	72.0	71.0	70.3	70.8	0.08
Adjusted + LVEF	71.1	71.1	70.4	69.5	69.8	0.02

Right ventricular (RV) data shown as least-square means. [a]Adjusted for age, sex, race/ethnicity, height, weight, smoking, hypertension, diabetes mellitus, impaired glucose tolerance, hypertension, and systolic and diastolic blood pressure. MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor 1;TNF-R1,tumour necrosis factor-α receptor 1; RVEDV,RV end-diastolic volume; RVSV,RV stroke volume; RVEF,RV ejection fraction; LV, left ventricular; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction.

associated with an increased risk of death in congestive heart failure (Deswal et al. 2001). In our study, the presence of subclinical myocardial inflammation could account for abnormal RV relaxation without an effect on total RV mass. Higher E-selectin levels were associated with lower RVSV; other studies have found that increased E-selectin levels are seen in congestive heart failure as well (Chong et al. 2006).

PAI-1 is a serine protease inhibitor of urokinase-type plasminogen activator and tissue-TPA and predisposes to fibrin deposition. Investigators have found not only increased plasma PAI-1 activity (Bakouboula et al. 2008) but also a gradient of increased PAI-1 activity across the pulmonary circulation (Hoeper et al. 1998) in PAH patients. Hypoxia stimulates the PAI-1-mediated reduction in fibrinolysis, particularly in the lung (Pinsky et al. 1998). HIF-1α-associated pulmonary artery smooth muscle cell proliferation is mediated by PAI-1 production (Diebold et al. 2008). Higher plasma levels of PAI-1 may be linked to subclinical increases in pulmonary vascular resistance or decreases in compliance, explaining the negative impact on RVEF and stroke volume, independent of LV function.

There are several potential limitations to our study. We performed cross-sectional analyses of observational data using assessments made at the baseline MESA examination. Therefore, it is not possible to prove temporality or causality, although we know of no data supporting a role of RV structure and function in determining the biomarker levels studied. Selection and/or information bias may be present. Confounding by either unmeasured covariates or residual confounding are possible;

however MESA included extensive measurements and assessments, which we used in multivariate analyses. The lack of large, epidemiological studies of RV structure and function in disease-free older adults make it difficult to judge whether the effect estimates amount to 'clinically important' associations but they were similar in magnitude to those of traditional cardiovascular risk factors with LV morphological changes. Of course, we analysed several biomarkers making type I error possible.

We have shown significant associations between MMP-9 and PAI-1 and RV morphology and function. Longitudinal studies of this and other cohorts may show whether these biomarkers predict the development of pulmonary vascular disease and RV failure due to obstructive or restrictive lung disease. Future studies of these molecules might help identify patients at highest risk of RV failure and suggest novel therapies to preserve RV structure and function, intervening before irreversible changes ensue.

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This manuscript has been reviewed by the MESA Investigators for scientific content and consistency of data interpretation with previous MESA publications and significant comments have been incorporated prior to submission for publication. The authors thank the other investigators, staff and participants of the MESA and MESA-Lung Studies for their valuable contributions. A full list of participating MESA Investigators and institutions can be found at http://www.mesa-nhlbi.org.

Declaration of interest

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