Inflammation and right ventricular structure and function in health and disease

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Abstract

Right ventricular structure, function, and contribution to health and disease

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Right heart failure is common and serious. For the vast majority of patients with right heart failure there are few if any treatments beyond diuretics, salt, and fluid management. The lack of drug development in this area may be anchored in the erroneous belief that the right heart is a passive observer in physiology that fails whenever right heart afterload increases. Case reports and clinical heterogeneity have shown in recent years that right ventricular function is not stereotyped and that adaptation is possible. In this dissertation, I discuss one paradigmatic approach to looking for right heart targeted therapy and show an association between Histamine H_2 receptor antagonist (H2RA) use and right ventricular structure and function in healthy men and women. Subsequent work described in this dissertation demonstrated that H2RA use is also associated with left ventricular morphology, the incidence of heart failure, and survival in individuals with pulmonary hypertension. I then describe other potential therapeutic targets in the form of inflammatory markers that are associated with right ventricular structure and function in healthy men and women and outcomes in individuals with pulmonary arterial hypertension. This work is the foundation of a five year randomized controlled trials of H2RAs in individuals with pulmonary arterial hypertension and it is hoped that with further validation will form the basis for additional therapeutic targets of inflammation in individuals with right heart failure.

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Introduction

Right heart failure is common and serious. Right heart failure complicates a range of diseases including left heart failure, pulmonary arterial hypertension, emphysema, interstitial lung disease, and sleep apnea.[1-4] When seen, right heart failure is a significant marker or mediator of morbidity and mortality. Routine principles of heart failure management (e.g. diuretics, fluid restriction, and a low salt diet) can help patients with right heart failure, but unlike left heart failure where neurohormonal therapy is a mainstay of treatment, no neurohormonal therapies are used in right heart failure. In fact, although some forms of pulmonary hypertension can be treated, there are no therapies that target the right heart directly for any form of right heart failure. Identifying appropriate mechanistic targets to promote or maintain right ventricular adaptation over failure have been elusive and only beta-blockade has been rigorously evaluated in randomized clinical study. Studies of beta-blockade to date have not looked promising for right heart failure.[5]

Although rare, pulmonary arterial hypertension (PAH) is the paradigmatic disease of isolated right heart failure. PAH begins as a disease of the pulmonary vasculature but progresses to become a disease of right heart failure by the time it is clinically recognized. Vascular remodeling in PAH includes endothelial cell proliferation (e.g. plexiform lesions), intimal fibrosis, and/or medial/intimal smooth muscle hypertrophy, all of which increase pulmonary vascular resistance.[6] Initially the right ventricle adapts to the increased vascular load by enhancing contractility. Ultimately the vascular load exceeds right ventricular adaptation or enhanced right ventricular contractility begins to diminish despite stable afterload. This uncoupling leads to right heart failure characterized by right ventricular dilation, progressive symptoms, and death.[7] *The time course, extent, and durability of right ventricular adaptation is variable and can lead to dramatic differences in the clinical course for patients with otherwise similar pulmonary vascular disease*.[8] This suggests that the right ventricular response is not passive and stereotyped but is potentially malleable.

The pulmonary vascular disease community has made progress in the treatment of PAH leading to improved outcomes over a relatively short duration of intense research effort. This has led to improvement in survival for patients with PAH from 34% five-year survival in the 1980s to 57% five-year survival, as estimated by the REVEAL cohort in the first decade of the 21st century.[9,10] This improvement is largely attributable to pulmonary vasodilators targeting three key pathways in the pulmonary vasculature: nitric oxide, endothelin receptors, and prostacyclin signalling.[11] Despite improvements, current five-year survival estimates suggest that much more ought to be done for PAH patients, and that novel targets and approaches are needed. There may be diminishing returns in additional therapies targeting vascular smooth muscle even in patients with PAH. In addition, patients with PAH account for fewer than 5% of patients with pulmonary hypertension. Pulmonary vasodilators are not efficacious in the vast majority of patients with pulmonary hypertension and right heart targeted therapy is needed, which may be agnostic to the underlying etiology of increased pulmonary arterial pressure.[12]

Targeting the right ventricle to promote adaptation over failure is desirable, but no medications target the right ventricle in PAH. Targeting inflammation, angiogenesis, and/or fibrosis have been broadly proposed as possible treatments for right heart failure in patients with PAH, but largely have not been further evaluated in man.[13,14] Although there is some evidence to support the importance of these pathways in animal models, evaluation is needed before these broad and complex pathways can be considered as viable therapeutic targets.

Targeting inflammation is particularly appealing because there are already readily available and relatively inexpensive medications that bear on many aspects of inflammation. Low-cost medications are needed in the management of cardiopulmonary diseases complicated by right heart failure and are particularly needed in pulmonary vascular disease. Traditional drug development remains vitally important; however, there is growing appreciation for the value of repurposing or repositioning existing compounds when possible. This can allow a drug to come to market at a fraction of the cost compared with typical drug development by avoiding

duplicative costs associated with compound development and phase I trials.[15-17] Caring for patients with PAH is particularly expensive, and average cost across the disease spectrum amounts to ~$11,000 per month in the United States.[18] Cost increases dramatically as the disease progresses. For example, the administration of selexipag alone - an add-on agent for severe disease – costs $17,000 per month, in addition to the cost of other agents that a patient is already taking.[19] In addition to the unmet clinical need for patients with right heart failure, there is an unmet societal need for low cost adjuncts in right heart failure and PAH care.

In this dissertation, I present a body of work in health and disease to better understand right heart adaptation with the hope that it will identify therapeutic targets to promote right heart resilience in diseases of increased afterload. This compendium begins with an evaluation of histaminic signaling. Histamine is an inflammatory mediator that is in part responsible for anaphylaxis and other allergic reactions. Histaminic signaling emerged in my early research as an easily targeted pathway that was associated with differences in cardiac structure and function in individuals without heart disease. As such I moved to analyses exploring the relationship between histamine H_2 receptor signaling and the development or progression of disease. Specifically, I explored the relationship between H_2 receptor antagonists or genetic variation in the H_2 receptor and heart failure incidence.[20,21] I also evaluated the relationship between H_2 receptor antagonists and mortality in individuals with pulmonary hypertension.[22] This work, and that in the animal models underpinning this effort, formed the basis for an ongoing five-year randomized controlled trial of famotidine (an H_2 receptor antagonist) in participants with PAH at the University of Washington sponsored by the National Heart, Lung, and Blood Institute.

Next the dissertation explores other aspects of inflammation. Specifically, I present work exploring the relationship between cardiac morphology and levels of inflammatory markers (pentraxin-3[23] & von Willebrand factor[24]) or pro-inflammatory environmental stimuli[25] in individuals without known cardiovascular disease. Finally, I present previously unpublished

analyses in a local University of Washington cohort evaluating inflammatory pathways. The pathways/markers were suggested by animal models, validated in healthy individuals, and evaluated for their relevance in participants with pulmonary vascular disease. The hope is that these and ongoing analyses of inflammatory pathways will identify other potential targets, akin to those in histaminic signaling, in some or all patients with PAH. Ultimately, I hope to extend these findings to the broader and much larger community of patients with right heart failure from non-PAH causes (e.g. left heart failure, emphysema or other lung disease).

Chapter 1

Histaminic signaling in the development or progression of heart failure

I begin with a summary of work linking pre-clinical data to human populations to evaluate the role of histaminic signaling in heart disease and right heart failure. As identified in rodent models as far back as the late 1970s, H_2 receptor activation in the heart may promote myocardial fibrosis, maladaptation, and heart failure. Similar to beta-receptors, H_2 receptors activate stimulatory G-proteins in the myocardium.[26,27] Pre-clinical models suggest that histaminic signaling is important in heart failure. Blockade of H_2 receptors or histamine release can prevent heart failure in rabbits exposed to doxorubicin and dogs with pacemaker-driven tachycardia.[28-30] Relative to mice with an intact H_2 receptor, mice with the H_2 receptor knock-out have improved cardiac function and develop less fibrosis when subjected to aortic banding.[31] H_2 receptor activation also increases mitochondrial permeability in cardiac myocytes and myocardial susceptibility to stress.[32]

My research sought to extend these observations to man, and provided data that suggest H_2 receptor antagonist use is associated with a substantial decrease in heart failure incidence and differences in left ventricular morphology.[20] A second study observed an association between H_2 receptor antagonist use and a 10-15% lower all-cause mortality among U.S. veterans with pulmonary hypertension established by right heart catheterization. [33,34] Similar to mice with aortic banding (who developed less myocardial fibrosis when they lacked a functional H_2 receptor), PAH-induced right heart failure is provoked by increased afterload and an approach intended to abrogate myocardial fibrosis for any given level of afterload could be beneficial.[31] While H_2 receptor antagonists have a mild impact on heart rate and inotropy, H_2 receptor antagonists are, in general, very well tolerated and widely available worldwide.[26] Based on this reinforcing story in animals, healthy individuals, and men and women with disease, I am now studying H_2-receptor antagonists for participants with PAH in a randomized controlled trial sponsored by the National Heart, Lung, and Blood Institute.

This chapter includes final submitted drafts (prior to journal editorial changes) used in four previous publications and shows (1) the relationship between histamine receptor antagonists and

the right heart in healthy individuals[33], (2) the relationship between histamine receptor antagonists and the risk of developing heart failure[20], (3) the relationship between genetic variation in the histamine H_2 receptor and the risk of developing heart failure[21], and (4) the relationship between histamine receptor antagonists and the risk of death in individuals with pulmonary hypertension[22]. The exploration of histamine receptor antagonists in individuals with pulmonary hypertension includes more details than seen in the publication, which was shortened to a research letter at the request of the journal editor.

H2 receptor antagonists and right ventricular morphology: The MESA Right Ventricle Study

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Abstract:

Rationale: Histamine H_2 receptor antagonist (H2RA) use is common and may act directly on the heart through myocardial H_2 receptors or indirectly through changes in pulmonary vascular resistance. This study aimed to determine the relationship between H2RA use and right ventricular (RV) morphology.

Methods: We studied 4,124 participants in the Multi-Ethnic Study of Atherosclerosis without overt cardiovascular disease who had magnetic resonance imaging (MRI) assessment of RV morphology and ascertainment of medication use. Multivariable linear regression estimated cross-sectional associations between H2RA use and RV morphology after adjusting for demographics, anthropometrics, smoking status, diabetes mellitus and hypertension. Further adjustment for co-medication use, left ventricular (LV) parameters, lung structure and function, renal function, or inflammatory markers were considered in separate models. Analyses in a sub-cohort restricted to H2RA or proton pump inhibitor (PPI) users accounted for confounding by the indication of gastroesophageal reflux disease.

Measurements and Main Results: H2RA use was associated with lower RV mass (-0.7 g, 95% confidence interval (CI): -1.2 to -0.2 g, p=0.004) and smaller RV end-diastolic volume (EDV) (-4.2 mL, 95% CI: -7.2 to -1.2 mL, p=0.006). This relationship was unchanged with adjustment for co-medication use, lung structure and function, renal function, and inflammation. The relationship with RV mass was independent of LV mass. Results were similar in a smaller sub-cohort restricted to PPI and H2RA users.

Conclusion: H2RA use was associated with lower RV mass and smaller RVEDV. This finding is novel. Additional study of histamine and H_2 receptors in cardiopulmonary diseases affecting the RV may have direct clinical relevance.

Introduction

Gastroesophageal reflux disease (GERD) is commonly treated with histamine H_2 receptor antagonists (H2RAs) [35,36]. H2RA use may have ramifications beyond GERD control [26,37,38]. Histamine is abundant in the myocardium and myocardial H_2 receptors activate stimulatory G-proteins in a mechanism similar to myocardial beta-receptors [26,27]. H2RA use prevents heart failure in animal models and is associated with decreased left ventricular size and improved symptoms in patients with left heart failure [29,30,39]. Mast cell activation and histamine release may also contribute to pulmonary vascular remodeling and lead to increased pulmonary vascular resistance and right ventricular (RV) afterload in pulmonary arterial hypertension [40].

Right heart failure is an important cause of morbidity and mortality and increased RV mass is an independent risk factor for heart failure and cardiovascular death in community dwelling adults without clinical cardiovascular disease [41,42]. H2RA use could contribute to changes in RV structure and function through impacts on the pulmonary vasculature, myocardium or both; however, relationships between H_2 receptor antagonism and the RV have not been previously evaluated.

We examined the relationship between H2RA use and magnetic resonance imaging (MRI) measures of RV structure and function in a multi-ethnic cohort of adults free of clinical cardiovascular disease. We hypothesized that H2RA use would be independently associated with lower RV mass and smaller RV end-diastolic volume (RVEDV).

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicenter prospective cohort study designed to investigate subclinical cardiovascular disease in whites, African-Americans, Hispanics and Chinese-Americans [43]. In 2000–2002, MESA recruited men and women aged 45–84 years old from six US communities. Exclusion criteria included clinical cardiovascular disease (physician diagnosed heart attack, stroke, transient ischemic attack, heart failure,

angina, current atrial fibrillation, any cardiovascular procedure), weight >136kg (300 lbs.), pregnancy, or impediment to long-term participation. The MESA-RV study was an ancillary study funded to interpret 4,204 cardiac MRIs for RV structure and function. The Institutional Review Boards of participating institutions and the National Heart Lung and Blood Institute approved the protocols of MESA and all studies described herein. All participants provided informed consent.

Cardiac magnetic resonance imaging measures

The cardiac MRI protocol has been previously described [44]. Methods for interpretation of left ventricular (LV) and RV parameters in MESA have been previously reported [45,46]. Briefly, endocardial and epicardial borders of the RV were traced on MRI short axis cine images at end-systole and end-diastole. The outflow tract was included in RV volume. Papillary muscles and trabeculae were included in RV volumes and excluded from RV mass, as is commonly done for LV mass [47,48]. RV end-systolic volume 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. The difference between epicardial and endocardial volumes of the RV free wall at end-diastole multiplied by the specific gravity of the heart (1.05g/mL) was used to estimate RV mass. RV stroke volume was calculated by subtracting RV end-systolic volume from the RVEDV. RV ejection fraction (RVEF) was calculated by dividing RV stroke volume by RVEDV.

Medication Use

A validated medication inventory was used to assess medication use [49]. Participants were asked to bring all medication used during the two weeks prior to a study visit. Study staff transcribed current medications and the use or non-use of medications. A participant was considered an H2RA user if they used either prescription or over-the-counter H2RAs at the first MESA examination. A participant was considered a proton pump inhibitor (PPI) user if they used

PPIs at the first MESA examination. Participants who used either H2RAs or PPIs were considered likely to have shared the indication of GERD. Other medication use considered as confounders included ace-inhibitor use (± diuretic), angiotensin-II-receptor blocker use (± diuretic), beta-blocker use (± diuretic), oral steroid use, non-steroidal anti-inflammatory use (including aspirin, COX-2 inhibitors and other non-steroidal anti-inflammatory medications) and leukotriene antagonist use.

Covariables

Covariables including age, sex, race/ethnicity, height, weight, body mass index (BMI) category, education, presence of hypertension or diabetes mellitus, time spent performing intentional exercise, smoking status and pack-years of smoking were recorded at the initial MESA exam [50]. Percent emphysema from chest CT, forced expiratory volume at one second (FEV1) and forced vital capacity (FVC), estimated glomerular filtration rate, impaired glucose tolerance, cholesterol, c-reactive protein, and interleukin-6 were measured as previously described [51-53].

Statistical Analysis

We used linear regression to characterize relationships between H2RA use and RV parameters. All models were adjusted for height and weight to account for differences in body size. Additional covariables were chosen *a priori* on the basis of known associations with ventricular size, heart disease and comorbidities. In limited models, we adjusted for age, sex, race/ethnicity, height and weight. In fully adjusted models, we also included MESA field center, education and cardiovascular risk factors including intentional exercise, smoking status, pack-years of smoking, hypertension, diabetes mellitus, cholesterol and impaired glucose tolerance. In pre-specified models, we further adjusted for co-medication use, LV parameters, markers of lung structure (% emphysema on chest CT) and function (FEV1 & FVC), renal function

(estimated glomerular filtration rate), or markers of inflammation (C-reactive protein and interleukin-6) in separate models to evaluate independence from these factors. In an attempt to further account for confounding by indication (in which the underlying disease of GERD, rather than treatment, could be linked to RV morphology), analyses were repeated in a cohort restricted to participants who used either H2RAs or PPIs.

The primary analyses examined cross-sectional relationships between RV parameters and H2RA use at the baseline MESA exam. Exploratory models evaluated whether age, sex, BMI category or beta-blocker use modified the association between H2RA use and RV parameters. Analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA).

Results

Of 6,814 participants enrolled in MESA, 5,098 underwent cardiac MRI and 4,634 participants were selected for the MESA-RV ancillary study. MRI reads were attempted in 4,484 participants before achieving the study goal of 4,204 participants (94% of attempted reads). H2RA use was ascertained in 4,198 of these participants. Seventy-four participants were excluded for missing covariables leaving 4,124 in the study sample (Figure 1). The mean age of the study sample was 63.2 years, 52.6% were women, 39.3% were white, 26.3% were African-American, 21.9% were Hispanic, and 12.5% were Chinese. Mean RV mass in the study sample was 21.0 ± 4.5 g, mean RVEDV was 124.1 ± 30.9 mL and mean RVEF was 70.5 ± 6.5 %.

Figure 1. Study Sample

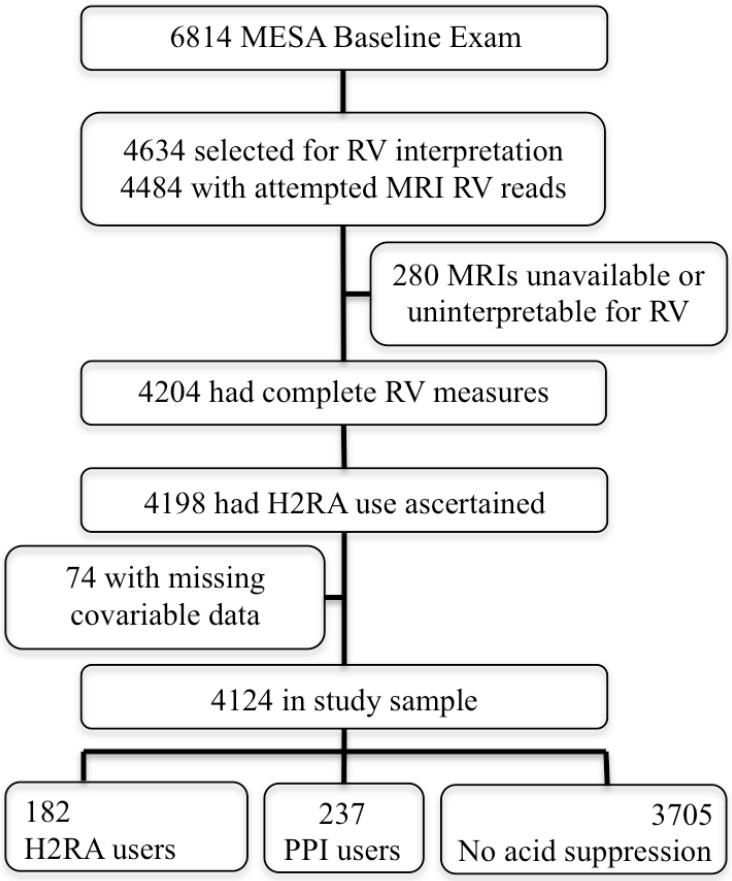


Table 1 shows characteristics of participants who used H2RAs, PPIs or who did not report use of medications for acid suppression. The prevalence of diabetes mellitus and hypertension appeared to be highest among H2RA users than participants in either of the other subgroups. Other characteristics of H2RA users were not different than those of participants in the other subgroups. Prevalence of beta-blocker use, ace-inhibitor use, and mean c-reactive protein in H2RA users which may be surrogates of cardiovascular disease severity were intermediate between participants who did not use acid suppression and those who used PPIs.

Table 1. Characteristics of the study sample

	No Acid Suppression (n=3705)	H2RA users (n=182)	PPI Users (n=237)
Age (years)	61.2 ± 10.1	64.0 ± 9.7	63.9 ± 9.9
Female (%)	51.8	53.9	64.1
Race (%)			
White	38.6	44.0	47.3
African-American	26.4	20.9	27.4
Hispanic	21.8	27.5	19.8
Chinese	13.2	7.7	5.5
Height (cm)	166.5 ± 10.0	165.4 ± 9.7	165.1 ± 9.6
Weight (kg)	77.1 ± 16.3	78.6 ± 15.4	80.4 ± 15.7
Body mass index (kg/m^2)	27.7 ± 4.9	28.7 ± 5.2	29.5 ± 5.1
Educational attainment (%)			
No high school degree	16.1	19.8	16.0
High school degree	17.9	22.0	24.1
Some college	16.1	14.8	18.6
Bachelor's Degree	18.3	20.9	13.1
Higher than bachelor's degree	18.9	13.7	19.8
Intentional exercise (MET-hrs/wk)	27.1 ± 40.4	19.2 ± 28.5	25.9 ± 39.4
Cigarette smoking status (%)			
Never	52.2	53.3	52.3
Former	34.9	36.8	40.5
Current	13.0	9.9	7.2
Pack-years	10.7 ± 22.8	13.6 ± 24.8	10.8 ± 20.3
Hypertension (%)	42.0	54.5	43.5
Systolic blood pressure (mmHg)	125.3 ± 21.2	126.6 ± 19.3	127.8 ± 18.0
Diabetes mellitus (%)	12.6	14.8	11.8
Cholesterol (mg/dL)	194.4 ± 35.3	197.8 ± 34.9	190.9 ± 31.3
Glucose (mg/dL)	96.4 ± 29.4	95.8 ± 25.3	93.2 ± 17.8
ACE-inhibitor or ARB use ± diuretic (%)	16.4	18.7	25.7
Beta-blocker use ± diuretic (%)	8.6	13.7	18.1
Anti-inflammatory use (%)	37.4	52.2	45.6
Forced Expiratory Volume at 1 second (L)	2.4 ± 0.7	2.4 ± 0.7	2.2 ± 0.7
Estimated glomerular filtration rate (mL/min)	81.5 ± 17.2	78.0 ± 18.6	77.3 ± 17.2
C-reactive Protein (mg/L)	3.4 ± 5.5	4.2 ± 5.6	4.6 ± 6.0
Interleukin-6 (pg/mL)	1.5 ± 1.2	1.6 ± 1.2	1.5 ± 1.1

Abbreviations: H2RA=H_2 receptor antagonist, PPI=proton pump inhibitor, cm=centimeters, kg=kilograms, m^2=meters squared, MET-hrs/wk=metabolic hours a week, mmHg=millimeters of mercury, mg=milligrams, dL=deciliter, ACE=angiotensin converting enzyme, ARB=angiotensin receptor blocker, Anti-inflammatory use included: oral steroids, non-steroidal anti-inflammatory use, and leukotriene antagonist use, L=liters, mL=milliliters, min=minute, pg=picograms

H2RA use was associated with lower RV mass (0.7 g less with H2RA use, 95% confidence interval (95% CI) -1.2 to -0.3 g, p=0.002) (Table 2). This relationship was unchanged with adjustment for cardiovascular risk factors, all considered medications, or separately by drug class (Table 2). The relationship between H2RA use and RV mass was only slightly changed after adjustment for LV mass (-0.6 g with H2RA use, 95% CI -1.0 to -0.1 g, p=0.009). Restricting the analyses to the 419 participants who used either PPIs or H2RAs (suggesting that all were treated for GERD) showed that H2RA use was associated with a decrease in RV mass relative to PPI use (0.7 g less with H2RA use, 95% CI -1.4 to 0.0 g, p=0.05) (Table 3). The decrease was similar to that seen in the unrestricted analyses.

Table 2. Multivariable linear regression estimating associations between H2RA use and right ventricular structure and function in the full study sample

Model (n=4,124)	H2RA use relative to non-use		
	Difference	(95% CI)	p-value
RV mass, g (Limited model*)	-0.7	(-1.2, -0.3)	0.002
RV mass, g (Full Model†)	-0.7	(-1.2, -0.2)	0.004
RV mass, g (Full Model† + Co-medication use)	-0.7	(-1.2, -0.2)	0.005
RV mass, g (Full Model† + LV mass)	-0.6	(-1.0, -0.1)	0.009
RVEDV, mL (Limited model)	-4.7	(-7.9, -1.6)	0.003
RVEDV, mL (Full Model)	-4.2	(-7.2, -1.2)	0.006
RVEDV, mL (Full Model + Co-medication use)	-4.2	(-7.3, -1.1)	0.007
RVEDV, mL (Full Model + LV EDV)	-0.7	(-3.0, 1.5)	0.53
RVEF, % (Limited model)	0.0	(-0.9, 0.8)	0.92
RVEF, % (Full Model)	-0.1	(-1.0, 0.8)	0.85
RVEF, % (Full Model + Co-medication use)	-0.1	(-1.0, 0.8)	0.76
RVEF, % (Full Model + LVEF)	-0.3	(-1.1, 0.5)	0.52

Abbreviations: H2RA=H_2 receptor antagonist, CI=confidence interval, RV=right ventricular, EDV=end-diastolic volume, and EF=ejection fraction, LV=left ventricular, g=grams, mL=milliliters

**Limited model: age, sex, race/ethnicity, height and weight*

†Full model: Limited + MESA field center, education, intentional exercise, smoking status, pack-years, hypertension, diabetes, cholesterol, and impaired glucose tolerance

Co-Medication use included: ace-inhibitor use (± diuretic), angiotensin-II-receptor blocker use (± diuretic), beta-blocker use (± diuretic), oral steroid use, non-steroidal anti-inflammatory use (including aspirin, COX-2 inhibitors and other non-steroidal anti-inflammatory medications) and leukotriene antagonist use.

Table 3. Multivariable linear regression estimating associations between H2RA use and right ventricular morphology among participants with a likely diagnosis of gastroesophageal reflux disease

Model (n=419)	H2RA use relative to PPI use		
	Difference	(95% CI)	p-value
RV mass, g (Limited model*)	-0.7	(-1.4, 0.0)	0.04
RV mass, g (Full Model†)	-0.7	(-1.4, 0.0)	0.05
RV mass, g (Full Model†+ Co-medication use)	-0.7	(-1.4, 0.0)	0.06
RV mass, g (Full Model† + LV mass)	-0.7	(-1.3, -0.1)	0.03
RVEDV, mL (Limited model*)	-4.4	(-8.6, -0.2)	0.04
RVEDV, mL (Full Model†)	-3.1	(-7.4, 1.1)	0.15
RVEDV, mL (Full Model†+ Co-medication use)	-2.9	(-7.4, 1.6)	0.21
RVEDV, mL (Full Model† + LV EDV)	-1.9	(-5.0, 1.1)	0.21
RVEF, % (Limited model*)	0.5	(-0.7, 1.7)	0.39
RVEF, % (Full Model†)	0.0	(-1.2, 1.2)	0.98
RVEF, % (Full Model†+ Co-medication use)	-0.1	(-1.3, 1.1)	0.85
RVEF, % (Full Model† + LVEF)	-0.1	(-1.2, 1.0)	0.82

Abbreviations: H2RA=H_2 receptor antagonist, PPI=proton pump inhibitor, CI=confidence interval, RV=right ventricular, EDV=end-diastolic volume, and EF=ejection fraction, LV=left ventricular, g=grams, mL=milliliters

**Limited model: age, sex, race/ethnicity, height and weight*

†Full model: Limited + MESA field center, education, intentional exercise, smoking status, pack-years, hypertension, diabetes, cholesterol, and impaired glucose tolerance

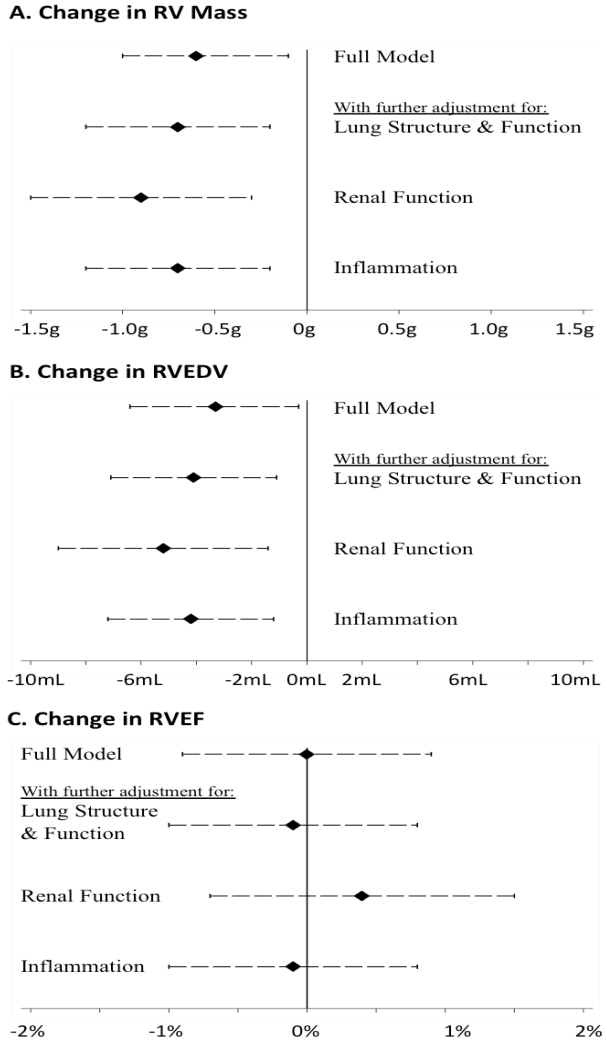
Co-Medication use included: ace-inhibitor use (± diuretic), angiotensin-II-receptor blocker use (± diuretic), beta-blocker use (± diuretic), oral steroid use, non-steroidal anti-inflammatory use (including aspirin, COX-2 inhibitors and other non-steroidal anti-inflammatory medications) and leukotriene antagonist use.

H2RA use was associated with a smaller RVEDV (-4.7 mL with H2RA use, 95% CI -7.9 to -1.6 mL, p=0.003) (Table 2). This relationship was not different with adjustment for cardiovascular risk factors, all considered medications, or separately by drug class (Table 2). The relationship between H2RA use and RVEDV was not present after adjustment for LVEDV (-0.7 mL with H2RA use, 95% CI -3.0 to 1.5 mL, p=0.53). Restricting analyses to the 419 participants who used either PPIs or H2RAs showed relationships with RVEDV qualitatively similar to the main analyses. In most cases the relationship was not statistically significant and may have been limited by small sample size (Table 3).

H2RA use was not associated with RVEF. Consideration of confounding by cardiovascular risk factors, co-medication use, or LV ejection fraction did not change this result. Additional control for confounding by indication in analyses restricted only to users of H2RAs or PPIs did not suggest a relationship between H2RA use and RVEF.

Further adjustment for lung structure and function, renal function (as estimated by the glomerular filtration rate) or markers of inflammation did not change the relationship between H2RA use and any RV parameter (Figure 2). In exploratory models, there was no significant effect modification by age, sex, body mass index or beta-blocker use for any RV parameter.

Figure 2. Relationship of histamine receptor antagonist use with (A) right ventricular (RV) mass, (B) RV end diastolic volume (RVEDV), and (C) RV ejection fraction (RVEF) after accounting for potential confounding arising from differences in lung structure & function (n=2,700), renal function (n=4,124), and inflammation (n=4,012)



Discussion

We have shown H2RA use is associated with lower RV mass and a smaller RVEDV in a multiethnic cohort of adults without clinical cardiovascular disease. MESA participants who used H2RAs had 0.7 g (3.3%) less RV mass and a 4.7 mL (3.8%) smaller RVEDV. Differences in RV mass are an independent risk factor for heart failure and cardiovascular death in individuals without cardiovascular disease, which may support the clinical relevance of our findings [42]. A similar magnitude of difference in LV mass has also been seen in other notable

modifiers of heart disease, such as the presence of diabetes mellitus or use of beta-adrenergic blockade [54,55].

This is the first report to suggest H2RA use may have implications on right heart morphology, but not the first to suggest H2RA use may impact the heart. Chronotropic effects of H_2 receptor antagonism were included in the initial description of H2RAs [56]. Later studies confirmed that stimulation of H2 receptors increased heart rate and further suggested positive inotropy with H_2 receptor stimulation [57]. Despite the recognition that H2RAs affect the heart, histamine-related cardiac signaling remains less well understood than cardiac adrenergic or renin-angiotensin signaling [58,59].

H2RA use may benefit the heart. H2RA use can prevent left heart failure and renal damage in rabbit models of anthracycline toxicity and in dogs with tachycardia-induced heart failure [28-30]. In Japanese men and women with existing LV failure, H2RA use is associated with improved heart failure symptoms and smaller LV end-diastolic volume [39]. Mechanisms to explain these associations have not been fully established, but could include histamine-related remodeling in the myocardium, alteration of renal blood flow, or altered systemic blood pressure [26,60-62].

Our data suggest H2RA use has cardiac implications in men and women without clinically apparent cardiovascular disease and is the first to suggest a relationship with the RV. In pulmonary arterial hypertension a smaller RV with less mass is a good prognostic sign and suggests adequate response to therapy [63]. We observed this pattern with H2RA use, which may suggest that H2RA use benefits the RV; however, further study with clinically relevant endpoints in diseased patients would be needed to advance this hypothesis.

Several possible mechanisms may explain our results. Histamine H_2 receptors are the predominant myocardial histamine receptor sub-type and histamine is abundant in the myocardium [27]. As has been suggested for the LV, it is possible that H2RAs may act directly on the myocardium to influence RV growth, adaptation, and development [30,39]. Alternatively, a

smaller RV with less mass may suggest lower pulmonary vascular resistance with less RV afterload in H2RA users. GERD has been implicated in lung damage and could lead to increased pulmonary vascular resistance; however, our associations with RV mass were specific to H2RA use[64]. Similar relationships were not seen in PPI users and adjustment for lung structure and function did not affect our results arguing against an explanation based on GERD or lung damage. Acute H_2 receptor agonism particularly with hypoxia may lead to pulmonary vasodilation[65]; however, over time mast cell degranulation and histamine release, which would be abrogated by H2RA use, may contribute to pulmonary vascular remodeling and increase pulmonary vascular resistance independent of parenchymal lung damage or GERD [40,66]. Prevention of mild pulmonary vascular remodeling with H2RA use could contribute to our findings.

We found that associations between H2RA use and the RV were independent of standard cardiovascular risk factors, such as diabetes, hypertension or obesity. The relationship between H2RA use and RV mass was independent of differences in LV mass suggesting that the relationship with mass was either specific or more pronounced in the RV. The relationship between H2RA use and a smaller RVEDV was not independent of LVEDV. This suggests that H2RA use is associated with biventricular decreases in end-diastolic volume of similar magnitude in each ventricle. H2RA use may impact renal blood flow, which could have implications for fluid balance and biventricular loading, but exploratory analyses do not suggest our results depend on differences in renal function (30).

This study has limitations. Confounding by indication is a significant concern. We feel that restriction to a sub-cohort who likely had the same disease indication (GERD) is a strength and the consistency of results using multiple approaches to account for confounding by indication is reassuring; however, residual or unmeasured confounding in this observational study is still possible. The low prevalence of H2RA use (5%) may suggest unreported H2RA use in those we classified as non-users. Such misclassification would be anticipated to be non-

differential with respect to RV morphology and the true relationship between H2RA use and RV differences may be diluted and therefore greater than we report. Additional information on duration and dose of H2RA, which may have informed an understanding of dose response, was not available and information about use of H_1 receptor antagonists was not collected. Several studies have been performed using the MESA-RV cohort and the likelihood of detecting a false positive association is elevated; however, the magnitude of statistical significance in our association argues against a relationship arising by chance alone even when considering the multiple comparisons of MESA-RV. Our understanding of the potential mechanism would have been enhanced through measurement of invasive pulmonary hemodynamics and evaluation of myocardial tissue, neither of which were feasible in this large study of community dwelling adults.

Conclusions

H2RA use is associated with lower RV mass and a smaller RVEDV. This is the first report to implicate H2RA use with RV morphology and the first to implicate H2RA use with differences in cardiac morphology in men and women without overt heart disease. Limitations in this study do not allow immediate extrapolation to disease treatment; however, future study of H_2 receptors in cardiopulmonary diseases affecting the RV may have direct clinical relevance.

Histamine H2-receptor antagonists, left ventricular morphology and heart failure risk: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Background: Myocardial H_2 receptor activation may promote cardiac fibrosis and apoptosis in preclinical models and histamine H_2 receptor antagonist (H2RA) use may improve symptoms in participants with heart failure; however, relationships between H2RA use, incident heart failure, and longitudinal change in left ventricular (LV) morphology are not known.

Objective: To determine whether H2RA use is associated with incident heart failure and change in LV morphology over time.

Methods: We included 6,378 men and women from the Multi-Ethnic Study of Atherosclerosis (MESA), a multi-center prospective observational cohort of participants without cardiovascular disease at baseline. We used Cox proportional hazards to estimate the association between H2RA use and incident heart failure in adjusted models. In participants with cMRI, relationships between H2RA use, baseline LV morphology (n=4,691), and longitudinal change in the LV (n=2,806) were estimated using linear regression.

Results: 313 participants used H2RAs and 6,065 did not. During a mean follow-up of 10.2 years, 236 participants developed heart failure. In adjusted models, baseline H2RA use relative to non-use was associated with 62% lower risk for incident heart failure (HR 0.38, 95% CI 0.17 – 0.86, p=0.02). H2RA use was associated with preserved stroke volume, LV end diastolic volume, and mass/volume ratio as measured by cMRI over approximately ten years (all $p<0.05$). There were no associations between H2RA use and LV mass or ejection fraction.

Conclusions: H2RA use was associated with reduced risk for incident heart failure. Changes in left heart morphology over time suggest less age-related change in H2RA users. These associations suggest histamine signaling may be important in the pathogenesis of heart failure.

Introduction

Histamine H_2 receptor antagonists (H2RAs) are commonly used to treat gastroesophageal reflux disease (GERD), have a relatively strong safety profile, and may have implications beyond GERD control.[36] Similar to beta-receptors, H_2 receptors activate stimulatory G-proteins in the myocardium.[26,27] H2RA administration prevents heart failure in animal models of heart failure and H_2 receptor activation contributes to myocardial apoptosis and fibrosis in response to aortic banding.[28,30,31]

In men and women with heart failure and reduced ejection fraction (HFrEF), H2RA use is associated with smaller left ventricular (LV) volumes and less severe symptoms, and we have previously shown H2RA use is associated with lower right ventricular mass and smaller volumes.[33,39] Alongside the observations in animals, these data suggest histamine signaling may be relevant to the pathogenesis of human heart failure.

We examined relationships between H2RA use and incident heart failure in a multi-ethnic cohort of adults free of clinical cardiovascular disease at baseline. We also evaluated relationships between H2RA use, LV morphology, and change in LV morphology over time. We hypothesized that H2RA use would be associated with a reduced risk for heart failure.

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study designed to investigate subclinical cardiovascular disease.[43] From 2000 to 2002, MESA recruited participants aged 45–84 years old from six US communities. Evaluation included five MESA examinations over approximately ten years. Exclusion criteria included clinical cardiovascular disease (physician diagnosed heart attack, stroke, transient ischemic attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure), weight >136kg, pregnancy, or impediment

to long-term participation. Institutional Review Boards of participating institutions approved MESA protocols. All participants provided informed consent.

Cardiac magnetic resonance imaging measures

Cardiac magnetic resonance imaging (cMRI) was obtained at the baseline examination for the majority of MESA participants. In a subset of these participants, cMRI was repeated approximately ten years later.[67] The cMRI protocol and interpretation of LV parameters in MESA have been previously described.[44,45]

Medication Use

A validated medication inventory was used to assess medication use.[33,49] Participants were asked to bring all medications used over the two weeks prior to a MESA examination. Participants were considered H2RA users in primary analyses if they used prescription or over-the-counter H2RAs at the baseline MESA examination. Other baseline medication use considered for confounding included proton pump inhibitors (PPIs), ACE-inhibitors (± diuretic), angiotensin-II-receptor blockers (± diuretic), beta-blockers (± diuretic), any diuretic alone, oral steroids, non-steroidal anti-inflammatory agents (including aspirin, COX-2 inhibitors and other non-steroidal anti-inflammatory medications), and leukotriene antagonists.

Ascertainment of events

Full details of event ascertainment and definition are available in MESA's manual of procedures (MOP).[50] Briefly, clinical outcomes were assessed at MESA study examinations and by telephone interview every 9 to 12 months. Records were obtained for approximately 99% of hospitalizations and 97% of outpatient cardiovascular diagnostic encounters through calendar year 2012. Incident heart failure required symptoms of heart failure, a physician diagnosis of heart failure and another objective feature of heart failure (dilated or poor LV function, pulmonary edema

by chest radiograph, treatment, or evidence of diastolic dysfunction). Two physicians from the MESA events committee independently reviewed all medical records for classification and dating of events. If reviewers disagreed, they adjudicated differences. If disagreement persisted, the full events committee made the final classification.

Statistical Analysis

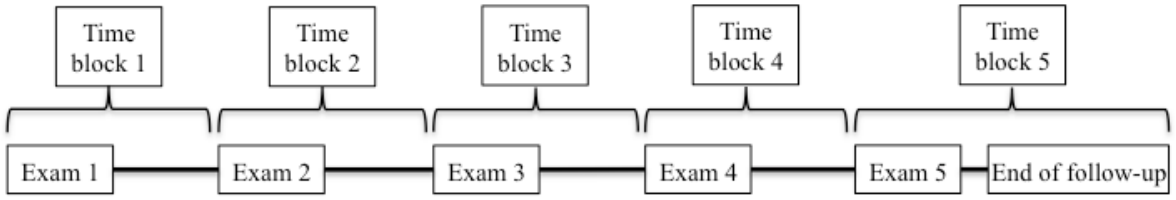
We used the methods of Kaplan & Meier to estimate unadjusted associations and Cox proportional hazards to estimate adjusted associations of H2RA use at the baseline exam with incident heart failure. Covariates were assessed at the initial MESA exam, described in MESA's MOP, and chosen *a priori*.[50] In limited models, we adjusted for age, sex, race, height, weight, and study site. In adjusted models, we included participants' education and cardiovascular risk factors including intentional exercise, smoking status, pack-years of smoking, hypertension, diabetes mellitus, cholesterol and fasting glucose. In separate models we further adjusted for co-medication use or level of the amino-terminal fragment of pro-B-type natriuretic peptide (NT-proBNP).

Linear regression was used to characterize relationships between H2RA use at the baseline exam, initial LV parameters, and change in LV parameters over time (e.g. baseline LV mass subtracted from LV mass at the follow-up cMRI). Models with limited adjustment, full adjustment, and full adjustment with co-medication use were considered. We adjusted for the sum of LV parameters (e.g. baseline LV mass added to LV mass at the follow-up cMRI) in all models of LV change to address concerns that change in LV parameters was confounded by baseline differences. This allowed inference on LV change independent of differences in the absolute magnitude of LV measures. We used consistency of H2RA use (non-use, use during any one MESA examination, use noted during at least four MESA examinations) as a plausible surrogate for cumulative dose over time in analyses of LV change and presented these results as marginal predictions from fully adjusted linear regression models.

Two restricted cohorts were considered in fully adjusted analyses to further address the possibility of confounding. To account for confounding by indication (in which the underlying disease of GERD, rather than treatment, could be linked to incident disease or LV morphology), analyses were repeated in a restricted cohort comparing participants who used H2RAs to those who used PPIs.[8] In a second cohort, to account for confounding accrued a participant's likelihood to use H2RAs, propensity scores were used to match H2RA users with non-users. The propensity to use H2RAs was calculated as a logit function including factors hypothesized to predict H2RA use, such as comorbidity, body mass index, and insurance status. Exploratory analyses evaluated relationships between H2RA use and heart failure in groups with differing predicted heart failure risk. A heart failure risk score derived from the Atherosclerosis Risk in Communities (ARIC) Study was estimated for each participant using baseline characteristics.[68] Observed heart failure relative to predicted risk was evaluated using locally weighted scatterplot smoothing (LOWESS). Incidence of heart failure, relative risk, and risk difference were presented in cohorts with high (ARIC risk score >11) and low (ARIC risk score ≤ 11) risk at baseline.

Exploratory models also evaluated whether age, sex, body mass index category, or beta-blocker use modified significant associations between H2RA use and clinical outcomes. In primary analyses, H2RA exposure was evaluated at baseline since the etiologic window of H2RA use relevant to heart failure is unknown, but may be protracted given potential biologic mechanisms involving cardiac fibrosis or remodeling. This is different from drugs like aspirin where pharmacologic effects are largely limited to times when an individual is actually taking the medication. To test this assumption for H2RA use and explore timing and duration of H2RA use, time-varying exposure models characterized relationships of active H2RA use with incident heart failure (Figure 1). Analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA).

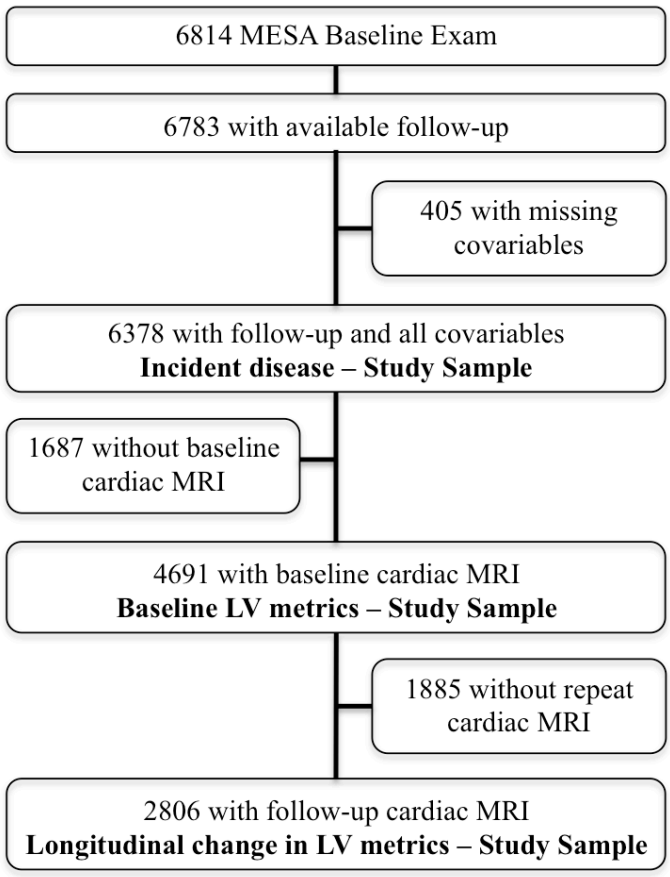
Figure 1. Discrete time intervals for the analysis of H_2 Receptor Antagonist Use as a time-varying exposure



Results

Of 6,814 participants enrolled in MESA, follow-up for incident heart failure was obtained for 6,783. Covariates were missing in 405 participants leaving 6,378 participants in the cohort evaluated for incident heart failure (94% of all participants). Of these 4,691 completed a baseline cMRI and were included in the cohort describing cross sectional relationships between H2RA use and the LV. A follow-up cMRI was obtained in 2,806 participants (107 H2RA users and 2,699 non-users) a mean of 9.5 ± 0.5 years after the first cMRI. These participants were included in the cohort describing relationships between H2RA use and longitudinal changes in the LV (Figure 2).

Figure 2. Study Sample



The mean age of the study sample was 62.3 years, 52.7% were women, and 37.9% were white. The 313 H2RA users in the cohort evaluated for incident heart failure tended to be older, more likely to be white, more likely to use other non-H2RA medications, and had a higher prevalence of hypertension than 6,065 non-users (Table 1). Mean follow-up was 10.2 ± 2.8 years and total follow-up was 65,082 person- years. In unadjusted analyses, six H2RA users developed heart failure in 3,082 person-years (1.9 events per 1,000 person years) and 230 non-users developed heart failure in 62,000 person-years (3.7 events per 1,000 person years.

Table 1. Characteristics of the study sample

	H2RA users (n=313)	Non-H2RA users (n=6065)
Age (years)	65.2 ± 9.9	62.1 ± 10.2
Female (%)	52.1	52.7
Race (%)		
White	43.8	37.6
Chinese	8.0	12.5
African-American	23.3	28.0
Hispanic	24.9	21.9
Height (cm)	165.8 ± 9.8	166.3 ± 10.0
Weight (kg)	80.3 ± 16.7	78.5 ± 17.4
Body mass index (kg/m^2)	29.2 ± 5.6	28.3 ± 5.5
Educational attainment (%)		
No high school degree	18.5	18.2
High school degree	22.4	17.8
Some college	17.6	16.2
Bachelor's Degree	17.6	17.5
Higher than bachelor's degree	13.4	18.2
Insurance Status (%)		
No insurance	5.8	9.2
Medicare	47.0	35.4
Private insurance	65.8	69.5
Cigarette smoking status (%)		
Never	48.9	51.0
Former	39.0	36.2
Current	12.1	12.8
Pack-years	16.3 ± 26.6	11.0 ± 20.4
Hypertension (%)	54.0	44.8
Systolic blood pressure (mmHg)	129.3 ± 20.4	126.5 ± 21.6
Diabetes mellitus (%)	14.4	13.6
Cholesterol (mg/dL)	191.9 ± 33.0	194.2 ± 36.0
Glucose (mg/dL)	97.0 ± 24.7	97.5 ± 30.7
NT-ProBNP (pg/mL)*	158.1 ± 737.8	99.5 ± 197.4
Medications (%)		
NSAIDs	54.3	40.1
Oral steroids	3.8	1.4
Beta-blockers	12.8	9.4
ACE-inhibitors/ARBs	25.6	18.0
Any diuretic	19.2	13.3

Data presented as mean ± standard deviation or percentage as appropriate
Abbreviations: cm=centimeters, kg=kilograms, m^2=meters squared, mmHg=millimeters of mercury, mg=milligram, dL=deciliter, pg=pictogram, mL=milliliter, NT-ProBNP=amino-terminal fragment of pro-B-type natriuretic peptide NSAIDs=non-steroidal anti-inflammatory medications, ACE-inhibitors=angiotensin converting enzyme inhibitors, ARBs=angiotensin II receptor blockers
** Available for 5,285 participants*

H2RA use at the baseline exam was associated with a 61% lower risk of heart failure (adjusted HR 0.39, 95% CI 0.17 – 0.87, p=0.02; Table 2). This relationship was stronger when

also accounting for NT-proBNP at baseline (adjusted HR 0.18, 95% CI 0.05 – 0.62, p=0.007; Table 2). Relationships were similar with adjustment for co-medication use, when the cohort was restricted to propensity-matched participants, or when the cohort was restricted to participants who used either H2RAs or PPIs (Table 2).

Table 2. Relationship of H2RA use at the baseline exam and cardiovascular outcomes (n=6,378)

	Adjusted risk of outcome in H2RA users relative to non-users		
	Hazard Ratio	95% CI	p-value
Heart failure			
Limited adjustment*	**0.40**	**0.18 to 0.90**	**0.03**
Full adjustment†	**0.39**	**0.17 to 0.87**	**0.02**
Full adjustment† + Co-medication use‡	**0.37**	**0.16 to 0.84**	**0.02**
Full adjustment† + NT-ProBNP (n=5,285)	**0.18**	**0.05 to 0.62**	**0.007**
Restricted to H2RA users & PPI users[x] (n=698)	0.41	0.15 to 1.14	0.09
Restricted to PS matched participants[x] (n=593)	**0.31**	**0.11 to 0.86**	**0.03**

Definition of abbreviations: CI-confidence interval; H2RA-H2 receptor antagonist; NT-ProBNP- amino-terminal fragment of pro-B-type natriuretic peptide; PPI-proton pump inhibitor; PS-propensity score

** Limited adjustment accounts for age, sex, race/ethnicity, height, weight, and study site*

† Full adjustment accounts for the limited model and education, cigarette smoking, pack-years, hypertension, diabetes, cholesterol, glucose, and daily exercise

‡ Co-medication use included non-steroidal anti-inflammatory medications (aspirin, cox-2 inhibitors, and other non-steroidal inflammatory medications), steroids, beta-blockers (± diuretics), ACE-inhibitors (± diuretics), angiotensin receptor blockers (± diuretics), any diuretic alone, and leukotriene antagonists

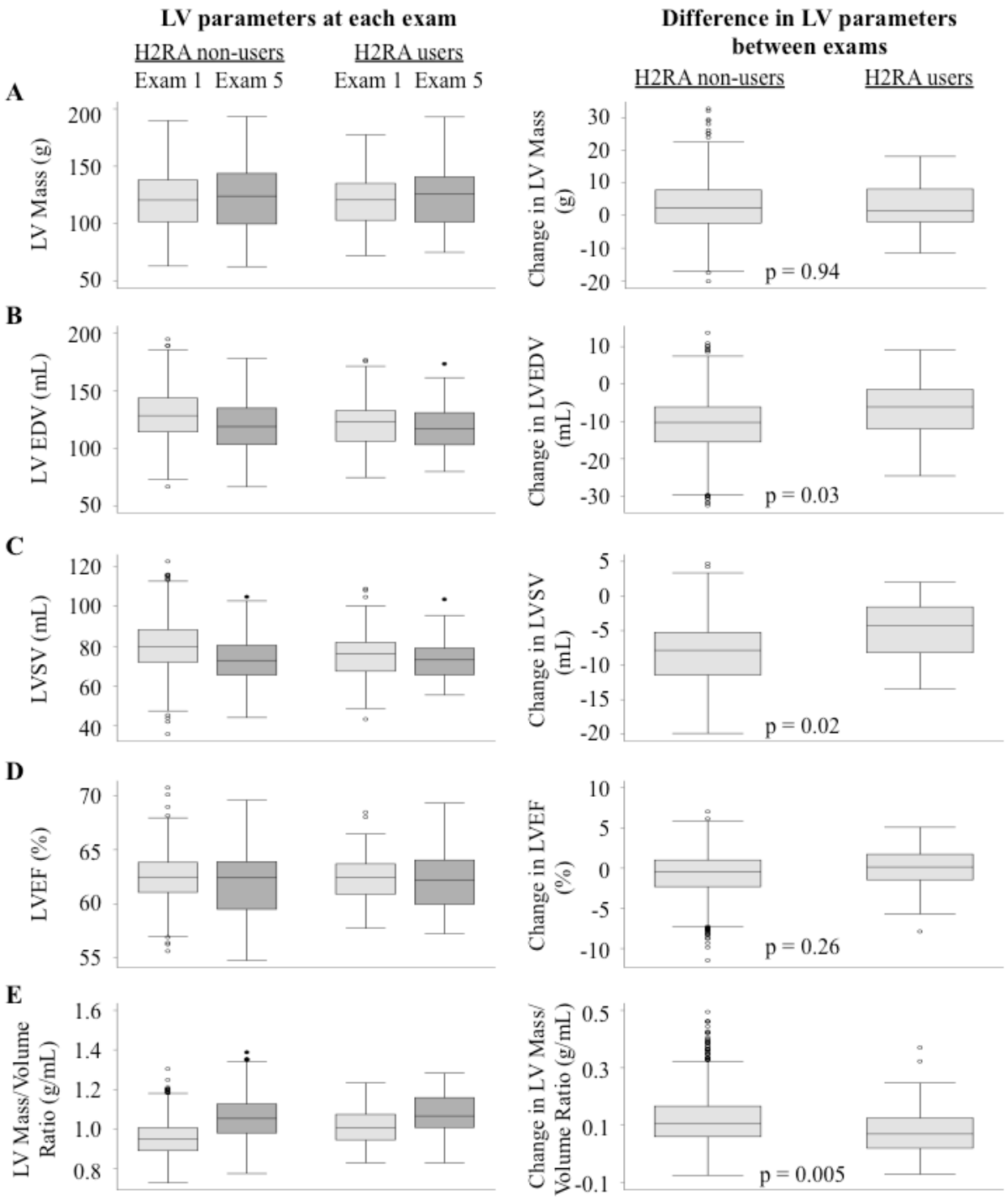
x Participants in the restricted cohorts were considered in models with full adjustment

H2RA use was associated with differences in baseline cMRI. After adjustment for covariates, H2RA use was associated with a smaller LVEDV (-5.9 mL, 95% CI -9.0 to -2.7 mL,

p<0.001), smaller stroke volume (-3.5 mL, 95% CI -5.7 to -1.4 mL, p=0.001), and a higher mass/volume ratio (0.04 g/mL, 95% CI 0.02 to 0.07 g/mL, p<0.001). Relationships were unchanged with adjustment for co-medication use and in the propensity-matched cohort. Restriction to participants who used H2RAs or PPIs attenuated relationships between H2RA use and baseline cMRI metrics. There was no relationship between H2RA use and baseline LVEF or mass.

H2RA use was associated with LV change over time. Among all participants who completed both cMRIs, LVEDV decreased, LVSV decreased, and mass/volume ratio increased over time. Compared to non-users, H2RA users had a smaller decline in LVEDV (4.5 mL, 95% CI 0.3 to 8.8 mL, p=0.03), a smaller decline in LVSV (3.8 mL, 95% CI 0.6 to 7.1 mL, p=0.02) and a smaller increase in mass/volume ratio (-0.06 g/mL, 95% CI -0.09 to -0.02 g/mL, p=0.005) over time (Figure 3).

Figure 3. Change in left ventricular (LV) (A) mass, (B) end-diastolic volume (EDV), (C) stroke volume (SV), (D) ejection fraction (EF), and (E) mass/volume ratio over approximately 10 years among users and non-users of H_2 receptor antagonists (H2RAs)



Results were not different after further adjustment for co-medication use and in the propensity-matched cohort. Restricting to participants with GERD attenuated relationships with LV change. Increasing consistency of H2RA use suggested progressively stronger relationships with change in LVEDV, LVSV and mass/volume ratio (Table 3).

Table 3. Relationship between consistency of H2RA use and change in the left ventricle between the initial and follow-up cardiac MRI (n=2,806)

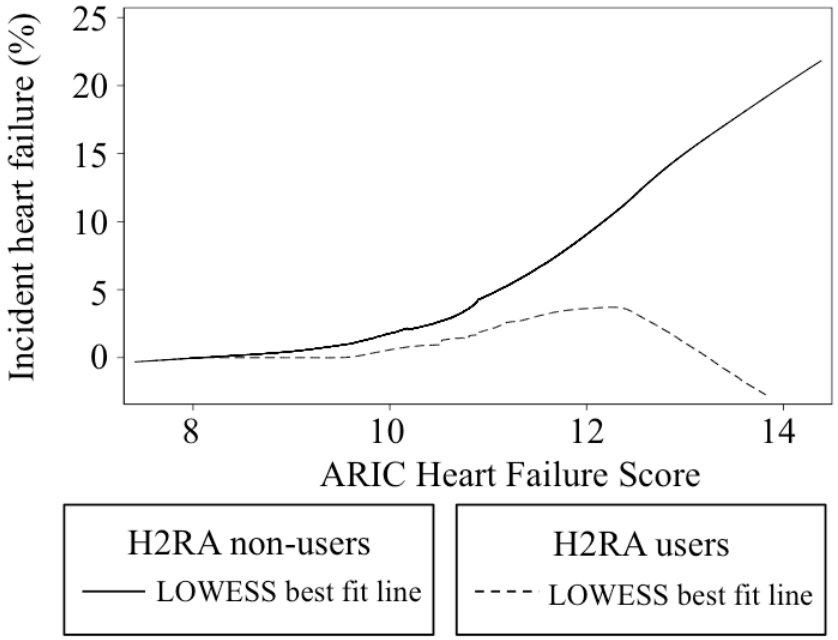
	Change in LV parameters relative to the baseline exam	
	Change	95% CI
Change in LV End Diastolic Volume		
No H2RA use	-10.9 mL	-11.7 to -10.0 mL
H2RA use at only one study exam	-8.8 mL	-14.4 to -3.2 mL
Consistent H2RA use at 4+ study exams	0.8 mL	-8.1 to 9.9 mL
	Test of trend:	**p = 0.01**
Change in LV Ejection Fraction (%)		
No H2RA use	-0.7 %	-1.0 to -0.4
H2RA use at only one study exam	-0.1 %	-1.9 to 1.8
Consistent H2RA use at 4+ study exams	0.0 %	-3.0 to 2.9
	Test of trend:	p = 0.61
Change in LV Stroke Volume (mL)		
No H2RA use	-8.3 mL	-9.0 to -7.7
H2RA use at only one study exam	-5.8 mL	-10.1 to 1.5
Consistent H2RA use at 4+ study exams	-0.8 mL	-7.6 to 5.9
	Test of trend:	**p = 0.04**
Change in LV Mass (g)		
No H2RA use	2.9 g	2.2 to 3.6 g
H2RA use at only one study exam	0.7 g	-3.7 to 5.2 g
Consistent H2RA use at 4+ study exams	6.2 g	-0.8 to 13.4
	Test of trend:	p = 0.53
Change in LV Mass/Volume Ratio (g/mL)		
No H2RA use	0.12 g/mL	0.11 to 0.13
H2RA use at only one study exam	0.08 g/mL	-0.10 to 0.00
Consistent H2RA use at 4+ study exams	0.04 g/mL	-0.17 to -0.07
	Test of trend:	**p = 0.02**

Definition of abbreviations: CI-confidence interval; H2RA-H2 receptor antagonist; LV-left ventricular
All models fully adjusted for age, sex, race/ethnicity, height, weight, study site, the sum of the LV parameter of interest at CMR 1 and CMR 2, education, cigarette smoking, pack-years, hypertension, diabetes, cholesterol, glucose, and daily exercise

In exploratory analyses, the largest unadjusted *risk difference* for heart failure was seen in participants with the highest predicted risk for heart failure at baseline (Figure 4). H2RA users

with low predicted heart failure risk (ARIC score ≤ 11) had a relative risk for heart failure of 0.5 and experienced 0.5 fewer episodes of incident heart failure per 1,000 person-years than non-users. H2RA users with higher heart failure risk (ARIC score > 11) had a relative risk for heart failure of 0.4 and experienced 5.3 fewer episodes of incident heart failure per 1,000 person-years.

Figure 4. Locally weight scatterplot smoothing (LOWESS) comparing observed heart failure relative to risk of heart failure (predicted by the ARIC heart failure score) between users and non-users of H_2 receptor antagonists (H2RAs)



Age, sex, body mass index and beta-blocker use did not modify relationships between H2RA use and heart failure (p-value for the interaction: 0.37, 0.13, 0.49, 0.25). Use of time varying exposure models to describe relationships between *active* H2RA use and clinical outcomes suggested a more modest relationship between H2RA use and heart failure (HR 0.73, 95% CI 0.36 – 1.50, p=0.39). The relationship was stronger but still not statistically significant when H2RA use was restricted to participants with at least one year of H2RA use (HR 0.48, 95% CI 0.20 – 1.18, p=0.11).

Discussion

We observed H2RA use to be associated with lower risk for incident heart failure in a multi-ethnic cohort without cardiovascular disease at baseline. We observed the strongest relationship (in absolute terms) among individuals at highest risk for heart failure. H2RA users had smaller LVEDV and stroke volume at baseline, but over time H2RA use was associated with a smaller decline in stroke volume, decline in LVEDV, and increase in the LV mass/volume ratio.

The strong relationship between baseline H2RA use and incident heart failure persisted regardless of the method used to account for confounding. The cohort matched by propensity to use H2RAs suggests H2RA use, and not characteristics of H2RA users, is responsible for the association. Comparing H2RA users to PPI users suggests H2RA use, and not the presence of GERD, is responsible for the association. Multivariate models argue that reduced heart failure risk is not explained by measured differences in cardiovascular risk factors between groups. In fact, we observed a particularly large association between H2RA use and heart failure when adjusting for NT-ProBNP (one of the strongest predictors of incident heart failure) and in participants with a high risk of heart failure as predicted by their ARIC heart failure risk score.[15,16]

This is the first report to suggest H2RA use is associated with decreased heart failure incidence in humans, but not the first to suggest H2RA use may be important for the heart. Among 318 Japanese participants with HFrEF, use of famotidine (an H2RA) was associated with better New York Heart Association functional class, lower BNP, and a smaller LVEDV.[39] A subsequent randomized trial of 50 participants with heart failure compared famotidine to teprenone use over 24 weeks and found improved functional class, decreased BNP, and decreased LV diameter in the famotidine arm.[39]

Improved heart failure symptoms may be related to our description of decreased heart failure incidence; however, we also observed important differences relative to these previous studies. Among participants with HFrEF, randomized famotidine use was associated with a smaller LV diameter. This agrees with our cross-sectional observation of a smaller LVEDV among H2RA users, but appears different than our finding of a smaller decline in LVEDV over time among

H2RA users. This discrepancy may reflect the difference in duration (10 years versus 24 weeks) and differences in the population being studied. The famotidine trial studied participants with HFrEF where decrease in the size of a pathologically enlarged LV is a marker of improvement.[17] In MESA we studied participants initially free of cardiovascular disease without a pathologically enlarged LV at baseline. Some developed HFrEF, some developed heart failure with preserved ejection fraction (HFpEF), and most did not develop heart failure. Decrease in LV size is not always a marker of improvement and in HFpEF decreased LV size is associated with disease progression, hospitalization, and death.[69] Protection from age-related decrease in LV size appears better aligned with our findings and protection from HFpEF may provide a rationale for our observations. Pathophysiology relevant to HFpEF may also be suggested by the less pronounced increase in mass/volume ratio over time in H2RA users, which may suggest less concentric remodeling.[19,20] We observed no relationship between H2RA use and ejection fraction. Currently there are no beneficial therapies for HFpEF, which is common, serious, and may support the importance of these observations.[21]

Animal models reinforce the importance of histamine signaling in heart failure pathogenesis. Blockade of histamine receptors or histamine release can prevent heart failure in rabbits exposed to doxorubicin and dogs with pacemaker-driven tachycardia.[4,5,22] Relative to mice with an intact H_2 receptor, mice with the H_2 receptor knock-out have improved cardiac function and develop less fibrosis when subjected to aortic banding.[31] H_2 receptor activation may increase mitochondrial permeability and myocardial susceptibility to stress.[23] These animal studies agree with our findings and, while speculative, may suggest biologically plausible mechanisms to explain our observations.

Timing and duration of H2RA use are likely important. Relationships with LV morphology were strongest in individuals who reported consistent H2RA use over several MESA exams. Also, while baseline H2RA use was strongly related to heart failure risk, “active use” was only weakly related to incident heart failure unless “active use” was restricted to participants who used H2RAs

for at least one year. This may suggest that there are benefits with early, prolonged, and/or consistent H2RA use that are not present with short-term use or use beginning during later stages of heart failure pathogenesis.

There are several limitations. Relationships with cardiac morphology were less consistent than those with heart failure, particularly after restricting to participants with GERD. Furthermore, H2RA users had smaller LVEDV and LVSV at baseline but less decline in these measures over time, which could represent regression to the mean. Regression to the mean would not explain associations with heart failure and small sample size likely contributed to inconsistency after restriction to participants with GERD; however, inference on cardiac structure, which informed our discussion of mechanism, should be more cautious. In addition, heart failure is a heterogeneous disease. Because of the small number of heart failure events among H2RA users, we could not reliably characterize important differences between types of heart failure relative to H2RA use. Finally, timing and duration of H2RA use may be important, but could not be fully evaluated as MESA did not assess medication use more frequently than every other year and medication use before the baseline exam was not known. Residual or unmeasured confounding and misclassification in a non-randomized study are always possible.

Conclusions

We observed lower risk for incident heart failure and better-preserved stroke volume, LVEDV and mass/volume ratio over time in H2RA users. Histamine signaling may be important in the pathogenesis of heart failure and could be an important target for therapy or prevention of heart failure.

Histamine H_2 Receptor Polymorphisms, Myocardial Transcripts and Heart Failure (From the Multi-Ethnic Study of Atherosclerosis and Beta-blocker Effect on Remodeling and Gene Expression Trial)

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Abstract

Myocardial H_2 receptor activation contributes to heart failure (HF) in preclinical models and H_2 receptor antagonists are associated with decreased HF incidence. This study evaluated whether H_2 histamine receptor (HRH2) single nucleotide polymorphisms (SNPs) are associated with HF incidence or myocardial transcript abundance is associated with recovery. The association of SNPs in HRH2 with incident HF were characterized using Cox proportional hazards regression among participants in the Multi-Ethnic Study of Atherosclerosis (MESA). Differences in myocardial HRH2 transcripts were characterized in participants with dilated cardiomyopathy comparing six "Super-responders" with six Non-responders to beta-blockade in the Beta-blocker Effect on Remodeling and Gene Expression (BORG) Trial. In MESA, no candidate SNP was associated with HF in Black, Hispanic, or White participants. The rs2241562 minor allele was only present in Chinese participants and the adjusted HF hazard among those with one or more copies of this allele was 3.7, 95% CI 1.0 to 13.4. In BORG, super-responders to beta-blockade had higher levels of myocardial HRH2 transcript at baseline compared with non-responders (Fragments per kilobase per transcript per million mapped reads: Variant 2, 5.5 ±1.1 compared with 3.2 ±0.8 in non-responders, p=0.002; variant 1+2, 32.1 ±7.4 compared with 23.3 ±4.2 in non-responders, p=0.04). In conclusion, the presence of a minor allele at rs2241562 was associated with increased HF incidence in Chinese participants. Differences in myocardial HRH2 transcript abundance were seen in participants with dilated cardiomyopathy who responded to beta-blockade. These observations support the hypothesis that HRH2 is involved in the pathogenesis of HF.

Introduction

We previously observed that H_2 receptor antagonist use was associated with favorable cardiac morphology and decreased heart failure (HF) incidence in community dwelling adults.[33,70] Although we attempted to account for confounding, residual or unmeasured confounding related to medication use could not be excluded as the basis for the association. Associations between HF and H_2 receptor (HRH2) genetic variants or HRH2 transcript abundance in the myocardium should not be subject to confounding from characteristics that influence medication use. These genetic and transcriptional characteristics of HRH2 might help to understand whether H_2 receptor signaling is important in myocardial dysfunction in human disease. In the current report, we further tested the general hypothesis that H_2 receptor mediated signaling is important in the establishment or progression of HF using data from two prospectively conducted studies. We tested the specific hypotheses that (1) HRH2 gene variants are associated with incident HF in the Multi-Ethnic Study of Atherosclerosis (MESA) and (2) that myocardial HRH2 gene expression is related to the ventricular response to beta-blocker therapy in the Beta-blocker Effect on Remodeling and Gene Expression (BORG) Trial of persons with dilated cardiomyopathy.[43,71]

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study designed to investigate subclinical cardiovascular disease. MESA recruited participants aged 45–84 years old without clinical cardiovascular disease from 6 US communities between 2000 and 2002.[43] Evaluation included 5 examinations over approximately 10 years. Clinical outcomes in MESA participants were assessed at study examinations and by annual telephone interview. Criteria for incident HF consisted of first-time HF symptoms, a physician diagnosis of HF, and an objective feature of HF (dilated left ventricle (LV) or poor LV function, pulmonary edema by chest radiograph, treatment, or evidence of diastolic dysfunction). Two physicians from the MESA

events committee independently reviewed all medical records. Differences were adjudicated or presented to the full events committee for a final determination. Full details of covariate measurement and event ascertainment are available in MESA's manual of procedures (MOP).[50]

The Beta-blocker Effect on Remodeling and Gene Expression Trial (BORG, NCT01798992) was conducted at the Universities of Colorado and Utah between 2000 and 2008.[71] Participants with idiopathic dilated cardiomyopathy (IDC) and HF with reduced left ventricular ejection fraction (LVEF) (defined as LVEF <40%) were randomized to carvedilol, metoprolol or metoprolol plus doxazosin, regimens that have in common blockade of beta-1 adrenergic receptors. All participants in the current H_2 receptor sub-study were evaluated with radionuclide SPECT ventriculography to estimate LVEF and had an endomyocardial biopsy before and after treatment with beta-blocking agents.[71] Six of 47 participants who completed the BORG Trial were classified as reverse remodeling "Super-responders" and 6 participants were classified as Non-responders. Super-responders were defined as participants whose LVEF increased ≥10 absolute % (EF Units) on follow-up study at either 3 or 12 months after starting beta-blockers. A random sample of gender matched non-responders had an LVEF change of <5 EF units.[71] Institutional Review Boards of participating institutions approved all protocols in both MESA and the BORG trial and all participants provided written informed consent.

For HRH2 genotypes, DNA was extracted from the peripheral leukocytes of MESA participants using a commercially available isolation platform (Puregene; Minneapolis, MN). Genotyping was performed by Illumina Genotyping Services using the GoldenGate Assay. After removal of failed SNPs and samples, the genotyping call rate was 99.93%. HRH2, located on chromosome 5q35.2, covers 28 kilobases and has two introns.[72,73] Sixteen SNPs in HRH2 were measured in MESA. After removal of variants with a minor allele frequency <1% and SNPs in high linkage disequilibrium, 4 SNPs remained for analysis (rs2241562, rs6864183, rs643586, rs647384). Rs2241562 is a non-coding variant in the 3' untranslated region within a half a kilobase of the 3'

end of the gene. Rs6864183 and rs643586 are intron variants. Rs647384 is an upstream non-coding variant within two kilobases of the 5' start of the gene.[74]

For transcript analyses, whole transcriptome shotgun RNA sequencing (RNA-Seq) was used to assess characteristics of myocardial HRH2 gene expression in failing compared with reverse remodeled, non-failing human ventricular myocardium.[71] RNA was extracted from RV distal septum endomyocardial biopsies taken from 12 IDC patients before and after treatment with beta-blocking agents for 3 (1 super-responder and 1 non-responder) or 12 (5 super-responders and 5 non-responders) months.[71] RNA-Seq methodology is described in the online supplement. For HRH2, 2 transcripts were identified by RNA-Seq. Variant 1 (ENST00000377291) is a 2561 base pair transcript with 2 retained introns and 3 exons, two of which are translated into a 397 amino acid protein.[74] Variant 2 (ENST00000231683) is a lower abundance, 1080 base pair intronless transcript that codes for a 359 amino acid protein that is homologous to that predicted from the originally cloned human HRH2 cDNA, considered to be the canonical sequence of HRH2.[73-75] Variant 2 is contained within exon 2 of Variant 1.

In the candidate gene analysis, Cox proportional hazards were used to estimate adjusted and unadjusted associations between SNPs and HF incidence. Given the low minor allele frequency for most SNPs, the risk for HF in individuals with one or more minor alleles was compared to those without a minor allele (dominant model). Minor allele frequencies for measured SNPs varied substantially by race/ethnicity and results were stratified by race/ethnicity to minimize potential bias from population stratification. In adjusted models, we included participants' age, gender, and the first three principal components of genetic ancestry. Principal components are a continuous measure of genetic ancestry with the potential to account for differences in race/ethnicity not apparent in self-identified racial/ethnic categories and were included to further minimize the impact of population stratification. A third *a priori* analysis explored associations between SNPs and HF incidence only in participants with a history of hypertension. This analysis intended to explore a 'multi-hit' hypothesis. Pre-clinical models suggest differences in myocardial

fibrosis with H_2 intervention are most pronounced following aortic banding, which has some similarity to the myocardial stress imposed by clinical hypertension.[31] In candidate gene analyses, p=0.05 was considered significant for primary inference; however, analyses involved 8 core evaluations of SNPs in racial/ethnic groups with a sufficient minor allele frequencies to permit analysis. A Bonferroni corrected p-value of 0.006 was also considered in the strength of inference on results.

In the myocardial gene expression analysis, HRH2 transcript levels were compared by Wilcoxon rank-sum and signed-rank tests. HRH2 myocardial transcript levels were compared between Super-responders and non-responders before and after beta-blocker treatment. In BORG analyses, p=0.05 was considered significant for primary inference; however, a Bonferroni corrected p-value of 0.004 for twelve comparisons was considered in the ultimate strength of inference on the results. All analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA) or GraphPad Prism 7.

Results

The MESA study population for the HRH2 candidate gene analysis consisted of 6,270 persons with available SNPs in HRH2 and principal components of genetic ancestry. There were 767 participants with Chinese ancestry, 1,598 Black participants, 1,425 Hispanic participants, and 2,480 White participants (Figure 1). The incidence of HF per 1000 person-years was 2.0 (n=16) among Chinese participants, 4.3 (n=68) among Black participants, 3.8 (n=55) among Hispanics, and 3.8 (n=100) among Whites. Traditional cardiovascular disease risk factors including cigarette smoking, hypertension, diabetes, medication use, cardiac morphology, and NT-pro-BNP differed between racial/ethnic groups at baseline (Table 1). Minor allele frequency of evaluated SNPs by race/ethnicity is presented in Table 2.

Figure 1. MESA Study Sample

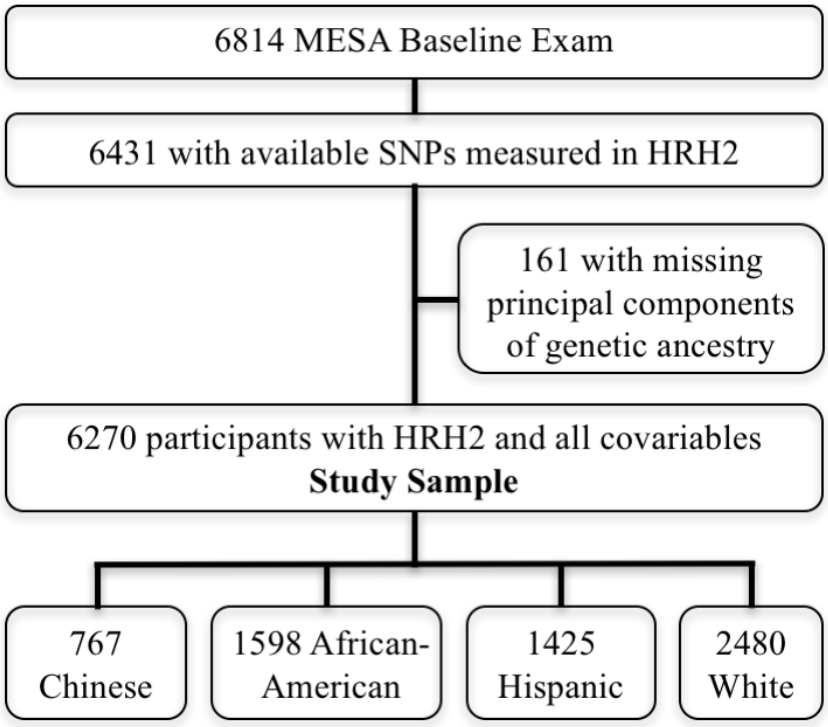

Table 1. Baseline characteristics of the MESA study sample

Variable	Chinese n=767	Black n=1,598	Hispanic n=1,425	White n=2,480	p-value
Age (years)	62.3 ± 10.4	62.1 ± 10.1	61.4 ± 10.3	62.7 ± 10.3	0.009
Women	50.6 %	54.4 %	51.7 %	52.3 %	0.06
Body mass index (kilograms/meter^2)	24.0 ± 3.3	30.1 ± 5.9	29.4 ± 5.1	27.8 ± 5.1	<0.001
Cigarette smoking					
Never	74.8 %	45.7 %	53.9 %	44.3 %	
Former	19.5 %	36.1 %	32.6 %	44.2 %	<0.001
Current	5.7 %	18.2 %	13.5 %	11.5 %	
Hypertension	38.7 %	59.6 %	42.2 %	38.5 %	<0.001
Diabetes mellitus	13.6 %	18.7 %	19.3 %	6.8 %	<0.001
Medications					
H_2 Receptor Blocker	3.4 %	3.6 %	5.4 %	5.7 %	0.004
Non-steroidal Anti-inflammatory Medications	22.7 %	41.3 %	35.7 %	55.4 %	<0.001
Oral steroids	0.5 %	1.8 %	1.4 %	1.9 %	0.05
Beta-blockers	10.4 %	10.4 %	8.6 %	9.6 %	0.19
Angiotensin Converting Enzyme Inhibitors or Angiotensin II receptor blockers	12.5 %	25.0 %	17.9 %	15.3 %	<0.001
Cardiac Morphology					
Left ventricular mass	123.5 ± 29.7	158.6 ± 41.6	146.6 ± 38.3	144.0 ± 38.2	<0.001
Left ventricular ejection fraction	72.1 ± 6.1	68.1 ± 7.8	68.9 ± 7.3	68.7 ± 7.3	<0.001
Left ventricular end diastolic volume	111.1 ± 22.9	130.9 ± 32.6	127.6 ± 30.3	128.5 ± 32.1	<0.001
NT-pro-BNP (pg/mL)	75.0 ± 110.3	92.3 ± 197.5	113.8 ± 432.0	113.2 ± 154.9	0.007

Results presented as mean ± SD or percentiles as appropriate and compared using analysis of variance

Table 2. The relationship of heart failure occurrence to the presence of minor alleles of SNPs in the H_2 receptor, by race

	Ancestry			
	Chinese	**African-American**	**Hispanic**	**White**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
rs2241562	MAF (C=3.7%)	MAF (C=0.0%)	MAF (C=0.0%)	MAF (C=0.0%)
Unadjusted	3.1 (0.9-11.0)	-	-	-
Adjusted for age, sex, and PC 1-3	**3.7 (1.0-13.4)***	-	-	-
Restricted to participants with hypertension†	**6.3 (1.6-25.7)****	-	-	-
rs6864183	MAF (T=37.5%)	MAF (T=46.6%)	MAF (T=49.3%)	MAF (T=48.6%)
Unadjusted	2.8 (0.8-9.9)	1.1 (0.6-1.8)	1.4 (0.7-2.8)	1.3 (0.8-2.2)
Adjusted for age, sex, and PC 1-3	2.9 (0.8-10.4)	1.1 (0.6-1.9)	1.6 (0.8-3.0)	1.3 (0.8-2.1)
Restricted to participants with hypertension†	3.7 (0.8-17.5)	1.3 (0.7-2.4)	1.2 (0.6-2.6)	1.3 (0.7-2.2)
rs643586	MAF (C=0.1%)	MAF (C=16.4%)	MAF (C=3.0%)	MAF (C=0.0%)
Unadjusted	-	1.3 (0.8-2.0)	0.6 (0.1-2.3)	-
Adjusted for age, sex, and PC 1-3	-	1.3 (0.8-2.1)	0.5 (0.1-2.1)	-
Restricted to participants with hypertension†	-	1.2 (0.7-2.1)	0.8 (0.2-3.7)	-
rs647384	MAF (A=0.0%)	MAF (A=5.4%)	MAF (A=0.9%)	MAF (A=0.0%)
Unadjusted	-	0.6 (0.3-1.6)	-	-
Adjusted for age, sex, and PC 1-3	-	0.7 (0.3-1.8)	-	-
Restricted to participants with hypertension†	-	0.5 (0.2-1.7)	-	-

Abbreviations: HR - hazard ratio associated with each additional minor allele; CI - confidence interval; MAF - minor allele frequency; PC - principal components; * $p<0.05$, ** $p<0.01$, † adjusted analyses

Sample Size: Chinese (n=764 or 287 when restricted to those with hypertension); African-American (n=1,598 or 954 when restricted to those with hypertension); Hispanic (n=1,425 or 601 when restricted to those with hypertension); White (n=2,480 or 965 when restricted to those with hypertension)

The minor allele for rs2241562 was present only in Chinese participants (Table 2). Chinese participants possessing one or more copies of the minor allele were at increased risk of HF in adjusted models compared with persons homozygous for the major allele (p=0.05). Of 764

Chinese participants, 708 were homozygous for the major allele, 55 were heterozygous, and 1 was homozygous for the minor allele. Thirteen Chinese participants homozygous for the major allele at rs2241562 developed HF over 7,395 person-years (1.8 events per 1,000 person years) and three participants who were heterozygous developed HF over 537 person-years (5.6 events per 1,000 person-years). The participant who was homozygous for the minor allele did not develop HF over eight years of follow-up. Restricting the analysis to Chinese participants with a history of hypertension, the hazard for HF with a minor allele at rs2241562 increased (p=0.01). Of 287 Chinese participants *with a history of hypertension* at the baseline MESA exam, 263 were homozygous for the major allele and 24 were heterozygous at rs2241562. No Chinese participant *with a history of hypertension* was homozygous for the minor allele at rs2241562. Nine participants who were homozygous for the major allele *and had a history of hypertension* developed HF over 2,669 person-years (3.4 events per 1,000 person-years) and three participants who were heterozygous *and had a history of hypertension* developed HF over 208 person-years (14.4 events per 1,000 person-years).

The presence of one or more minor alleles in rs643586 or rs647384 was not associated with HF incidence in the ethnic groups in which these genotypes were evaluable (Table 2). Among Chinese participants there was a trend toward increased HF incidence among individuals with one or more copies of rs6864183 (p=0.10), but this trend was not evident in other ethnic groups (Table 2). When considering a conservative Bonferroni correction, no association in MESA was significant.

The study population for HRH2 gene expression in ventricular myocardium consisted of 6 super-responder and 6 non-responder IDC patients treated with beta-blockers (Figure 2). Baseline LVEF was similar between super-responders and non-responders (Table 3). By design, the end-of-study LVEF was higher among super-responders with an LVEF change among super-responders of 31 ± 9 % compared with a non-responder change of 1 ± 6 % (p=0.005). Levels of Variant 1 were not significantly different between super-responders and non-responders at

baseline, but Variant 2 and total HRH2 (variant 1 + 2) transcript levels were higher in super-responders (Table 4). Non-responders increased expression of Variant 1 and total HRH2 (Variant 1 + 2) transcripts over the follow-up period, while super-responders did not; however, change over time was not statistically different between the two groups. Super-responders achieved an end of study LVEF of 55 ± 6 %, which is within the normal range. Comparing HRH2 gene expression for biopsies of hearts with normal (follow-up for super-responders) versus abnormal function (baseline for super-responders, baseline for non-responders, and follow-up for non-responders) did not suggest differences in Variant 1 ($p = 0.89$) or Variant 2 ($p = 0.75$) (Table 4).

Figure 2. BORG Study Sample

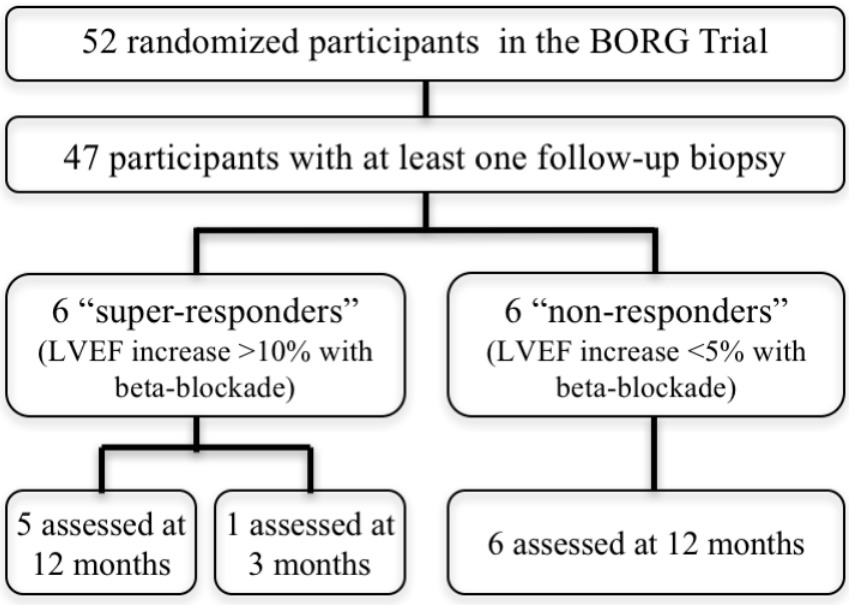

Table 3. Baseline characteristics of the BORG Trial study sample

Variable	**Super-Responders** n=6	**Non-Responders** n=6	**p-value**
Age (years)	38 ± 20	55 ± 11	0.09
Women	50%	50%	1.00
Race/Ethnicity			
White	67%	67%	
Black	16.5%	0%	0.36
Hispanic	0%	16.5%	
Other	16.5%	16.5%	
Hypertension	33%	0%	0.17
Creatinine clearance (milliliters/minute)	95 ± 17	77 ± 16	0.09
Medications			
Carvedilol	33%	33%	1.00
Metoprolol	33%	33%	1.00
Metoprolol + Doxazosin	33%	33%	1.00
Metoprolol Equivalent Dose (milligrams)	142 ± 67	117 ± 52	0.55
Change in systolic blood pressure (millimeters of mercury)	14 ± 21	-6 ± 12	0.13
Change in diastolic blood pressure (millimeters of mercury)	2 ± 13	0 ± 13	0.81
Left ventricular ejection fraction (baseline)	24 ± 7	31 ± 12	0.34
Left ventricular ejection fraction (follow-up)	55 ± 6	32 ± 12	0.008

Results presented as mean ± SD or percentiles as appropriate. Compared using a 2-sample t-test.

Table 4. RNA transcripts from the HRH2 gene in human ventricular myocardium before and after beta-blocker therapy.

Group and RNA Transcript (Ensembl#)	FPKM		P value		
	Baseline	Follow-up	Fold Change	Within group	Between groups
Non-Responders					
ENST00000377291 (Variant 1)	20.2 ±3.8	26.4 ±8.3	1.3 ±0.3	0.03	–
ENST00000231683 (Variant 2)	3.2 ±0.8	3.5 ±1.4	1.1 ±0.4	0.84	–
Variant 1 + Variant 2	23.3 ±4.2	29.9 ±9.3	1.3 ±0.3	0.03	–
Super-Responders					
ENST00000377291 (Variant 1)	†26.6 ±6.8	24.6 ±7.5	1.0 ±0.5	0.56	0.18
ENST00000231683 (Variant 2)	‡5.5 ±1.1	4.2 ±1.1	0.8 ±0.3	0.18	0.13
Variant 1 + Variant 2	§32.1 ±7.4	28.8 ±8.6	0.9 ±0.4	0.56	0.13
Normal versus abnormal heart function	**Normal**	**Abnormal**			
ENST00000377291 (Variant 1)	24.6 ± 23.0	24.9 ± 25.8			
ENST00000231683 (Variant 2)	4.2 ± 4.0	4.4 ± 4.5			

Normal heart function includes samples from super responders at follow-up and abnormal heart function includes samples from super-responders at baseline and samples from non-responders at both baseline and follow-up. *Within group* comparisons evaluate change in transcript abundance between baseline and follow-up for non-responders or super-responders separately; *Between group* comparisons evaluate whether the change between baseline and follow-up was different in super-responders compared with non-responders. Abbreviations: FPKM - Fragments Per Kilobase of transcript per Million mapped reads; †p = 0.13 vs. non-responders; ‡p = 0.002 vs. non-responders; §p 0.04 vs. non-responders.

Discussion

Of 4 evaluated SNPs in the H_2 receptor coding region, three were not associated with the occurrence of HF. The presence of a minor allele at rs2241562 in HRH2 was associated with increased risk for HF in participants of Chinese ancestry, particularly among those with hypertension. RNA-Seq confirmed the HRH2 receptor is expressed in human ventricular myocardium and suggested differences in pre-treatment HRH2 expression between participants who ultimately did and did not respond to beta-blockade by normalizing their LV function. These results suggest H_2 receptor signaling may be important in the pathogenesis and progression of

HF. These results are unlikely to be confounded by factors that had the potential to influence our previous work on the pharmacoepidemiology of H_2 receptor antagonist use.

Sequence ontology, rule-based mathematical models based on SNP position, suggests rs2241562 is an intron variant, could be involved in non-sense mediated decay, or may be an enhancer.[76] A non-sense mediated decay variant or enhancer may have functional significance by allowing mRNA coding for dominant negative or deleterious gain-of-function proteins to persist; however, there is no direct evidence that this is true for rs2245162.[77-79] If the association between rs2241562 and HF incidence is true and reproducible, we can suggest, but not conclude the mechanism may involve a gain of function in the H_2 receptor in individuals with the minor allele. The alternative exists that genotypes at rs2241562 may merely be correlated with those at a SNP in a different mechanistically-relevant gene.

Previous candidate gene analyses related to histaminic signaling identified associations between HF and genotypes for the H_3 receptor or histidine decarboxylase.[80,81] Similar to our study, associations with HF were identified, but the relationship between genotype and protein activity was not evaluated. Previous studies found an association with Chinese participants and in our study, the link between HF and HRH2 gene variants was only observed in participants with Chinese ancestry living in the United States.

H_2 signaling is relatively understudied in HF and the association between genetic variation and H_2 receptor function is not known. In pre-clinical models, H_2-receptor knock-out mice had less fibrosis and remodeling in response to increased left heart afterload with aortic banding.[31] In our study, at least among Chinese participants, we observed the largest hazard of HF in individuals with a minor allele at rs2241562 *and* hypertension. Although there are differences between aortic banding and clinical hypertension, the core impact of each is increased load on the left ventricle and an increase in ventricular fibrosis.

We confirmed HRH2 is expressed in human ventricular myocardium and identified two transcripts using RNA-seq. Variant 2 is an intronless variant homologous to the first cloned human HRH2 cDNA and considered to be the canonical sequence. Variant 1 has two retained introns and three exons.[73] Interestingly, participants who responded to beta-blocker therapy had higher baseline levels of the intronless HRH2 transcript (variant 2) and the summary product of both variants. Non-responders subsequently increased expression of the larger transcript (Variant 1) and the summary of both variants after beta-blocker therapy while super-responders experienced little change. Associations between HRH2 transcript levels and protein activity are not known and the associations between myocardial HRH2 transcript, protein levels and/or cardiac remodeling would need confirmation in subsequent investigations.

Cardiac beta-adrenergic and histaminic systems are known to cross-regulate, and it is possible that HRH2 receptor expression could play a role in the remodeling process or in the response to beta-blockers.[82] Furthermore, HRH2 transcript abundance did not differ between failing and non-failing human hearts, which agrees with previous data on H_2-receptor mediated stimulation of adenylate cyclase or muscle contraction in failing and non-failing hearts.[83,84] Unlike beta-adrenergic signaling in the failing heart, H_2 histamine receptor signaling may remain intact in the failing heart and continue to mediate adverse effects throughout the disease course.[84]

One core limitation is that both cohorts were small. In the MESA cohort, only three participants carrying a minor allele at rs2241562 developed HF. Multiple comparisons further increase the possibility that associations may have occurred by chance, which is underscored by the loss of significance with a Bonferroni correction in the MESA population. Thus, the current hypothesis-generating study needs to be repeated in a larger cohort enriched in East Asian ancestry participants. While we showed HRH2 is expressed in human ventricular myocardium and may be altered or predictive of changes in ventricular remodeling, the associations between SNPs and H_2 receptor activity was not evaluated. Also, DNA and RNA was derived from separate

studies, the gene expression BORG cohort was too small to detect the low frequency variants identified in MESA, and there were no East Asian ancestry participants in BORG.

At least in part, our results support the hypothesis that the H_2 receptor plays a role in HF pathogenesis. While our observations are not definitive, given the limitations imposed by the sample size, multiple comparisons, and population, the hypothesis is important given the clear potential for existing well-tolerated drugs to target the H_2 receptor. This work joins previous work to suggest randomized study of H_2 receptor antagonism in men and women with or at risk for HF may be justified.

Association between Histamine H2-receptor antagonist use and mortality among veterans with pulmonary hypertension: Insights from the VA-CART Program

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Abstract

Rationale: Pulmonary hypertension contributes to right heart failure and predicts or mediates poor outcome in many diseases. There are no medications that target *right* heart failure. H_2 receptor signaling contributes to cardiac fibrosis and H_2 receptor antagonist use is an effective treatment for left heart failure. Relationships between H_2 receptor antagonist use and outcomes in individuals with pulmonary hypertension are not known. This study sought to characterize the associations between H_2 receptor antagonist use and mortality or frequency of heart failure admission in individuals with pulmonary hypertension.

Methods: We included 17,529 veterans with a mean pulmonary artery pressure ≥ 25 mmHg confirmed by right heart catheterization and enrolled in the Veterans Affairs Clinical Assessment, Reporting, and Tracking Program. Cox proportional hazard models estimated the association between H_2 receptor antagonist use and all-cause mortality. Poisson generalized linear mixed models estimated the association between H_2 receptor antagonist use and frequency of heart failure admission.

Measurements and Main Results: 1,518 participants had an H_2 receptor antagonist prescription filled within 90 days of meeting the criterion for pulmonary hypertension and 16,011 did not. During mean follow-up of 2.9 ± 2.0 years, 6,930 participants died. H_2 receptor antagonist use was associated with a 10% lower risk of all-cause mortality compared to non-use ($p=0.02$). There were 7,977 hospitalizations for heart failure. In adjusted models, H_2 receptor antagonist use was not associated with frequency of admission for heart failure.

Conclusions: H_2 receptor antagonist use was associated with reduced all-cause mortality among veterans with pulmonary hypertension.

Introduction

Pulmonary hypertension is common and predicts or mediates poor outcome across a wide range of medical disorders.[12,85] The World Health Organization (WHO) classification scheme combines diseases complicated by pulmonary hypertension that have a similar pathogenesis, hemodynamic characteristics, and treatment approach into five core groups. Pulmonary vasodilators have been an intense focus of research and clinical development for pulmonary arterial hypertension (WHO Group 1), but pulmonary vasodilators are beneficial in some forms of pulmonary hypertension (e.g. pulmonary arterial hypertension), harmful in some forms of pulmonary hypertension, and have no impact in others.[86]

Independent of the underlying basis for a patient's pulmonary hypertension, persons affected by this condition have an increased load on the right ventricle. Increased right ventricular afterload, the manifestation of disparate pathologic changes in different WHO groups, contributes to increased right heart strain and right heart failure.[7] Even though right heart adaptation determines the outcome in several types of pulmonary hypertension, there are no therapies that improve the fitness of the right ventricle.[87] This is in contrast to left heart failure, where neurohormonal modulation intended to improve the fitness of the left ventricle has been shown to improve morbidity and mortality.

There is reason to believe that histaminic signaling in the myocardium could be important in the pathogenesis and progression of heart failure. Similar to beta-receptors, H_2 receptors activate stimulatory G-proteins in the myocardium and H_2 receptor activation contributes to myocardial apoptosis, fibrosis, and heart failure in animal models.[26-28,30,31] We have also shown that H_2 receptor antagonists (H2RAs) are associated with reduced heart failure incidence and differences in right ventricular morphology in community dwelling adults.[33,70] Taken together, this suggests H_2 receptor antagonism may be cardioprotective and relevant in diseases associated with the development of right heart failure.

To explore this possibility, we examined the relationship between H2RA use and mortality in a large cohort of veterans with pulmonary hypertension confirmed at right heart catheterization. We hypothesized H2RA use would be associated with reduced risk for death and a lower rate of hospital admission for heart failure among veterans with a range of pulmonary hypertension phenotypes.

Methods

The Veterans Affairs Clinical Assessment, Reporting, and Tracking Program (VA-CART) is a comprehensive national program evaluating veterans who received a cardiac catheterization. This includes veterans who received a heart catheterization at VA and non-VA centers identified by means of VA administrative and fee-based surveillance.

The VA-CART program has been previously described.[85] The VA-CART cohort has been validated for clinical accuracy and uses embedded software in the integrated VA health care system to collect patient data, procedural characteristics, and longitudinal outcomes. For the current analysis, we included veterans who received a right heart catheterization at a VA-center between fiscal year 2008 and 2014, had a mean pulmonary artery (mPAP) of $\geq$ 25 mmHg, and a recorded value for pulmonary artery wedge pressure (PAWP). The Colorado Multiple Institution Review Board approved this study (#14-1449).

Medication Use

Medication use was ascertained using the VA medical record. Participants were considered to have "used" a medication if an *outpatient* prescription was filled and sent to a patient within 90 days of the right heart catheterization. Participants were excluded if they died within the first 90 days after their heart catheterization or had a hospital stay lasting longer than 60 days following that catheterization. This provided a minimum of 30 days to detect outpatient medication use.

H2RA use was the primary exposure in all analyses. Because H2RAs are available inexpensively and over-the-counter, we assumed that substantial misclassification might exist when these medications were not recorded in the pharmacy record. Some participants characterized as H2RA "non-users" may have actually taken over-the-counter H2RAs. This misclassification of a common over-the-counter medication is unlikely to be systematically related to mortality and would tend to dilute any signal related to H2RA use toward the null.

A patient who uses H2RAs may be more likely to take other medications as well. Other baseline medication use considered for confounding included documentation of a filled prescription for aldosterone antagonists, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, beta-receptor antagonists, calcium-channel blockers, digoxin, diuretics, lipid lowering medications, medications for asthma or chronic obstructive pulmonary disease, medications for diabetes mellitus, oral anticoagulants, platelet inhibitors, systemic vasodilators (including nitrates, $alpha_1$-antagonists, $alpha_2$-agonists, and direct vasodilators), and proton pump inhibitors. A full list of medications is included in the online supplement.

Covariates

In addition to concurrent medication use, characteristics that might confound the relationship between H2RA use and mortality were collected from the VA electronic medical record and included (1) demographic characteristics including age (modeled continuously), sex, race/ethnicity (black or African American, white, or other), body mass index (modeled as a natural spline with fixed degrees of freedom); (2) markers of socioeconomic status and health behaviors including current or previous history of smoking, current or previous history of alcohol abuse, income (as quartiles of income based on the median income of a participant's home zip code according to 2006-2010 census data), marital status; and (3) comorbid medical conditions at the time of the right heart catheterization including the presence or absence of end-stage renal disease, diabetes, cirrhosis, sleep apnea, chronic obstructive pulmonary disease (COPD),

asthma, interstitial lung disease, prior myocardial infarction, prior coronary intervention, congestive heart failure, valvular disease, congenital heart disease, and atrial fibrillation or flutter.

Outcomes

The primary outcome was the rate of all-cause mortality. Risk time accrued after the 90-day window that was used to establish exposure status. Separation of exposure ascertainment and outcome assessment was used to avoid immortal time bias and ensure all participants had an equal chance for exposure to H2RAs. Mortality was determined using the combined VA vital status file, which has a 97.6% exact agreement with the National Death Index.[88] The secondary outcome was admission at a VA hospital or non-VA hospital in which the primary diagnosis was recorded as heart failure or pulmonary hypertension (a full list of administrative codes is included in the online appendix). Follow-up time for hospital readmission was truncated at three years and a given participant could contribute more than one hospital admission to the analysis.

Statistical Analysis

We used methods of Kaplan & Meier to estimate unadjusted associations and Cox proportional hazards models to estimate adjusted associations of H2RA use within 90 days of right heart catheterization and mortality. A generalized linear mixed model with a generalized Poisson function for the response variable was used to estimate the association between H2RA use within 90 days of right heart catheterization and the rate of admission for heart failure over the subsequent three years (additional details in the online supplement). A generalized Poisson distribution was used to account for overdispersion and an offset was included to account for differing exposure times. A random intercept (frailty term) was included in all models to account for within hospital correlation in all primary models.

A series of planned *a priori* adjustments were performed to evaluate whether results from unadjusted analyses were robust. In the limited model, we adjusted for age, sex, race, and body

mass index. In the adjusted model, we also accounted for participants' markers of socioeconomic status and health behaviors including income, tobacco use, alcohol abuse, and marital status. In separate models, we further adjusted for comorbid medical conditions or a veteran's co-medication use.

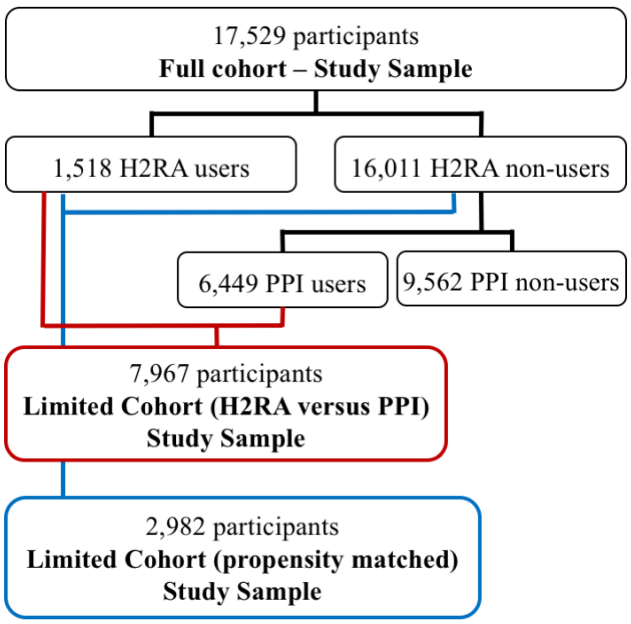
Two restricted cohorts were considered in fully adjusted analyses to further address the possibility of confounding. To account for confounding by indication (in which the underlying disease of GERD, rather than treatment, was associated with mortality), analyses were repeated in a cohort comparing participants who used H2RAs to those who used PPIs. In a second cohort, propensity scores were used to match H2RA users with non-users using the MatchIt package in R.[89,90] The propensity to use H2RAs was calculated as a logit function including factors hypothesized to predict H2RA use, such as comorbidity, body mass index, and co-medication use. Nearest-neighbor matching was used to create pairs of H2RA users and non-users who had an otherwise similar likelihood of use. Only H2RA users with a propensity score less extreme than non-users were matched (common support). Matched pairs were included only if propensity scores differed by less than 0.05 standard deviations to ensure similarity.

Pre-specified exploratory models also evaluated whether age, sex, race, or the veteran's home region (West, Midwest, Northeast, or South) modified the associations between H2RA use and mortality. Because this was a large administrative cohort, careful phenotyping of participants by World Health Organization pulmonary hypertension group was not feasible; however, the PAWP was available for all participants. PAWP less than or equal to 15 mmHg versus greater than 15 mmHg was evaluated as an effect modifier in the association between H2RA use and mortality in veterans with pulmonary hypertension. A second analysis evaluated whether a more stringent cut-off (PAWP of 12 mmHg) modified the association between H2RA use and mortality. This second analysis addressed the suspicion that a PAWP of 12 mmHg, while less sensitive for diagnosing PAH, is more specific for excluding left heart dysfunction.[91] Analyses were performed using SAS 9.4 and R 3.3.1.

Results

During the study period, 34,227 veterans had a right heart catheterization. The final cohort included 17,529 men and women who met the study criterion for the presence of pulmonary hypertension. Of these, 1,518 had a prescription for H2RAs filled and 16,011 had not (Figure 1). Of H2RA non-users, 6,449 veterans had a prescription for proton pump inhibitors filled during the corresponding 90 day period and were included in analyses comparing H2RA and proton pump inhibitor users. A minority of participants received both H2RAs and proton pump inhibitors. Participants on dual acid suppression therapy were analyzed as H2RA users. Of 1,518 H2RA users, 26 did not meet matching criteria leaving 1,491 H2RA users to be matched with 1,491 non-users with an otherwise similar propensity to use H2RAs.

Figure 1. Study sample.



The mean age of the full study sample was 66.2 ± 9.9 years, 96.6% were men, and 77.1% were white. The 1,518 H2RA users in the cohort were slightly more likely to be white, more likely to use additional non-H2RA medications (especially antiplatelet agents), and tended to have a higher prevalence of diabetes, obstructive lung disease, and coronary artery disease (Table 1). Hemodynamic characteristics obtained during right heart catheterization were similar between H2RA users and non-users (Table 2). Mean follow-up was 2.9 ± 2.0 years and total follow-up was 50,848 person-years.

Table 1. Characteristics of the study sample

	H2RA users (n=1,518)	Non-H2RA users (n=16,011)
Age (%)		
< 45 years-old	1.7	2.0
45 – 55 years-old	7.8	8.6
55 – 65 years-old	40.4	37.3
65 – 75 years-old	30.0	31.8
75 – 85 years-old	16.8	17.0
≥ 85 years-old	3.2	3.3
Male (%)	95.8	96.7
Race (%)		
White	80.6	76.8
African-American	17.5	21.1
Other	1.8	2.1
Body mass index (%)		
Underweight, <18.5 kg/m^2	0.8	0.7
Normal, 18.5 – 25 kg/m^2	15.2	17.1
Overweight, 25 – 30 kg/m^2	30.4	28.8
Obese, 30 – 35 kg/m^2	25.0	25.0
Severely obese, ≥ 35 kg/m^2	28.6	28.4
Income ($)	49,035 ± 16,690	49,848 ± 17,428
Marital Status		
Single	13.7	13.6
Married	50.1	48.4
Divorced	28.6	30.5
Widowed	7.6	7.5
Documented current or previous alcohol abuse (%)	10.0	10.4
Documented current or previous smoking (%)	61.9	60.7
Comorbidity		
Diabetes mellitus (%)	55.7	51.7
End-stage renal disease/dialysis (%)	35.3	33.2
Cirrhosis (%)	6.3	7.2
Obstructive sleep apnea (%)	14.4	14.6
Chronic Obstructive Pulmonary Disease (%)	40.6	37.3
Asthma (%)	6.9	5.6
Interstitial lung disease (%)	0.6	0.5
Prior MI, PCI, or CABG (%)	48.7	39.9
Congestive heart failure (%)	67.1	68.1
Valvular heart disease (%)	40.6	38.7
Congenital heart disease (%)	0.5	0.6
Atrial fibrillation / flutter (%)	30.1	33.1

Abbreviations: kg=kilograms, m^2=meters squared, MI=myocardial infarction, PCI=percutaneous intervention, CABG=coronary artery bypass graft

Table 2. Hemodynamic characteristics of the study sample at right heart catheterization

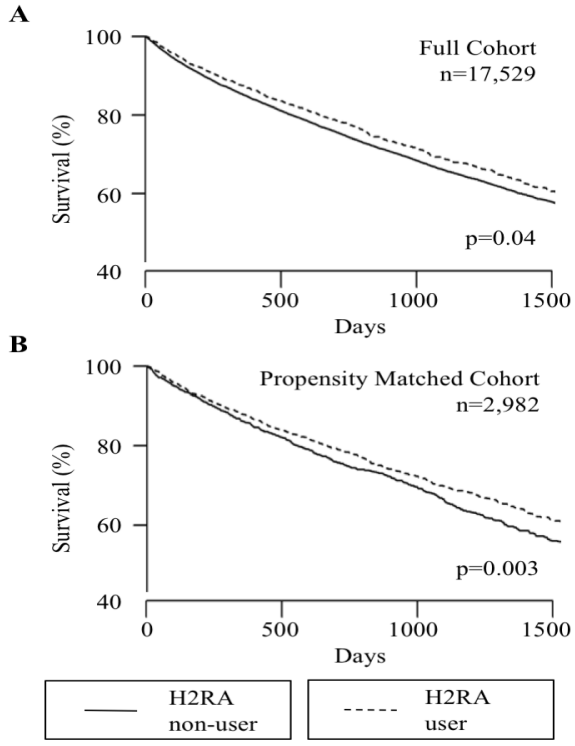
	H2RA users (n=1,518)	Non-H2RA users (n=16,011)
Systemic Pressures		
Systolic systemic blood pressure (mmHg)	130 ± 15	131 ± 16
Diastolic systemic blood pressure (mmHg)	73 ± 10	74 ± 10
Pulmonary Arterial Pressures		
Pulmonary artery systolic pressure (mmHg)	53 ± 14	53 ± 14
Pulmonary artery diastolic pressure (mmHg)	24 ± 7	24 ± 7
Mean pulmonary arterial pressure (mmHg)	35 ± 9	35 ± 9
Pulmonary artery wedge pressure (mmHg)	22 ± 7	22 ± 8
Cardiac Output and Calculated Variables		
Cardiac output (L/min)	5.2 ± 1.7	5.2 ± 1.9
Pulmonary vascular resistance (Wood units)†	2.8 ± 2.0	3.0 ± 2.1

Abbreviations: mmHg=millimeters of mercury, L=liters, min=minute

† Pulmonary vascular resistance calculated as (mean pulmonary artery pressure – pulmonary artery occlusion pressure)/cardiac output for each participant and then averaged

Five hundred eighty-nine H2RA users died in 4,719 person-years (12.5 deaths per 100 person-years) and 6,341 non-users died in 46,129 person-years (13.7 deaths per 100 person-years) (See Figure 2 for the unadjusted Kaplan-Meier survival curve). H2RA use within 90 days of right heart catheterization in veterans with pulmonary hypertension was associated with a 10% lower risk for all-cause mortality (adjusted HR 0.90, 95% CI 0.83 – 0.98, p=0.02). This relationship was slightly stronger in models accounting for co-medication use, comorbid medical conditions, in the cohort comparing H2RA users to proton-pump inhibitor users, and when H2RA users were compared to propensity-matched non-users (Table 3).

Figure 2. Unadjusted Kaplan-Meier survival curves for mortality among veterans with pulmonary hypertension who did and did not use H_2 receptor antagonists (H2RA) in (A) the full cohort and (B) and analysis restricted to participants who used H2RAs and those who did not, but had an otherwise similar propensity to use H2RAs.



Age, sex, race, and the veteran's home region did not modify associations between H2RA use and mortality (p-value for the interaction in adjusted models: 0.42, 0.36, 0.85, and 0.90 respectively). There also was no evidence that pulmonary artery wedge pressure modified the relationship between H2RAs and mortality (p-value for the interaction in adjusted models using a PAWP cut-off of 15 mmHg=0.75, and using the more restrictive cut-off of 12 mmHg cut-off, p=0.25). When relationships between H2RA use and mortality were evaluated in smaller cohorts limited to veterans with COPD or heart failure, effect estimates were similar although the precision in these smaller populations was diminished (Figure 3).

Table 3. Relationship of H2RA use within 90 days of right heart catheterization to mortality in veterans with pulmonary hypertension (n=17,529)

	Adjusted risk of mortality in H2RA users relative to non-users		
	Hazard Ratio	95% CI	p-value
Unadjusted	0.90	0.83 to 0.98	0.02
Limited adjustment†	0.91	0.83 to 0.99	0.03
Full adjustment‡	0.90	0.83 to 0.98	0.02
Full adjustment‡ + Co-medication use	0.88	0.80 to 0.96	0.003
Full adjustment‡ + Comorbidity‖	0.86	0.79 to 0.94	0.001
Restricted to H2RA users & PPI users# (n=7,967)	0.86	0.79 to 0.94	0.001
Restricted to PS matched participants# (n=2,982)	0.82	0.74 to 0.92	0.001

Definition of abbreviations: CI-confidence interval; H2RA-H2 receptor antagonist; PPI-proton pump inhibitor; PS-propensity score

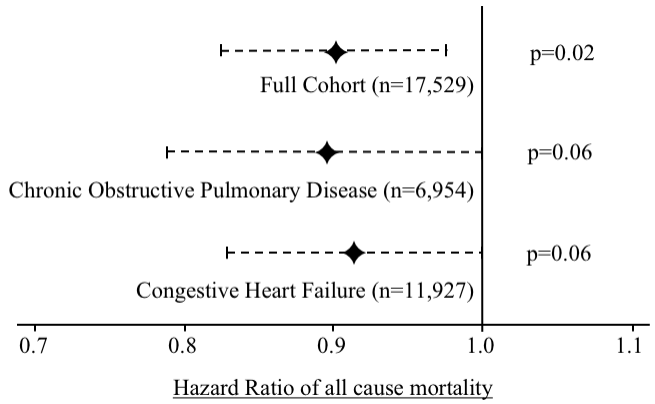
† Limited adjustment accounts for age, sex, race/ethnicity, and body mass index

‡ Full adjustment accounts for the limited model and income, tobacco use, alcohol abuse, and marital status

|| Comorbidity included the presence or absence of end-stage renal disease / dialysis, diabetes mellitus, cirrhosis, sleep disordered breathing, chronic obstructive pulmonary disease or asthma, interstitial lung disease, prior myocardial infarction, prior percutaneous coronary intervention, prior coronary artery bypass graft, congestive heart failure, valvular heart disease, congenital heart disease, atrial fibrillation, and/or atrial flutter

 # Participants in the restricted cohorts were considered in models with full adjustment

Figure 3. Horizontal plot depicting the hazard of mortality and 95 % confidence interval for veterans who used H_2 receptor antagonists relative to those who did not in the full cohort, in a limited cohort that only included veterans with chronic obstructive pulmonary disease, and a limited cohort that only included veterans with heart failure.



In unadjusted analyses, H2RA users were hospitalized 752 times for heart failure or pulmonary hypertension over 3,374 person-years (22.3 hospitalizations per 100 person-years) and non-users were hospitalized 7,225 times over 33,709 person-years (21.4 hospitalizations per 100 person-years). There was no difference in the rate of admission for heart failure or pulmonary hypertension among individuals who had a prescription for H2RAs filled relative to those who did not (adjusted readmission rate

ratio 1.08, 95% CI 0.98 – 1.20, p=0.13). The lack of a relationship between H2RA use and rate of hospitalization persisted in models further accounting for a participant's co-medication use (adjusted readmission rate ratio 0.95, 95% CI 0.86 – 1.06, p=0.38) or comorbid medical conditions (adjusted readmission rate ratio 0.98, 95% CI 0.88 – 1.08, p=0.66).

Discussion

We observed that H2RA use was associated with a 10% lower risk for all-cause mortality in a large cohort of veterans with pulmonary hypertension confirmed by right heart catheterization. This is the first report of reduced all-cause mortality associated with any medication in a cohort defined by invasively confirmed pulmonary hypertension. Similar relationships were seen in unadjusted analyses, adjusted analyses, comparisons between participants who used H2RAs relative to those who used PPIs, and comparisons between those who used H2RAs relative to non-users with a similar propensity to use H2RAs, which reinforces the robustness of our results.

Pulmonary hypertension is common and associated with decreased survival in many diseases, including COPD, left heart failure, and pulmonary arterial hypertension.[1,92,93] Conventional treatment focuses on the underlying cause of the pulmonary hypertension, including pulmonary vasodilators in pulmonary arterial hypertension.[94] Because VA-CART is a large administrative cohort, participants could not be reliably grouped into World Health Organization Groups; however, improved survival with H2RA use was similar in several subgroups including veterans with COPD or heart failure. In addition, participants above or below key thresholds of PAWP (12 or 15 mmHg) did not have a different association between H2RA use and mortality.[1] This finding is unique among therapies for pulmonary hypertension and in contrast to pulmonary vasodilators like epoprostenol, where treatment may improve mortality in selected groups of patients with pre-capillary pulmonary hypertension, but worsen mortality in post-capillary pulmonary hypertension.[95,96]

This report is part of a growing literature suggesting histaminic signaling may be important to cardiovascular health. A previous randomized controlled trial found improved symptoms, lower brain natriuretic peptide, and favorable cardiac morphology in participants with heart failure and reduced ejection fraction treated with the H2RA famotidine.[39] In addition, H2RA use is associated with reduced heart failure incidence and favorable cardiac morphology in community dwelling adults without clinical cardiovascular disease at baseline.[70] These clinical and population-based studies are reinforced by animal models suggesting biologically plausible mechanisms for a beneficial impact. Modulating histaminic signaling in animal models can abrogate heart failure from tachycardia, doxorubicin, and aortic banding.[28-31] Furthermore, H_2 receptor activation contributes to mitochondrial permeability, myocardial susceptibility to stress, and myocardial fibrosis in experimental models.[32]

We did not observe a difference in the frequency of hospital admission for heart failure or pulmonary hypertension with H2RA use despite a mortality benefit. One interpretation for reduced all-cause mortality without a reduction in the frequency of admissions for heart failure or pulmonary hypertension could be that H2RAs reduce mortality in veterans with pulmonary hypertension through a non-cardiovascular intermediary (e.g. reduced risk for a gastrointestinal bleed). A mortality benefit explained by acid suppression should also have been apparent in individuals who used proton pump inhibitors, but this was not seen. Alongside the accumulating basic and observational evidence, a non-cardiovascular explanation for our findings in a group at high risk for poor cardiovascular outcomes would be unexpected, but plausible. Alternatively, the impact on survival may have confounded the results in non-mortality outcomes. Frequency of admission for heart failure or pulmonary hypertension may not have been an adequate surrogate for cardiovascular disease severity. This inadequacy could be related to misclassification of the admission diagnosis imposed by the use of administrative data (e.g. an admission for pneumonia in a patient with controlled heart failure that was recorded as a “heart failure” admission) or could

suggest that admission for heart failure may not be strongly related to disease severity and instead hospital admission may reflect social preferences and regional differences.[97-100]

There are several limitations. Unmeasured confounding can complicate the inference in observational studies, and confounding by indication is a particular concern in pharmacoepidemiology.[101] In addition, all-cause mortality, while expedient, relatively insensitive to misclassification, and inherently patient-oriented, is by definition not "cause-specific."[102] Cause-specific mortality was not available for the VA-CART cohort. As highlighted by the analysis of hospitalization frequency, alternative explanations for a reduction in mortality with H2RA use are possible.

In addition, H2RAs are available over-the-counter and over-the-counter medications may be poorly enumerated in an integrated medical record.[103] In addition, the exposure was assessed only during the first 90 days. It is likely that misclassification of H2RA use was present. Associations between H2RA use and mortality may be diluted by H2RA use in participants analyzed as non-users and associations with mortality may be stronger than reported.

These results are suggestive of a benefit with H2RA use in the management of pulmonary hypertension and justify the conduct of a randomized controlled trial. H2RAs are inexpensive and have a favorable side effect profile. If our results can be confirmed in prospective, randomized studies H2RAs may represent an attractive treatment strategy for a large number of patients with pulmonary hypertension.

Conclusions

We observed a lower risk for all-cause mortality in a large cohort of American veterans with invasively confirmed pulmonary hypertension who used H_2 receptor antagonists. Histaminic signaling may be important in diseases complicated by right heart failure and could be a therapeutic target.

Chapter 2

Inflammation and the heart in individuals without heart disease

In this chapter, I present three manuscripts (previously published and completed during my time as a student in the epidemiology program). All papers include participants from the Multi-Ethnic Study of Atherosclerosis (MESA) who were free of cardiovascular disease at the time of these cross-sectional evaluations. We discovered that increased pentraxin-3 levels and exposure to air pollution were associated with larger RV volumes and greater RV mass.[25,104] Increased von Willebrand Factor (vWF) activity was associated with smaller RV volumes and mass.[24,105]

Previous work has established that increased RV mass in MESA participants was associated with an increased risk for heart failure or cardiovascular death.[106] This would seem to argue against our hypothesis that pro-inflammatory phenotypes are associated with maladaptive RV phenotypes. We found that a pro-inflammatory marker (vWF) is associated with a smaller RV mass and an anti-inflammatory molecule (pentraxin 3) is associated with higher RV mass.[104] Only the relationship between increased exposure to air pollution (a pro-inflammatory environmental exposure) and increased RV mass would seem to agree with the underlying hypothesis that pro-inflammatory states are associated with maladaptive RV phenotypes.

The explanation for this possible divergence from our hypothesis is not entirely clear; however, it is important to consider the limitations of the data. In these cross-sectional analyses the temporality of the association is not evident and the possibility for non-causal associations or reverse confounding is quite possible.[107] In particular, these analyses focus on single aspects of the inflammatory response. There is a strong likelihood that the balance of endogenous inflammatory and anti-inflammatory responses is more strongly associated with any cardiac phenotype than any single aspect of the response in isolation. Interestingly, all the inflammatory markers evaluated were associated with cardiac morphology-- albeit in divergent directions. Although more work clearly needs to be done before these aspects of inflammation can be targeted therapeutically, the consistency of the association between inflammatory markers and cardiac morphology joins previous animal research to highlight the importance of the inflammatory microenvironment with regards to cardiac structure and function.

Pentraxin-3 and the Right Ventricle: The MESA-Right Ventricle Study

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Abstract

Background: Pentraxin-3 (PTX3) is a protein mediator of innate immunity that is elevated in the setting of left heart disease and pulmonary arterial hypertension. The relationship between PTX3 and right ventricular (RV) structure and function is not known.

Methods: We included men and women with magnetic resonance imaging (MRI) assessment of RV structure and function and measurement of PTX3 from the Multi-Ethnic Study of Atherosclerosis, a study of individuals free of clinical cardiovascular disease. Multivariable linear regression estimated the associations between PTX3 protein levels and RV measures after adjusting for demographics, anthropometrics, smoking, diabetes mellitus, hypertension and the corresponding left ventricular (LV) parameters. Instrumental variable analysis exploiting Mendelian randomization was attempted using two-stage least squares regression.

Results: The study sample included 1,779 participants with available PTX3 levels, RV measures, and all covariables. Mean PTX3 level was 2.1 ng/mL. Higher PTX3 was independently associated with greater RV mass and larger RV end-diastolic volume with and without adjustment for the corresponding LV parameters or C-reactive protein (all $p < 0.05$). There was no association between PTX3 and RV ejection fraction or stroke volume. Single nucleotide polymorphisms were not associated with PTX3 protein levels or RV measures after accounting for race. Instrumental variable analysis could not be reliably performed.

Conclusions: Higher PTX3 protein levels were associated with greater RV mass and larger RV end-diastolic volume. These associations were independent of common cardiovascular risk factors and LV morphologic changes. Inflammation is associated with differences in the pulmonary circulation-right ventricular axis in adults without clinical cardiovascular disease.

Introduction

Pentraxin-3 (PTX3) is a protein mediator of innate immunity produced by vascular smooth muscle cells, endothelial cells and fibroblasts. PTX3 may dampen the immune response through interruption of the complement cascade, apoptotic clearance, and mitigation of atherosclerosis.[108] PTX3 is found in atherosclerotic plaques and increased circulating levels are seen in diastolic dysfunction, metabolic syndrome, and myocarditis.[109-112] High PTX3 levels also suggest poor prognosis in chronic heart failure and other diseases.[113-115]

PTX3 may also be important in pulmonary vascular disease and right ventricular (RV) dysfunction. High PTX3 levels are reported in patients with pulmonary hypertension,[116] and local control of inflammation is hypothesized to influence RV adaptation.[14] Furthermore, specific PTX3 genotypes are associated with increased risk for primary graft dysfunction after lung transplant for which pulmonary hypertension is also a known risk factor.[117,118] The relationship between PTX3 and RV structure and function has not been studied.

We examined the relationship of PTX3 with magnetic resonance imaging (MRI) measures of RV structure and function in a multiethnic cohort of adults free of clinical cardiovascular disease. We hypothesized that higher PTX3 protein levels would be independently associated with greater RV mass, larger end-diastolic volume, and reduced RV ejection fraction.

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicenter prospective cohort study designed to investigate the prevalence, correlates and progression of subclinical cardiovascular disease in whites, African-Americans, Hispanics and Chinese-Americans.[43] 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 diagnosed heart attack, stroke, transient ischemic

attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure), weight >136kg (300 lbs.), pregnancy, or impediment to long-term participation. The Institutional Review Boards of all participating institutions approved the protocols of MESA and all studies described herein. The MESA-Right Ventricle Study, which includes the present investigation, is an ancillary study with funding to interpret approximately 4,200 cardiac MRIs for RV morphology in MESA participants at the baseline examination.

Cardiac magnetic resonance imaging measures

The cardiac MRI protocol has been previously described.[44] All imaging was performed on 1.5 Tesla magnets. Imaging consisted of fast-gradient echo cine images with temporal resolution ≤50 ms. Cardiac MRI examinations were analyzed at the reading center at Johns Hopkins University in Baltimore, MD. Image analysis was performed by two independent analysts on Windows workstations using QMASS software (Medis, Leiden, the Netherlands). Images were magnified to 250%, contrast and brightness were set to 55, window width and level were set with auto function in QMASS.

Methods for interpretation of LV and RV parameters have been reported.[45,119] Endocardial and epicardial borders of the RV were manually traced on MRI short axis cine images at end-systole and end-diastole. Full visualization of the correct placement of RV contours relied on evaluation of cine images to determine the demarcation between the right atrium and the RV. Contours were modified at basal slices of the heart by careful identification of the tricuspid valve to exclude the right atrium and to avoid over-estimation of the volumes. The outflow tract was included in RV volume. Papillary muscle and trabeculae were included in RV volumes and excluded from RV mass, as is commonly done for LV mass.[47,48]

RV end-systolic volume and RV end-diastolic volume (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 of the RV free wall multiplied by the specific gravity of the heart (1.05g/mL). RV stroke volume was calculated by subtracting RV end-systolic volume from the RVEDV. RVEF was calculated by dividing RV stroke volume by RVEDV.

Our protocol included random blinded rereads by the same reader. The intra-reader intra-class correlation coefficient for RV mass was 0.94 (229 scans), for RVEDV was 0.99 (230 scans) and for RVEF was 0.89 (230 scans). In addition, blinded rereads by a second reader were performed. The inter-reader intra-class correlation coefficients on 240 scans for RV mass, RVEDV and RVEF were 0.89, 0.96 and 0.80 respectively.

Biomarkers and Covariables

Fasting blood samples were drawn, processed and stored using standardized procedures.[120] PTX3 was measured as part of another ancillary study in which 2,880 MESA participants. Participants underwent novel biomarker testing and were selected to achieve balanced representation from all four race/ethnic groups to allow sufficient power to conduct subgroup analyses in each racial/ethnic group. PTX3 was measured at the Laboratory for Clinical Biochemistry Research at University of Vermont (Burlington, VT, USA) using a sandwich ELISA (PTX3 (human) detection set, Alexis Biochemicals, San Diego, CA). The analytic coefficient of variation was 10.2%.

Genotyping was attempted in a random sample of participants in MESA who gave consent for genetic analysis and from whom DNA was extracted from peripheral leukocytes using a commercially available isolation platform (Puregene; Minneapolis, MN).[121] Genotyping was performed by Illumina Genotyping Services (San Diego, California) using the GoldenGate Assay. After removal of failed single nucleotide polymorphisms (SNPs) and samples, the genotyping call rate was 99.93%. The human PTX3 gene, located on chromosome 3q25.32, consists of three exons separated by two introns and covering 7 kilobases.[122] Several SNPs have been described in the PTX3 gene including SNPs with potential functional significance. The G-A-G haplotype for

sites rs2305619, rs3816527 and rs1840680 in particular has been associated with increased PTX3 protein levels[123], a reduced risk of tuberculosis[124], a reduced risk of pseudomonas colonization in cystic fibrosis[122] and improved female fertility.[123] There were six available SNPs in the PTX3 gene locus considered for analysis.

Other covariables including race/ethnicity, height, weight, presence of hypertension or diabetes mellitus, C-reactive protein (CRP), smoking, spirometry, and emphysema were measured as previously described.[24]

Statistical analysis

We used linear regression to characterize the relationship between PTX3 and RV parameters. PTX3 was log transformed to achieve normality. All models were adjusted for height and weight, so it was not necessary to index RV parameters to account for differences in body size. Generalized additive models were used to assess possible non-linearity. Covariables were chosen on the basis of known associations with ventricular size, heart disease and comorbidities. In limited models, we adjusted for age, sex, race/ethnicity, height and weight. In fully adjusted models, we included participant's educational level, income, smoking, hypertension, diabetes mellitus, cholesterol and impaired glucose tolerance. We adjusted for LV parameters to evaluate for independence from LV abnormalities (e.g. increased LV mass causing pulmonary venous hypertension leading to increased RV mass) and to better account for differences in body size. Because RVSV and LV stroke volume are interdependent, we adjusted for LV mass in this case. We adjusted for both structural and functional lung disease using % emphysema on chest CT, forced expiratory volume at 1 second (FEV1) and forced vital capacity (FVC) when available (n=1,360). Because of theoretical antagonism between CRP and PTX3, we performed further analyses adjusted for CRP (n=1,772).

In participants with available SNPs in the PTX3 gene locus (n=1,582) departures from Hardy-Weinberg Equilibrium were assessed using Fisher's exact test. Multiple linear regression

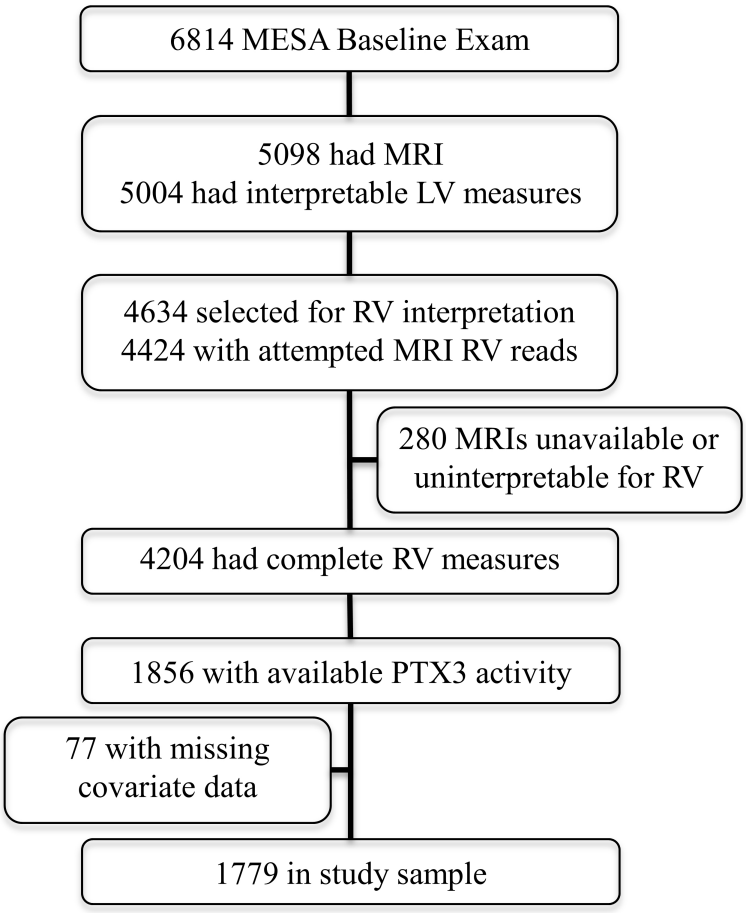
was used to characterize the relationship between candidate genotypes/haplotypes and PTX3 protein levels or RV measures in models with and without adjustment for race and principal components.[125] As the true nature of inheritance is unknown, both additive and dominant models were assessed. Instrumental variable analysis exploiting Mendelian randomization was attempted using two-stage least squares regression.[126]

Statistical significance was defined as $p<0.05$ for all non-genetic analyses. For genetic analyses, we used a Bonferroni correction for twelve comparisons so that $p<0.004$ was considered significant. Analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA). Results of genetic analyses were confirmed using the Golden Helix Software Package (Bozeman, MT).

Results

There were 6,814 men and women enrolled in MESA (Figure 1) of whom 5,098 underwent cardiac MRI and 5,004 (98%) had interpretable exams for the LV. The RV was successfully interpreted in 4,204 participants (95% of 4,424 attempted). PTX3 was measured in 1,856 of these participants under another ancillary study. Seventy-seven participants were excluded for missing covariables, leaving 1,779 in the study sample. Table 1 shows characteristics of the study sample compared with those excluded. The mean age of the study sample was 60.7 years

Figure 1. Study design



and 46.1% were men. As compared with MESA participants not included in the analysis, the study sample had a larger proportion of Chinese race and fewer whites, which reflects oversampling of this racial group in the PTX3 ancillary study design. Formal statistical testing is not appropriate for such comparisons, which are descriptive rather than inferential. Mean RV mass in the study sample was 20.9±4.5g, mean RVEDV was 121.3±30.5mL, mean RVSV was 84.6±20.2mL and mean RVEF was 70.3±6.6%. Mean PTX3 level was 2.1ng/mL.

Table 1. Characteristics of the study sample compared with participants not included in the analysis

	Study Sample (n=1779)	Excluded (n=5035)
Age (years)	60.7±10.0	62.7±10.3
Male (%)	46.1	47.5
Race (%)		
White	26.6	42.7
Chinese	26.5	6.6
African-American	23.5	29.3
Hispanic	23.4	21.4
Educational attainment (%)		
No high school degree	19.2	17.6
High school degree	17.6	18.4
Some college	14.4	17.0
Bachelor's Degree	18.1	17.0
Higher than bachelor's degree	17.9	18.1
Height (cm)	165.1±9.8	166.8±10.1
Weight (kg)	74.5±16.3	80.1±17.4
Body mass index (kg/m^2)	27.2±5.0	28.7±5.6
Cigarette smoking status (%)		
Never	57.2	47.9
Former	30.0	38.9
Current	12.8	13.2
Hypertension (%)	40.7	46.5
Systolic blood pressure (mmHg)	124.6±21.0	127.3±21.6
Diastolic blood pressure (mmHg)	71.7±10.0	72.0±10.3

Diabetes mellitus (%)	12.9	13.8
PTX3 (ng/mL)	2.1±1.2	2.2±1.6*

Abbreviations: PTX3=pentraxin-3, cm=centimeters, kg=kilogram, m^2=meters squared, mmHg=millimeters of mercury

Higher PTX3 was associated with greater RV mass (0.3 g increase per log increase in PTX3, p=0.04)(Table 2, Figure 2). This relationship was not altered by adjustment for cardiovascular risk factors (p=0.03) or LV mass (p=0.02). Further adjustment for lung structure and function did not alter the relationship between PTX3 and RV mass in the 1,360 participants with these measures (0.4 g increase per log increase in PTX3, p=0.03). Adjustment for CRP did not change the relationship between PTX3 and RV mass (0.4g increase per log increase in PTX3, p=0.02)(Table 3).

Figure 2. Association between the log of pentraxin-3 (PTX3) protein level and right ventricle (RV) parameters. Linear regression adjusted for age, sex, race/ethnicity, education, income, height, weight, smoking status, presence of hypertension or diabetes mellitus, cholesterol, and impaired glucose tolerance.

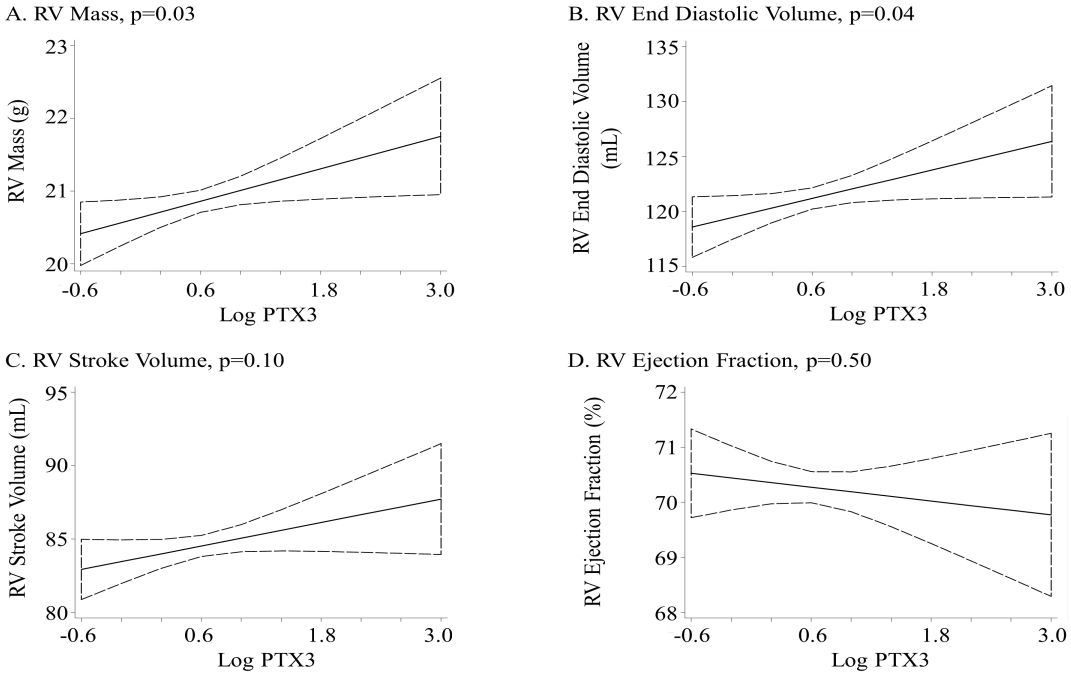


Table 2. Change in RV measures per log increase in PTX3 protein level

	per log increase in PTX3		
	Coefficient	95% CI	p-value
RV mass, g (Limited model)	0.3	0.0, 0.7	0.04
RV mass, g (Adjusted model)	0.4	0.0, 0.7	0.03
RV mass, g (Adjusted + LV mass)	0.4	0.1, 0.7	0.02
RVEDV, mL (Limited model)	1.8	-0.3, 3.9	0.09
RVEDV, mL (Adjusted model)	2.2	0.1, 4.3	0.04
RVEDV, mL (Adjusted + LV-EDV)	2.3	0.8, 3.8	0.003
RVSV, mL (Limited model)	1.0	-0.5, 2.6	0.19
RVSV, mL (Adjusted model)	1.3	-0.2, 2.9	0.10
RVSV, mL (Adjusted + LV mass)	1.3	-0.2, 2.8	0.08
RVEF, % (Limited model)	-0.2%	-0.9, 0.4	0.44
RVEF, % (Adjusted model)	-0.2%	-0.8, 0.4	0.50
RVEF, % (Adjusted + LV-EF)	-0.2%	-0.7, 0.4	0.55

Limited model: age, gender, race/ethnicity, height and weight

Adjusted model: limited model +education, income, smoking, presence of hypertension or diabetes mellitus, cholesterol and impaired glucose tolerance

Higher PTX3 protein level had a borderline association with larger RVEDV after limited adjustment (1.8 mL increase per log increase in PTX3, p=0.09)(Table 2, Figure 2). Full adjustment accounting for confounding by cardiovascular risk factors strengthened the relationship (p=0.04), as did further adjustment for LVEDV (p=0.003). Additional adjustment for lung structure and function attenuated the relationship (2.0 mL increase per log increase in PTX3, p=0.09) and further adjustment for CRP did not change the relationship between PTX3 and RVEDV (2.4mL increase per log increase in PTX3, p=0.03)(Table 3). PTX3 protein level was not associated with RVSV or RVEF (Tables 2, Figure 2). Adjustment for cardiovascular risk factors including race/ethnicity, LV mass or ejection fraction, CRP and lung structure and function did not alter these results (Table 3). In exploratory analyses, there was no effect modification by age, gender,

race or LV parameters. No significant non-linearity was detected using generalized additive models (all p>0.2).

Table 3. Change in RV measures per log increase in PTX3 protein level accounting for differences in lung structure, function and C-reactive protein

	per log increase in PTX3		
	Coefficient	95% CI	p-value
Adjusted for lung structure and function (n=1,360)			
RV mass, g	0.4	0.0, 0.8	0.03
RVEDV, mL	2.0	-0.3, 4.4	0.09
RVSV, mL	1.1	-0.7, 2.8	0.23
RVEF, %	-0.3%	-1.0, 0.4	0.39
Adjusted for C-reactive protein (n=1,772)			
RV mass, g	0.4	0.1, 0.7	0.02
RVEDV, mL	2.4	0.3, 4.5	0.03
RVSV, mL	1.4	-0.1, 2.9	0.07
RVEF, %	-0.2%	-0.9, 0.4	0.45

Lung structure and function adjustment: adjusted model + forced expiratory volume at 1 second, forced vital capacity and % emphysema on Chest CT
C-reactive protein adjustment: adjusted model + C-reactive protein

Of 1,779 participants, 1,582 had genotypes available for analysis (86.4%). Of these, rs3845978, rs9289983 and rs2614 were not in Hardy-Weinberg Equilibrium (Table 4). When stratified by race/ethnicity only rs2614 was not in Hardy-Weinberg Equilibrium (Table 5). None of the 5 candidate genes with a minor allele frequency greater than 5% (rs3845978, rs2305619, rs2120243, rs1456099 and rs9289983) were associated with PTX3 protein levels, RV mass or RVEDV after adjustment for principle components (Table 6). Further adjustment for race/ethnicity did not change these results. There was no significant interaction between race/ethnicity and gene. Exploratory relationships stratified by race approached but did not achieve significance for some evaluated SNPs (Table 7). The first stage F-statistic in all instrumental variable analyses

was less than 10, which suggests a poor instrument. The weak association between selected SNPs and PTX3 protein level precluded meaningful analysis using this technique (Table 8).[127]

Table 4. Hardy-Weinberg Equilibrium of the SNPs in the PTX3 gene locus

	(n)	Observed genotype counts			Observed allele counts		Minor Allele Frequency (%)	Expected counts under HWE			Exact p-value
rs3845978		**AA**	**AG**	**GG**	**A**	**G**		**AA**	**AG**	**GG**	
	1585	34	306	1245	374	2796	12	22	330	1233	0.01
rs2305619		**AA**	**AG**	**GG**	**A**	**G**		**AA**	**AG**	**GG**	
	1583	335	764	484	1434	1732	45	325	784	474	0.31
rs2120243		**AA**	**AC**	**CC**	**A**	**C**		**AA**	**AC**	**CC**	
	1580	237	740	603	1214	1946	38	233	748	599	0.71
rs1456099		**AA**	**AT**	**TT**	**A**	**T**		**AA**	**AT**	**TT**	
	1582	330	744	508	1404	1760	44	312	781	490	0.06
rs9289983		**AA**	**AG**	**GG**	**A**	**G**		**AA**	**AG**	**GG**	
	1582	311	719	554	1341	1827	42	284	773	525	0.01
rs2614		**AA**	**AG**	**GG**	**A**	**G**		**AA**	**AG**	**GG**	
	1582	5	55	1522	65	3099	2	1	64	1518	0.00

Table 5. Hardy-Weinberg Equilibrium of the SNPs in the PTX3 gene locus stratified by race

	Exact P-value			
	White (n=412)	*Chinese (n=441)*	*African-American (n=341)*	*Hispanic (n=391)*
rs3845978	0.99	0.31	0.48	0.41
rs2305619	0.49	0.60	0.38	0.61
rs2120243	0.77	0.74	0.49	0.52
rs1456099	0.43	0.45	0.27	0.76
rs9289983	0.43	0.57	0.86	0.61
rs2614	0.00	0.00	0.06	0.00

Table 6. SNP association with PTX3, RV mass, and RV End Diastolic Volume

	Association with PTX3 protein level Genetic model (p-values)		Association with RV mass Genetic model (p-values)		Association with RV End Diastolic Volume Genetic model (p-values)	
	Additive	*Dominant*	*Additive*	*Dominant*	*Additive*	*Dominant*
rs3845978						
Unadjusted	0.03	0.07	0.007	0.02	0.03	0.08
Adjusted	0.24	0.46	0.23	0.38	0.48	0.76
rs2305619						
Unadjusted	0.57	0.37	0.008	0.03	0.005	0.01
Adjusted	0.85	0.83	0.76	0.80	0.53	0.46
rs2120243						
Unadjusted	0.75	0.70	0.11	0.10	0.07	0.04
Adjusted	0.68	0.83	0.95	0.82	0.75	0.44
rs1456099						
Unadjusted	0.49	0.73	0.32	0.27	0.14	0.29
Adjusted	0.55	0.75	0.96	0.97	0.56	0.94
rs9289983						
Unadjusted	0.36	0.59	0.19	0.10	0.07	0.10
Adjusted	0.37	0.57	0.97	0.87	0.59	0.84

Adjusted model includes principal components

Table 7. Adjusted SNP association with PTX3, RV mass, and RV End Diastolic Volume stratified by race using an additive genetic model of inheritance

	Adjusted association between SNP and PTX3, Mass, EDV by race				
	Interaction p-value	*White (n=412)*	*Chinese (n=441)*	*African-American (n=341)*	*Hispanic (n=391)*
PTX3					
rs3845978	0.19	0.18	0.01	0.68	0.96
rs2305619	0.35	0.46	0.84	0.20	0.69
rs2120243	0.67	0.92	0.24	0.83	0.66
rs1456099	0.38	0.62	0.04	0.59	0.89
rs9289983	0.45	0.62	0.04	0.80	0.96
RV Mass					
rs3845978	0.57	0.77	0.14	0.40	0.27
rs2305619	0.80	0.35	0.75	0.15	0.96
rs2120243	0.76	0.34	0.49	0.38	0.60
rs1456099	0.37	0.33	0.14	0.10	0.32
rs9289983	0.36	0.33	0.13	0.05	0.41
RV EDV					
rs3845978	0.77	0.74	0.13	0.97	0.82
rs2305619	0.99	0.59	0.42	0.54	0.86
rs2120243	0.91	0.51	0.65	0.99	0.43
rs1456099	0.73	0.67	0.55	0.24	0.33
rs9289983	0.76	0.67	0.51	0.11	0.47

Adjusted model includes principal components

Table 8. Mendelian Randomization using SNPs in the PTX3 gene locus as the instrumental variable in 2-stage least squares regression of the relationship with RV mass and end diastolic volume.

	Relationship with RV mass	Relationship with RV EDV

	p-value	First Stage F-statistic	Bias likely	p-value	First Stage F-statistic	Bias likely
Unadjusted						
rs3845978	0.06	3.2	Yes	0.09	3.2	Yes
rs2305619	0.42	0.5	Yes	0.40	0.5	Yes
rs2120243	0.71	0.1	Yes	0.70	0.1	Yes
rs1456099	0.64	0.3	Yes	0.47	0.3	Yes
rs9289983	0.57	0.5	Yes	0.39	0.5	Yes
Adjusted						
rs3845978	0.28	1.4	Yes	0.30	1.4	Yes
rs2305619	0.93	0.2	Yes	0.84	0.2	Yes
rs2120243	0.77	0.1	Yes	0.79	0.1	Yes
rs1456099	0.92	0.2	Yes	0.56	0.2	Yes
rs9289983	0.87	0.5	Yes	0.58	0.5	Yes

Adjusted model includes principal components

Discussion

We have shown that higher PTX3 protein levels are associated with greater RV mass and larger RVEDV in a multi-city cohort of adults without clinical cardiovascular disease. The relationship with RV mass was not strongly affected by adjustment for differences in pulmonary structure and function or by differences in respective LV mass suggesting an RV-specific relationship. MESA participants had a 0.3 g (1.4%) increase in RV mass per log increase in PTX3. This is similar to the difference seen in LV mass (2.4%) in MESA participants with and without diabetes and may support the clinical and biological relevance of this association.[55] In addition, RV hypertrophy in MESA is associated with a three-fold higher risk for cardiovascular death or heart failure.[42]

Our findings suggest the importance of PTX3 in the pulmonary circulation-right ventricular axis. An increasingly nuanced understanding of inflammation in the pulmonary circulation and right ventricle is necessary as immunomodulators such as imatinib are trialed for diseases of the pulmonary circulation.[128] Inflammation is broadly implicated in pulmonary vascular disease and recent work confirms that PTX3 is elevated in pulmonary arterial hypertension.[116,129] Our findings

may reflect subclinical pulmonary vascular disease with decreased ability to recruit the pulmonary vasculature, leading to increased afterload, RV hypertrophy and dilation. The left ventricle undergoes similar hypertrophy even in response to mild systemic hypertension; hypertrophy in the thin walled RV may be more pronounced.[130] Unlike systemic hypertension, however, small changes in the pulmonary vasculature typically do not result in increased resting pulmonary arterial pressure due to the large flow volume reserve of the pulmonary vasculature.[131]

A direct relationship between PTX3 and RV structure independent of pulmonary vascular disease may also contribute to our findings. Myocardial inflammation can promote LV hypertrophy both in the presence and absence of pathologic afterload.[132-134] The pathophysiologic role of inflammation in determining RV structure may explain the variable susceptibility to right heart failure that exists among patients with identical afterload.[135]

While CRP and PTX3 plasma levels are both elevated in pulmonary hypertension, their relationship with the RV differs in adults without clinical cardiovascular disease. High levels of CRP are associated with less RV mass and a smaller RVEDV, which is opposite to the greater mass and larger RVEDV that we currently describe with high levels of PTX3.[51,116,136] Animal models have found that CRP potentiates and PTX3 dampens myocardial inflammation and, in patients with heart failure, rosuvastatin decreases CRP while increasing PTX3 protein levels.[114,137-139] The different relationship of CRP and PTX3 to RV structure cautiously support this paradigm of distinct and opposing biologic roles for CRP and PTX3 in cardiac pathophysiology and expands this paradigm to the RV.

Finally, we evaluated several SNPs in the PTX3 gene locus and found no association with PTX3 protein level or RV structure after accounting for race/racial stratification. Previous analyses of similar PTX3 genotypes have shown increased risk for pseudomonas in cystic fibrosis patients and increased PTX3 protein levels and primary graft dysfunction in patients undergoing lung transplant.[117,122] Both cystic fibrosis and lung transplant are pathologically inflamed states and the strong relationship of PTX3 genotype to aspects of these diseases, not seen in our largely healthy

cohort, may suggest that studied PTX3 genotypes may have a greater effect on PTX3 levels only in the setting of robust inflammation. The lack of association with previously reported genotypes may therefore reflect the lack of pathologic inflammation in the relatively healthy MESA cohort and the relevance of these genotypes only in the setting of such inflammation.

This study has limitations. As is possible in all observational studies, residual or unmeasured confounding could account for the results. Our study was cross-sectional and causality cannot be assessed. Unfortunately, measurement of invasive pulmonary hemodynamics, which may have informed the mechanism underlying our results, was not feasible in this large study of community dwelling participants free of cardiovascular disease. Finally, this cohort is reasonably healthy, leading to relatively small effect estimates. However, such estimates are similar in scale to other clinically significant associations in MESA.[55]

Summary

Higher PTX3 levels are associated with greater RV mass and larger RVEDV. These relationships are independent of left sided cardiovascular disease. This study contributes to the understanding of inflammation in the pulmonary circulation-right ventricular axis at a time when immunomodulators are being considered for use in pulmonary hypertension.

Traffic Related Air Pollution and the Right Ventricle: The Multi-Ethnic Study of Atherosclerosis

Peter J Leary, Joel D Kaufman, R. Graham Barr, David A Bluemke, Cynthia L Curl, Catherine L Hough, Joao A Lima, Adam A Szpiro, Victor C Van Hee, Steven M Kawut

Abstract

Rationale: Right heart failure is a cause of morbidity and mortality in common and rare heart and lung diseases. Exposure to traffic-related air pollution is linked to left ventricular hypertrophy, heart failure and death. The relationship between traffic-related air pollution and right ventricular (RV) structure and function is not known. The objective of this study is to characterize the relationship between traffic-related air pollutants and RV structure and function.

Methods: We included men and women with magnetic resonance imaging (MRI) assessment of RV structure and function and estimated outdoor nitrogen dioxide (NO_2) concentrations at their residence from the Multi-Ethnic Study of Atherosclerosis, a study of individuals free of clinical cardiovascular disease at baseline. Multivariable linear regression estimated associations between NO_2 exposure (averaged over the year prior to MRI) and measures of RV structure and function after adjusting for demographics, anthropometrics, smoking, diabetes mellitus and hypertension. Adjustment for the corresponding left ventricular (LV) parameter, traffic-related noise, markers of inflammation and lung disease were considered in separate models.

Measurements and Main Results: The study sample included 3,896 participants with available NO_2 predictions, RV measures, and all covariables. In fully adjusted models, higher NO_2 was associated with greater RV mass and larger RV end-diastolic volume with and without further adjustment for corresponding LV parameters, traffic-related noise, inflammatory markers, or lung disease (all $p < 0.05$). There was no association between NO_2 and RV ejection fraction.

Conclusion: Higher levels of NO_2 exposure were associated with significantly greater RV mass and larger RV end-diastolic volume.

Introduction

Right heart failure is a cause of morbidity and mortality in obstructive and restrictive lung disease, left ventricular dysfunction, and pulmonary arterial hypertension.[9,41,140] Right ventricular (RV) hypertrophy is also associated with increased risk for heart failure and cardiovascular death in community dwelling adults without known cardiac disease at baseline.[42] Despite important epidemiologic and clinical roles of the RV, little is known about modifiable determinants of RV structure and function.[141]

Traffic-related air pollution is linked to left ventricular hypertrophy, heart failure and cardiac death.[142,143] Mechanisms for effects of air pollution on the left ventricle may include inflammation, oxidative stress, and autonomic dysfunction, which could also affect the RV.[144-146] The lungs have substantial exposure to traffic-related air pollution and inhalants, which may directly increase RV afterload and lead to disproportionately greater changes in the RV compared to the left ventricle.[147,148] The impact of traffic-related air pollution on the RV, however, is not well-studied. We examined the relationship between nitrogen dioxide (NO_2), a surrogate for traffic-related air pollution, and magnetic resonance imaging (MRI) measures of RV structure and function in a multi-ethnic cohort of adults free of clinical cardiovascular disease. We hypothesized that increased exposure to traffic-related air pollution would be independently associated with greater RV mass and larger RV end-diastolic volume.

Methods

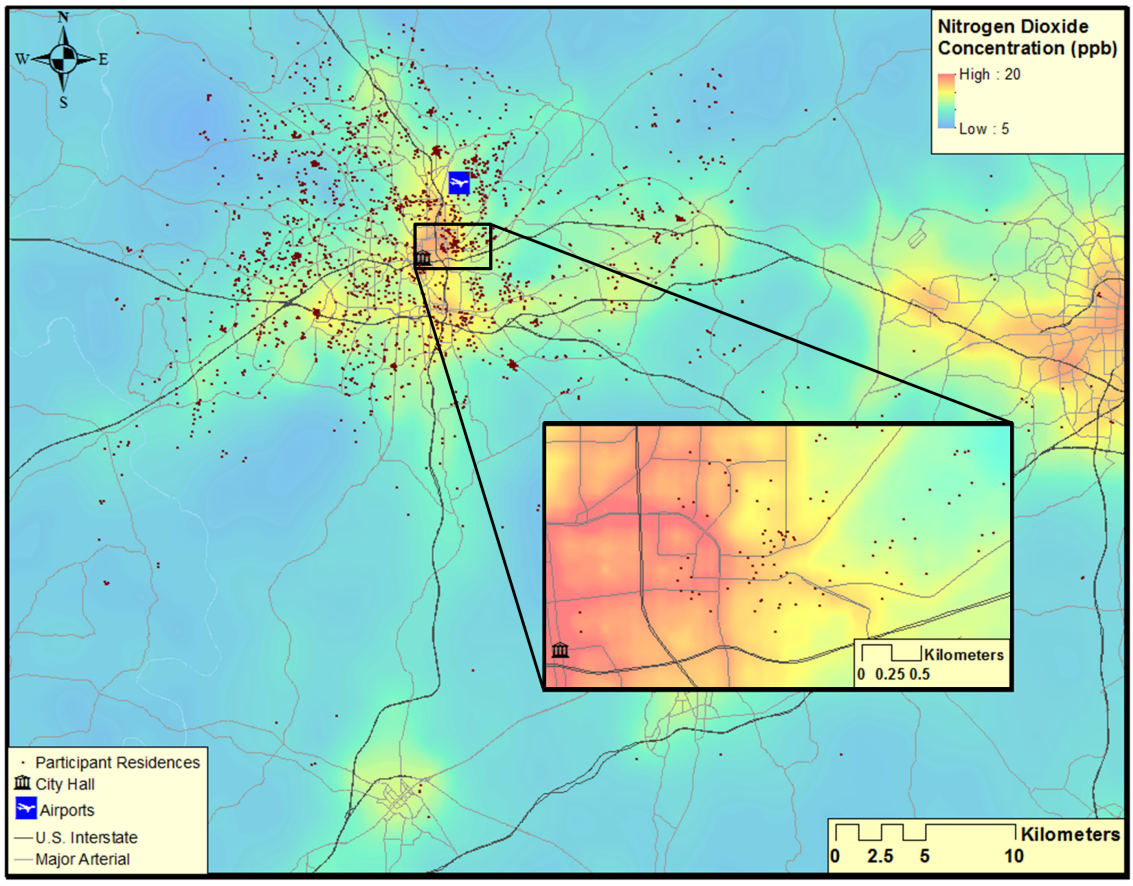
The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicenter prospective cohort study designed to investigate subclinical cardiovascular disease in whites, African-Americans, Hispanics and Chinese-Americans.[43] Exclusion criteria included clinical cardiovascular disease (physician diagnosed heart attack, stroke, transient ischemic attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure), weight >136kg (300 lbs.), pregnancy, or impediment to long-term participation. The Environmental Protection Agency funded a large

ancillary study to MESA, the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air), which added cohort-specific air pollution monitoring and modeling.[149] The MESA-RV study was an ancillary study funded to interpret cardiac MRIs for RV function. Individual participants gave informed consent and the Institutional Review Boards of participating institutions approved the protocols of MESA and all studies described herein.

Traffic-related air pollution exposure

Participants' residential address was assigned geographic coordinates using ArcGIS 9.1 software (ESRI, Redlands, CA) in conjunction with Dynamap/2000 street network and geocoding database (Tele Atlas, Boston, MA). Using weighted averages of residential addresses over the year prior to cardiac MRI, individual outdoor home exposure to NO_2 was estimated using spatio-temporal modeling and maximized via maximum likelihood (Figure 1).[150,151] Estimates were fit using monitoring data from the Environmental Protection Agencies Air Quality System database and extensive cohort-specific air monitoring conducted as part of MESA Air.[152] Geographical variables incorporated into the model included information on land use (e.g., industrial, residential), vegetative index, distance to various features (e.g., airports, coastline), population density, elevation, urban topography, emissions sources, and dispersion model outputs integrating road position, traffic volume, diurnal traffic patterns and meteorology.

Figure 1 Representative map of Winston-Salem showing coarse and fine details of Nitrogen Dioxide predictions in parts per billion (ppb) from the spatio-temporal model including approximate MESA participant locations (jittered for privacy).



Cardiac magnetic resonance imaging measures

Methods for acquisition and interpretation of LV and RV MRI parameters MRI have been previously reported.[45,119] Endocardial and epicardial borders of the RV were manually traced on short axis cine images at end-systole and end-diastole. The outflow tract was included in RV volume. Papillary muscle and trabeculae were included in RV volumes and excluded from RV mass, as is commonly done for LV mass.[47,48] RV end-systolic volume and RV end-diastolic volume (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 end-diastole

as the difference between RV free wall end-diastolic epicardial and endocardial volumes multiplied by the specific gravity of the heart (1.05g/mL). RVEF was calculated by subtracting RV end-systolic volume from RVEDV and dividing this difference by RVEDV.

Covariables

Covariables including age, race/ethnicity, height, weight, education, income, presence of hypertension or diabetes mellitus, cholesterol, smoking status and pack-years, percent emphysema (obtained by chest CT), and self-reported lung disease (asthma and/or emphysema) were measured as previously described.[24] Because levels of air pollution within a neighborhood are correlated over time, self-reported time a participant lived in the index neighborhood (the residential neighborhood used to determine one year NO_2 estimates) was used as a surrogate for exposure duration.[144] Participants reported roadway noise as a: "very serious problem", "somewhat serious problem", "minor problem", or "not really a problem."

Statistical Analysis

We used linear regression to characterize relationships between NO_2 and RV parameters. All models were adjusted for height and weight, so it was not necessary to index RV parameters to account for differences in body size. Covariables were chosen *a priori* on the basis of known associations with ventricular size, heart disease and comorbidities. In limited models, we adjusted for age, sex, race/ethnicity, height and weight. In fully adjusted models, we also included MESA field center, markers of socioeconomic status (self-reported income and education), and cardiovascular risk factors including smoking status, smoking pack-years, hypertension, diabetes mellitus, cholesterol and impaired glucose tolerance. In pre-specified models, we further adjusted for LV parameters, self-reported roadway noise, markers of inflammation (C-reactive protein and interleukin-6), or lung structure (% emphysema) and self-reported lung disease in separate models to evaluate independence from these factors.

The primary analysis examined the relationship between RV parameters and NO_2 averaged over the year prior to cardiac MRI. Sensitivity analyses used fixed-year estimates of NO_2 in 2000, 2001 and 2002 to ensure there was no artifice in timing of the MRI in relation to secular exposure trends.

Several exploratory models were performed. Duration and timing of exposure were considered using a sliding time window analysis.[153] We estimated associations between NO_2 and RV parameters in 5-year 'time windows' (e.g. participants who lived in the index neighborhood for between 1 and 6 years). The time window was then shifted by one year (e.g. participants who lived in the neighborhood between 2 and 6 years) and new estimates of association and 95% confidence intervals were calculated. Overlapping 5-year periods avoid unstable estimates based on sparse data for a single calendar year and may more appropriately characterize the biologically relevant duration of exposure. Further exploratory models evaluated whether age, gender, or study site modified the association between NO_2 and RV parameters. Analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA).

Results

There were 6,814 men and women enrolled in MESA of whom 5,098 underwent cardiac MRI and 5,004 (98%) had interpretable exams for the LV. Of 4,634 participants selected for MESA-RV, MRI reads were attempted in 4,484 participants before achieving the study goal of 4,204 participants (94% of attempted reads). Outdoor exposure to NO_2 was estimated in 4,095 of these participants (97%). One hundred ninety-nine participants were excluded for missing covariables leaving 3,896 in the study sample. Table 1 shows characteristics of the study sample compared with those excluded. The mean age of the study sample was 61.4 years and 52.6% were women. Mean RV mass in the study sample was 21.1 ± 4.4 g, mean RVEDV was 124.2 ± 30.8 mL and mean RVEF was 70.5 ± 6.4 %. Mean NO_2 was 21.8 ± 10.3 ppb with an interquartile

range (IQR) from 13.9 to 31.0 ppb (IQR 17.1 ppb). Mean NO_2 ranged from 10.1 to 32.7 ppb between cities and within city IQR ranged from 3.1 to 5.0ppb (see Figure 2).

Table 1. Characteristics of the study sample compared with the non-study subjects

	Study Sample (n=3896)	Non-Study Sample (n=2910)
Age (years)	61.4 ± 10.1	63.2 ± 10.4
Female (%)	52.6	53.2
Race (%)		
White	39.9	36.6
Chinese	12.5	10.8
African-American	25.7	30.6
Hispanic	21.9	22.0
Height (cm)	166.4 ± 9.9	166.3 ± 10.2
Weight (kg)	77.3 ± 16.2	80.3 ± 18.6
Body mass index (kg/m^2)	27.8 ± 5.0	29.0 ± 6.0
Educational attainment (%)		
No high school degree	15.8	21.1
High school degree	18.1	18.4
Some college	16.1	16.6
Bachelor's Degree	18.6	15.5
Higher than bachelor's degree	19.1	16.6
Cigarette smoking status (%)		
Never	52.6	47.2
Former	35.1	38.7
Current	12.3	14.1
Hypertension (%)	42.6	48.3
Systolic blood pressure (mmHg)	125.3 ± 20.9	128.3 ± 22.1
Diabetes mellitus (%)	12.3	15.3
Stable residential neighborhood (%)		
> 5 years	79.7	76.1

> 10 years	63.7	61.9
NO_2 (ppb)	22.6 ± 10.3	22.2 ± 9.2*

Abbreviations: NO_2=nitrogen dioxide, cm=centimeters, kg=kilograms, m^2=meters squared, mmHg=millimeters of mercury, ppb=parts per billion

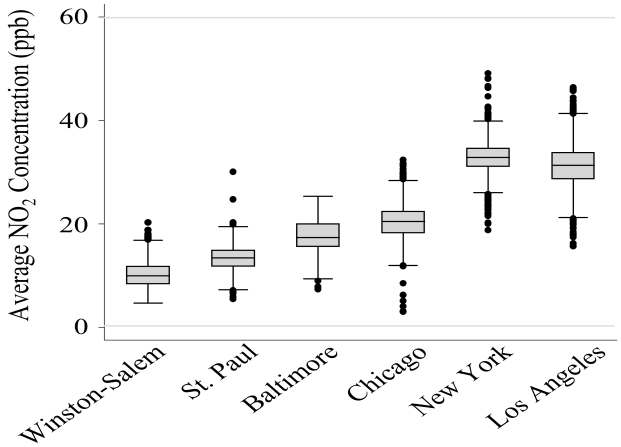


Figure 2. Box plot of between and within city gradients of nitrogen dioxide (NO_2) in parts per billion (ppb) for participants in the year prior to their MRI in the Multi-Ethnic Study of Atherosclerosis and Air Pollution.

Higher NO_2 was associated with greater RV mass (0.4 g for an interquartile increase in NO_2)(Table 2, Figure 3). This relationship became stronger after adjustment for city (0.9 g for an interquartile increase in NO_2) and after full adjustment for cardiovascular risk factors (1.0 g for an interquartile increase in NO_2). This amounted to ~5% increase in RV mass per interquartile increase in NO_2. This significant association did not change with further adjustment for LV mass, traffic-related noise, inflammatory markers, or lung disease (see Table 2 & 3).

Table 2. Difference in right ventricular morphology in groups of differing NO_2 exposure

Model	per interquartile increase in NO_2

	difference	95% CI	p-value
RV mass, g (Limited model*)	0.4	0.2, 0.7	<0.001
RV mass, g (Limited model + city)	0.9	0.3, 1.4	0.002
RV mass, g (Full Model†)	1.0	0.4, 1.5	0.001
RV mass, g (Full Model† + LV mass)	0.9	0.3, 1.4	0.001
RVEDV, mL (Limited model*)	2.9	1.4, 4.7	<0.001
RVEDV, mL (Limited model + city)	2.7	-0.9, 6.2	0.14
RVEDV, mL (Full Model†)	4.1	0.5, 7.7	0.03
RVEDV, mL (Full Model† + LVEDV)	2.7	0.0, 5.4	0.05
RVEF, % (Limited model*)	-0.1	-0.5, 0.4	0.80
RVEF, % (Limited model + city)	-0.2	-1.2, 0.8	0.69
RVEF, % (Full Model†)	-0.2	-1.2, 0.8	0.72
RVEF, % (Full Model† + LVEF)	0.0	-1.0, 0.9	0.92

Abbreviations: NO_2=nitrogen dioxide, CI=confidence interval, RV=right ventricle, LV=left ventricle, EDV=end-diastolic volume, and EF=ejection fraction

**Limited model: age, gender, race/ethnicity, height and weight*

†Full model: Limited + city, education, income, smoking, pack-years, hypertension, diabetes, cholesterol, and impaired glucose tolerance

Table 3. Difference in right ventricular mass in groups of differing NO_2 exposure adjusting for differences in roadway noise, inflammation, and lung disease

Model	per interquartile increase in NO_2

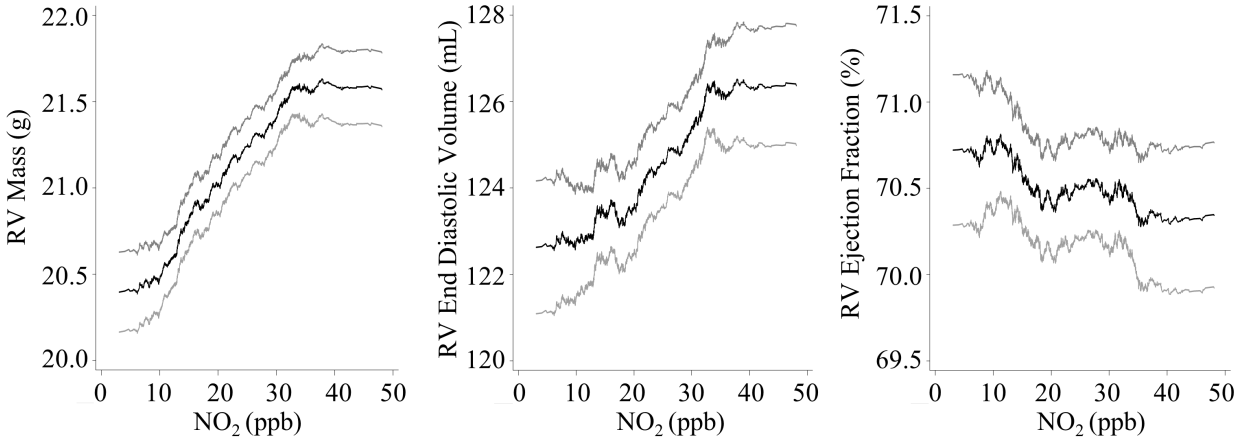
	difference	95% CI	p-value
RV Mass, g, Full Model (n=3,896)	**1.0**	**0.4, 1.5**	**0.001**
Full + roadway noise (n=3,890)	1.0	0.4, 1.5	0.001
Full + CRP and IL-6 (n=3,797)	1.1	0.5, 1.6	<0.001
Full + % emphysema (Chest CT) & self reported asthma or emphysema (n=3,892)	0.9	0.4, 1.5	0.001
RVEDV, mL, Full Model (n=3,905)	**4.1**	**0.5, 7.7**	**0.03**
Full + roadway noise (n=3,890)	3.9	0.3, 7.6	0.04
Full + CRP and IL-6 (n=3,797)	4.2	0.5, 7.8	0.03
Full + % emphysema (Chest CT) & self reported asthma or emphysema (n=3,892)	3.7	0.1, 7.3	0.04

Abbreviations: NO_2=nitrogen dioxide, RV=right ventricle, CI=confidence interval, CRP=c-reactive protein, IL-6=interleukin-6

**Full model: age, gender, race/ethnicity, height and weight, city, education, income, smoking, pack-years, hypertension, diabetes, cholesterol, and impaired glucose tolerance*

Higher NO_2 was associated with larger RVEDV (2.9 mL for an interquartile increase in NO_2)(Table 2, Figure 3). This relationship became stronger after full adjustment for potential confounding by cardiovascular risk factors (4.1 mL for an interquartile increase in NO_2). This amounted to ~3% increase in RVEDV per interquartile increase in NO_2. The significant association remained with further adjustment for LV end-diastolic volume, traffic-related noise, inflammatory markers, or lung disease (see Table 2 & E1 in the online supplement). NO_2 was not associated with RVEF (Table 2, Figure 3).

Figure 3. Multivariable non-parametric smoothed relationship between nitrogen dioxide (NO_2) in parts per billion (ppb) and right ventricular (RV) parameters including covariables in the full model. Grey lines represent 95% confidence bounds.



For participants with residential stability estimates (3,892 of 3,905 participants), sliding time window analyses indicated that participants who lived in the neighborhood several years before the MRI had incrementally stronger associations between NO_2 and RV mass then did those in lived in the neighborhood for a shorter duration (Figure 4 and Table 4). An incremental increase in RVEDV with participant duration in the neighborhood was less clear (Figure 4 and Table 4). Choice of the NO_2 reference period (calendar year 2000, 2001 or 2002) did not meaningfully impact the relationship between NO_2 and RV parameters.

Figure 4. The relationship between the number of years a participant lived in their neighborhood and the change in right ventricular (RV) mass or end diastolic volume (EDV) per interquartile increase in nitrogen dioxide (NO_2): a sliding time window analysis of the full model (* $p \leq 0.05$,

** $p \leq 0.01$).

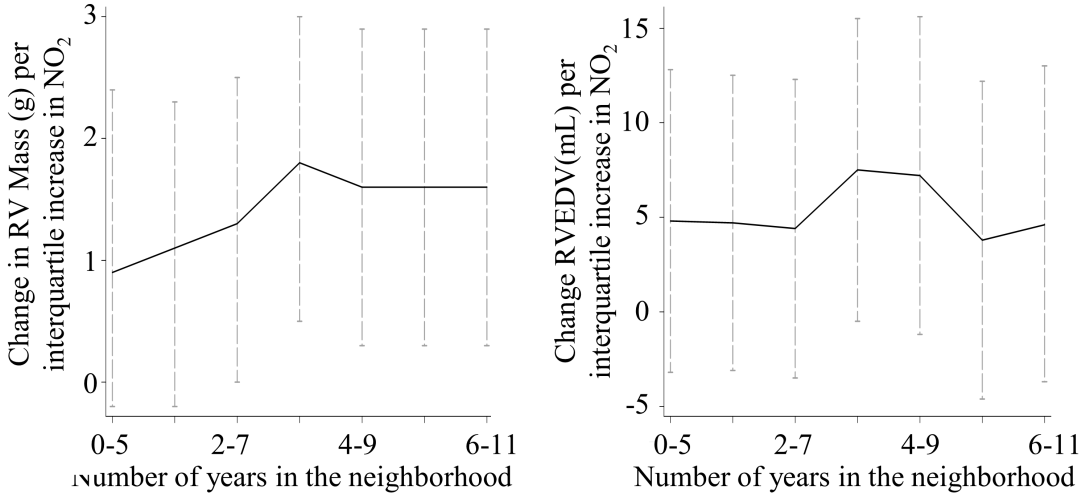


Table 4. Residential stability as a proxy for the timing of exposure to traffic related air pollution: a sliding time window analysis of the full model*

Number of years lived in the same neighborhood	n	RV mass (g) per interquartile increase in NO2	95% CI	p-value
0 to 5 years	785	1.0	(-0.2, 2.4)	0.09

1 to 6 years	834	1.1	(-0.2, 2.3)	0.09
2 to 7 years	848	1.3	(0.0, 2.5)	0.04
3 to 8 years	816	1.8	(0.5, 3.0)	0.005
4 to 9 years	743	1.6	(0.3, 2.9)	0.02
5 to 10 years	773	1.6	(0.3, 2.9)	0.02
6 to 11 years	727	1.6	(0.3, 2.9)	0.01
All years (full model)	**3,896**	**1.0**	**(0.4, 1.5)**	**0.001**
	n	RVEDV (mL)	95% CI	p-value
0 to 5 years	785	4.8	(-3.2, 12.8)	0.24
1 to 6 years	834	4.7	(-3.1, 12.5)	0.24
2 to 7 years	848	4.4	(-3.5, 12.3)	0.28
3 to 8 years	816	7.5	(-0.5, 15.5)	0.07
4 to 9 years	743	7.2	(-1.2, 15.6)	0.10
5 to 10 years	773	3.8	(-4.6, 12.2)	0.37
6 to 11 years	727	4.6	(-3.7, 13.0)	0.28
All years (full model)	**3,896**	**4.1**	**(0.5, 7.7)**	**0.03**

Abbreviation: RV=right ventricle, NO_2=nitrogen dioxide, CI=confidence interval, EDV=end-diastolic volume
** Full model: age, gender, race/ethnicity, height and weight, city, education, income, smoking, pack-years, hypertension, diabetes, cholesterol, and impaired glucose tolerance*

Participant age did not meaningfully modify relationships between NO_2 and RV parameters. The relationships of NO_2 with RV mass and EDV may have been stronger in men (1.3 g, 95% CI: 0.4 to 2.2 g; 5.5 mL, 95% CI: -0.3 to 11.2 mL per interquartile increase in NO_2) then women (0.6 g, 95% CI: -0.1 to 1.3 g; 2.1 mL, 95% CI: -2.2 to 6.5 mL per interquartile increase in NO_2)(interaction p-values: 0.03 for RV mass and 0.04 for RVEDV). Participant city modified the relationship between NO_2 and RV mass, but not RVEDV (interaction p-values: <0.001 and 0.33 respectively). Qualitative associations between NO_2 and RV mass were in the same direction as the main association in St. Paul (6.4 g per interquartile increase in NO_2, 95% CI: 4.1 to 8.8 g), Los Angeles (0.9 g per interquartile increase in NO_2, 95% CI: -0.1 to 1.9 g), Baltimore (0.4 g per interquartile increase in NO_2, 95% CI: -1.2 to 1.9 g) and Chicago (0.3 g per interquartile increase in NO_2, 95% CI: -1.0 to 1.6 g), with weaker findings in New York (-0.2 g per interquartile increase

in NO_2, 95% CI: -1.5 to 1.1 g) and Winston-Salem (-0.4 g per interquartile increase in NO_2, 95% CI: -2.6 to 1.8 g). Because of large estimates of association for St. Paul, we then excluded participants in cities with the greatest (St. Paul) and smallest (Winston-Salem) estimates of association between NO_2 and RV mass. The estimate of association in this four–city sample was smaller but qualitatively similar to the main analysis (0.5 g increase per interquartile increase in NO_2, 95% CI -0.1 to 1.1 g, n=2738). Restricting this four-city sample to the sliding time window with the strongest association strengthened the relationship (1.3 g increase per interquartile increase in NO_2, 95% CI -0.1 to 2.7 g, n=476).

Discussion

We have shown that higher estimates of long-term outdoor residential NO_2 exposure are associated with greater RV mass and larger RVEDV in a multiethnic, multicity cohort of adults without clinical cardiovascular disease. MESA participants had a 1.0 g (5%) increase in RV mass and 4.1 mL (3%) increase in RVEDV per interquartile increase in NO_2. This difference in RV mass is quantitatively similar to that seen in LV mass in MESA participants with and without diabetes (2.4%) and in current smokers (5.3%), supporting potential clinical and biologic relevance.[55,154] In addition, RV hypertrophy in MESA participants is associated with a three-fold increased risk for heart failure or cardiovascular death[42]. This is the first report to suggest traffic-related air pollutants, of which NO_2 is a well-recognized surrogate for the pollutant mix, is associated with morphologic changes in the right ventricle of the heart.

Our study provides initial insight into timing of this association. Duration of exposure to traffic-related air pollutants appears to be important. Participants who lived in the same neighborhood for several years had the strongest associations between NO_2 and RV mass. This suggests a dose-response, may provide insight for duration of necessary exposure, and supports a causal relationship.

The finding of both increased RV mass and RVEDV may suggest the exposure of interest increased RV afterload.[155] Previous studies have suggested air pollution increases endothelin-1, a potent pulmonary vasoconstrictor[156], which could lead to increased pulmonary vascular resistance, increased RV afterload and ultimately RV hypertrophy and dilation. Alternatively, air pollutants can irritate the respiratory epithelium and lead to heterogeneous ventilation with decreased regional ventilation.[157] Regional hypoxia can cause hypoxic pulmonary vasoconstriction, increased resistance and RV enlargement.[158] Increases in afterload may compound oxidative stress and autonomic dysfunction, which have been implicated in the relationship between air pollution and left ventricular mass and could directly contribute to right ventricular pathology.[14,144-146]

Other mechanisms are possible as well. Air pollution may up-regulate myocardial inflammatory genes and proteins in the right ventricle.[159] While it is not feasible to study myocardial gene and protein profiles in such a large study of the general population, our findings remained after adjustment for C-reactive protein and interleukin-6 blood levels, which suggests our findings may be independent of systemic inflammation. Roadway noise, which accompanies traffic-related air pollution and may disrupt sleep, could mediate some aspects of the relationship between roadway proximity and heart disease.[160] Adjusting for traffic-related noise did not attenuate relationships between NO_2 and RV morphology in our analyses.

Air pollution has also been linked to obstructive lung disease severity, which could increase RV afterload leading to RV hypertrophy.[161,162] However, we have previously shown that increasing airflow obstruction is associated with decreased RVEDV in MESA.[163] In addition, adjustment for structural or self-reported lung disease did not change relationships between NO_2 and RV morphology in this analysis. Finally, LV mass may increase with traffic-related air pollution and LV hypertrophy can contribute to diastolic dysfunction and increased RV afterload, potentially explaining our results[142,164]. We found no difference in relationships between NO_2 and the RV when adjusting for LV parameters (e.g. comparing participants of similar LV mass or volume).

We found that associations between NO_2 and RV mass was modified by city of residence and gender. Heterogeneity by city is very common in air pollution research and was also seen in studies of LV mass and endothelial dysfunction.[142,165] Two factors that may contribute to heterogeneity include: variability in the relative contribution of an individuals outdoor residential exposure to their total air pollution exposure and variation in the composition of traffic-related air pollution. Validity of outdoor assessment of NO_2 as a surrogate for individual exposure to traffic-related pollutants depends on the degree to which outdoor pollution contributes to indoor pollution (e.g. home infiltration coefficient, indoor sources) and the proportion of time that a participant spends indoors, outdoors, and in different micro-environments.[166] These complex relationships vary between cities as a function of culture, climate, cooking/ventilation patterns, and average building age, among other factors. Second, our estimates of NO_2 are best conceptualized as a pattern of spatial decay consistent with some but not all traffic-related pollutants. For example, participants' exposure to NO_2 also reflects exposure to other hazardous air pollutants such as benzene and several volatile organic compounds, relative levels of which may vary by city.[167]

This study has limitations. While we consider our exposure models to be a significant improvement over roadway proximity and nearest monitor analyses, measurement error and misclassification are likely present. Because error in exposure assignments is unlikely to be dependent on RV measurements, these errors may be non-differential with bias toward the null, so that the actual relationships may even be stronger than we have shown. In addition, residual or unmeasured confounding, particularly at the neighborhood level, could contribute to the results. Similarly, our study was cross-sectional and causality cannot be confirmed. Finally, measurement of invasive pulmonary hemodynamics, which may have informed the mechanism underlying our results, was not feasible in almost 4,000 community dwelling participants free of cardiovascular disease.

Summary

Higher estimated exposure to NO_2 is associated with greater RV mass and larger RVEDV. This relationship is independent of markers of socioeconomic status, cardiovascular risk factors, left sided cardiovascular disease, markers of inflammation and lung disease. This is the first report to implicate traffic-related air pollution with changes in right ventricular morphology. Air pollution may therefore play a role determining the RV response and outcomes in cardiopulmonary disease.

Von Willebrand Factor and the Right Ventricle: the MESA-Right Ventricle Study

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Abstract

Elevation in plasma activity of von Willebrand Factor (vWF) reflects endothelial dysfunction and predicts death in pulmonary arterial hypertension (PAH). Higher vWF activity is also associated with lower right ventricular (RV) ejection fraction in PAH. Little is known about the relationship between vWF and RV structure and function in adults without cardiovascular disease. In the current investigation, we included 1,976 participants with MRI assessment of RV structure and function and measurement of vWF activity from the Multi-Ethnic Study of Atherosclerosis. Multivariable linear regression was used to estimate the associations between vWF activity and measures of RV structure and function after adjusting for demographics, anthropometrics, smoking, diabetes mellitus, hypertension and the corresponding left ventricular (LV) parameter. The average vWF activity was 140.7 ± 57.2%. Elevated vWF activity was independently associated with lower RV mass, RV end-diastolic volume and RV stroke volume in models with and without adjustment for the corresponding LV parameter (all $p < 0.05$). There was no association observed between vWF activity and RV ejection fraction. In conclusion, higher vWF activity is associated with lower RV mass, RV end-diastolic volume and RV stroke volume. These associations are independent of common cardiovascular risk factors and LV morphologic changes.

Introduction

Von Willebrand Factor (vWF) is principally released by endothelial cells and mediates the initiation and progression of thrombus formation via platelet aggregation at a site of vascular injury.[168] vWF reflects endothelial dysfunction which can both influence and be affected by left ventricular (LV) dysfunction.[169,170] Elevated vWF predicts poor prognosis in several diseases including heart failure,[171] hypertension[172] and coronary artery disease.[168] Prior work by our group has suggested an independent association between vWF and mortality in patients with pulmonary arterial hypertension (PAH).[173] We have also shown that elevated vWF activity is associated with lower right ventricular ejection fraction (RVEF) in patients with PAH.[174] As a marker of endothelial dysfunction, vWF may reflect pulmonary vascular disease severity. However, the relationship between vWF and RV structure and function has not been studied in adults free of cardiovascular disease. A better understanding of the relationship of vWF with RV structure and function in adults without pulmonary hypertension may help to clarify the cardiac role of vWF in disease. We examined the relationship of vWF and measures of RV structure and function in a multiethnic cohort of adults free of clinical cardiovascular disease. We hypothesized that elevated vWF activity would be associated with greater RV mass, larger end-diastolic volume, and decreased RV ejection fraction.

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicenter prospective cohort study designed to investigate the prevalence, correlates and progression of subclinical cardiovascular disease in whites, African-Americans, Hispanics and Chinese-Americans. In 2000–2002, MESA recruited 6814 men and women aged 45–84 years old from 6 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 diagnosed heart attack, stroke, transient ischemic

attack, heart failure, angina, current atrial fibrillation, any cardiovascular procedure), weight >136kg (300 lbs.), pregnancy, or impediment to long-term participation. The Institutional Review Boards of all participating institutions and the National Heart Lung and Blood Institute approved the protocols of MESA and all studies described herein. The MESA-Right Ventricle Study, which includes the present investigation, is an ancillary study focused on RV morphology in MESA participants eligible for MRI at the baseline examination.

The cardiac MRI protocol has been previously described[44] and methods for interpretation of LV and RV parameters have been previously reported.[119] Cardiac MRI examinations were transmitted to the reading center at Johns Hopkins University in Baltimore, MD, and image analysis was performed by 2 independent analysts using QMASS software (Medis, Leiden, the Netherlands). Images were magnified to 250%, contrast/brightness set to 55, and window width/level were set with the QMASS auto function.

Endocardial and epicardial borders of the RV were manually traced on short axis cine images at end-systole and end-diastole. Correct placement of RV contours relied on evaluation of cine images to determine the demarcation between the right atrium and the RV. Contours were modified at basal cardiac slices by careful identification of the tricuspid valve so as to exclude the right atrium and to avoid over-estimation of the volumes. The outflow tract was included in RV volume. Papillary muscle and trabeculae were included in the RV volumes and excluded from RV mass, as is commonly done for LV mass. RV end-systolic volume and RV end-diastolic volume (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 end-diastole as the difference between epicardial and endocardial volumes of the RV free wall multiplied by the specific gravity of the heart (1.05g.mL). RV stroke volume (RVSV) was calculated by subtracting RV end-systolic volume from the RVEDV. RVEF was calculated by dividing RVSV by RVEDV. The intra-reader intra-class correlation coefficient for RV mass was 0.94 (229 scans), RVEDV was 0.99 (230 scans) and RVEF was 0.89 (230 scans). The blinded inter-reader intra-class

correlation coefficients on 240 scans for RV mass, RVEDV and RVEF were 0.89, 0.96 and 0.80 respectively.

Fasting blood samples were drawn, processed and stored using standardized procedures.[120] The MESA parent study originally measured baseline vWF in 1,000 participants of whom 733 also had RV data from cardiac MRI. We selected 1290 additional participants with sufficient plasma and available RV data for measurement of vWF. vWF activity was measured in the Laboratory for Clinical Biochemistry Research at University of Vermont (Burlington, VT, USA). vWF activity was measured by the Liatest (latex immunoturbidometric) assay on the STA analyzer (Diagnostica Stago, Parsippany, NJ). In this assay the degree of agglutination (measured by light absorption) between vWF in the sample and vWF monoclonal antibody in the assay is directly proportional to vWF activity. The intra-assay and inter-class coefficient of variation was 3.7 and 4.5% respectively.

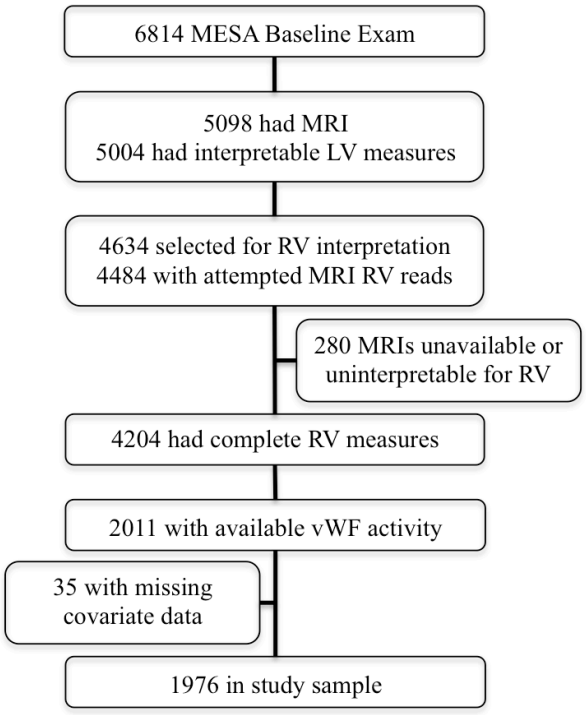
Race/ethnicity was self-reported during the baseline MESA examination according to 2000 US Census criteria. Height was measured to the nearest 0.1 cm with the subject in stocking feet and weight was measured to the nearest pound with the subject in light clothing using a balanced scale. Hypertension was accounted for with systolic blood pressure (mmHg) and current use of anti-hypertension medication. Presence of diabetes mellitus was based on self-reported physician diagnosis, use of medication for hyperglycemia, or fasting glucose value ≥126 mg dl^{-1}, 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 and computed tomographic (CT) lung density measures of % emphysema were available for a subset of participants.[175] Flow-mediated dilation (FMD) as a marker of systemic endothelial dysfunction has been previously described.[176] The brachial artery was occluded by a sphygmomanometer at a pressure of 50mmHg above systolic blood pressure for 5 minutes. Maximal brachial artery dilation over the last 2 minutes of this occlusion was recorded. Percent FMD (%FMD) was the maximum brachial artery diameter minus

the baseline diameter divided by the baseline diameter expressed as a percentage. The MESA Typical Week Physical Activity Survey (TWPAS) had been previously described and includes intentional exercise as the sum of metabolic equivalents (METS) from activities that were consciously done for exercise. Intentional exercise was most strongly associated with LV structure and function.[177]

We used linear regression to characterize the relationship between vWF and RV parameters. Covariates were chosen on the basis of known associations with ventricular size, heart disease and comorbidities. In limited models, we adjusted for age, sex, race/ethnicity, height and weight and study site. Adjustment for height and weight 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. In fully adjusted models we included smoking, hypertension, diabetes mellitus, cholesterol, impaired glucose tolerance, systolic blood pressure, treatment for hypertension, and CRP. We then adjusted the RV mass analysis by LV mass, the RVEDV analysis by LVEDV and the RVEF analysis by LVEF to evaluate for independence from LV abnormalities (e.g. increased LV mass causing pulmonary venous hypertension leading to increased RV mass) and to better account for differences in body size. Because RVSV and LV stroke volume are interdependent, we adjusted for LV mass in this case. In separate analyses, we adjusted for intentional exercise, lung function (forced expiratory volume at 1 second (FEV1), forced vital capacity (FVC) and % emphysema on chest CT)(n=1,826) and % FMD (n=1,620). Generalized additive models were used to assess possible non-linearity in the associations between vWF and RV parameters. Statistical significance was defined as $p<0.05$. Analyses were performed using STATA 11.0 (StataCorp, College Station, TX, USA). We anticipated 99% power to detect a difference of 0.3 standard deviations in RV mass, volumes and ejection fraction from the top quintile to the bottom quintile of vWF in the full sample.

Results

Figure 1. Study Sample.



There were 6814 men and women enrolled in MESA (Figure 1). Of them, 5098 underwent cardiac MRI and 5004 (98%) had interpretable exams for the LV. We selected 4634 scans and attempted reads in 4484 scans before reaching our goal of 4204 participants with successful RV interpretation (94% of 4484 attempted). vWF was available for 2011 of these participants. Thirty-five participants were excluded for missing covariates leaving 1976 in the study sample. Table 1 shows characteristics of the study sample compared with those excluded. The mean age was 60.2 years and 47.2% were men. As compared with MESA participants not included in the analysis, the study sample was slightly younger with a slightly lower prevalence of hypertension and diabetes. Mean RV mass in the sample was 21.4 ± 4.4 g, the mean RVEDV was 127.2 ± 31.5mL and mean RVSV was 88.7 ± 20.9mL. The mean RVEF was 70.2 ± 6.6%. All analyses were adjusted for height and weight (accounting for differences in body size), so that indexing to certain measures (e.g., body surface area) was not necessary and was not performed.

Table 1. Characteristics of the study sample compared with excluded MESA subjects

Variable	Study Sample (n=1976)	Excluded (n=4838)
Age (years)	60.2±9.9	62.9±10.3
Male	47.2%	47.1%
Race/Ethnicity		
White	43.3%	36.5%
Chinese	10.9%	12.1%
African-American	25.6%	28.6%
Hispanic	20.1%	22.7%
Educational attainment		
No high school degree	14.5%	19.5%
High school degree	16.2%	19.0%
Some college	16.6%	16.2%
Bachelor's Degree	19.7%	16.2%
Higher than bachelor's degree	22.0%	16.4%
Height (cm)	167.0±10.1	166.1±10.0
Weight (kg)	77.8±16.6	79.0±17.6
Body mass index (kg/m^2)	27.8±5.0	28.6±5.7
Cigarette smoking status		
Never	52.1%	49.6%
Former	35.2%	37.2%
Current	12.7%	13.2%
Hypertension	40.6%	46.6%
Systolic blood pressure (mmHg)	123.6±20.2	127.8±21.9
Diastolic blood pressure (mmHg)	71.7±10.1	72.0±10.3
Diabetes mellitus	10.4%	14.9%
vWF activity	140.7±57.2%	145.2±57.5%*

cm indicates centimeters; kg, kilogram; m^2, meters squared; mmHg, millimeters of mercury; and vWF, von Willebrand Factor

**309 subjects with measured vWF not included in the study sample because of missing MRI or covariates*

vWF was independently associated with RV mass (Table 2, Figure 2). Higher vWF was associated with lower RV mass. This relationship was not meaningfully altered by adjustment for cardiovascular risk factors or LV mass. Adjustment for intentional exercise, %FMD (n=1620) (as a proxy for systemic endothelial function) or FEV1, FVC and radiographic % emphysema (n=1826) did not alter the relationship between vWF and RV mass in participants with these measures (Table 3).

Table 2. Multivariable linear regression for von Willebrand Factor activity and right ventricular parameters

Right Ventricular Parameter	Per 50% increase in vWF activity		
	Coefficient	(95% CI)	p-value
Mass (g)			
Limited model	-0.2	(-0.4 to -0.1)	<0.001
Adjusted model	-0.2	(-0.4 to -0.1)	<0.001
Adjusted model + LV mass	-0.2	(-0.4 to -0.1)	<0.001
End-diastolic volume (mL)			
Limited model	-1.2	(-2.1 to -0.4)	0.005
Adjusted model	-1.2	(-2.1 to -0.4)	0.005
Adjusted model + LV end-diastolic volume	-0.7	(-1.3 to -0.1)	0.03
Stroke volume (mL)			
Limited model	-0.8	(-1.4 to -0.1)	0.02
Adjusted model	-0.7	(-1.4 to -0.1)	0.02
Adjusted model + LV mass	-0.6	(-1.2 to 0.0)	0.04
Ejection fraction (%)			
Limited model	0.1%	(-0.1 to 0.3)	0.4
Adjusted model	0.1%	(-0.1 to 0.4)	0.4
Adjusted model + LV ejection fraction	0.1%	(-0.1 to 0.3)	0.4

The limited model included age, gender, race/ethnicity, study site, height and weight. The adjusted model included the covariables in the limited model plus education, smoking, systolic blood pressure, treatment for hypertension, diabetes mellitus, cholesterol, impaired glucose tolerance, and c-reactive protein.

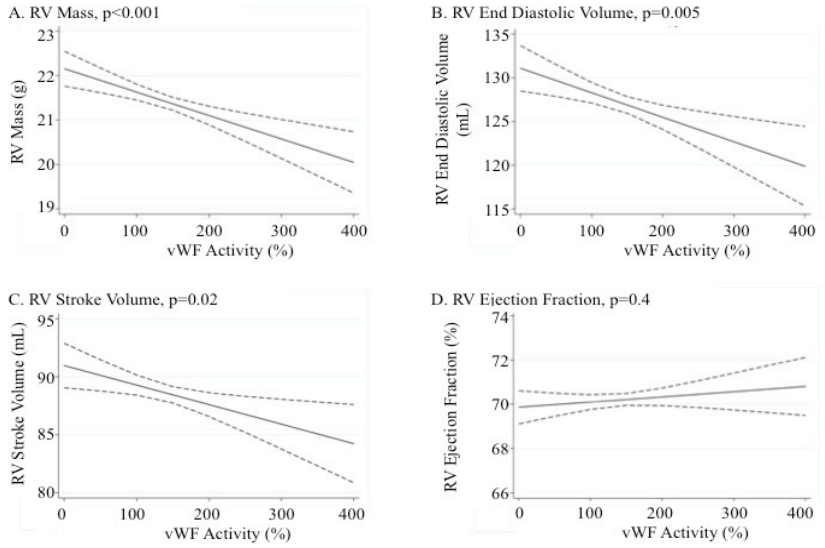


Figure 2. The association between vWF and RV parameters. Linear regression adjusted for age, gender, race/ethnicity, study site, height, weight, education, smoking, systolic blood pressure, treatment for hypertension, diabetes mellitus, cholesterol, impaired glucose tolerance, and c-reactive protein.

Table 3. Multivariable linear regression for von Willebrand Factor and right ventricular parameters accounting for differences in lung function, systemic endothelial dysfunction and exercise

Right ventricular parameter	Per 50% increase in vWF activity		
	Coefficient	(95% CI)	p-value
Adjusted for lung function			
Mass (g)	-0.2	(-0.4, -0.1)	<0.001
End-diastolic volume (mL)	-1.1	(-2.0, -0.2)	0.01
Stroke volume (mL)	-0.7	(-1.3, -0.1)	0.04
Ejection fraction (%)	0.2%	(-0.1, 0.4)	0.2
Adjusted for systemic endothelial dysfunction			
Mass (g)	-0.3	(-0.4, -0.1)	<0.001
End-diastolic volume (mL)	-1.4	(-2.3, -0.4)	0.006
Stroke volume (mL)	-0.8	(-1.5, -0.1)	0.03
Ejection fraction (%)	0.1%	(-0.2, 0.4)	0.5
Adjusted for Exercise			
Mass (g)	-0.2	(-0.4, -0.1)	<0.001
End-diastolic volume (mL)	-1.2	(-2.0, -0.3)	0.007
Stroke volume (mL)	-0.7	(-1.3, -0.1)	0.03
Ejection fraction (%)	0.1%	(-0.1, 0.4)	0.4

Lung function adjustment: adjusted model+ forced expiratory volume at 1 second, forced vital capacity and % emphysema on CT
Systemic endothelial dysfunction adjustment: adjusted model+ flow mediated dilation of the brachial artery
Exercise adjustment: adjusted model+ intentional exercise (METS)

Higher vWF was associated with lower RVEDV (Table 2, Figure 2). This relationship was unchanged after adjustment for cardiovascular risk factors and diminished but remained significant after adjustment for LVEDV. Adjustment for intentional exercise, systemic endothelial dysfunction or lung function did not alter or attenuate the inverse relationship between vWF and RVEDV (Table 3).

vWF was independently associated with RVSV (Table 2, Figure 2). Higher vWF was associated with a lower RVSV. This relationship was unchanged after adjustment for cardiovascular risk factors. The relationship persisted after adjustment for LV mass. Adjustment for intentional exercise, systemic endothelial dysfunction or lung function did not alter or attenuate the inverse relationship between vWF and RVEDV (Table 3).

Elevated vWF activity was not associated with RVEF (Table 2, Figure 2). Adjustment for cardiovascular risk factors, intentional exercise, systemic endothelial dysfunction and lung function did not change this result (Table 3). There was no effect modification between vWF and age, gender, race or respective LV parameters (all $p>0.25$). All analyses were repeated using generalized additive models. No significant non-linearity was detected (all $p>0.3$).

Discussion

We have shown that elevated vWF activity is associated with lower RV mass, RVEDV, and RVSV. This finding is opposite from that reported for the left ventricle. Previous publications have reported that elevated vWF and systemic endothelial dysfunction are associated with increased LV volumes and mass.[178] Our finding of the inverse relationship in the RV reinforces the limited ability to extrapolate findings from left to right ventricles.

The association between vWF and LV hypertrophy is likely mediated by the strong correlation between vWF and hypertension.[179] We found instead that the RV has smaller volumes and lower mass with increasing vWF. vWF was recently described as a potent angiogenesis inhibitor and our findings may represent a paucity of angiogenesis with impaired blood flow to active myocardium and restricted RV growth.[180] Alternatively, elevated vWF activity may indicate coronary endothelial dysfunction accompanied by the inability of coronaries to dilate and provide increased blood flow to support cardiac growth.[168,181] Inhibiting angiogenesis and/or coronary dysfunction leads to decreased cardiac mass and volumes in otherwise healthy animal models.[182]

If elevated vWF activity does reflect the inability to augment blood flow in active cardiac muscle, this may contribute to the increased mortality we previously described in patients with pulmonary hypertension and elevated vWF activity.[173,174] Increased perfusion is part of normal myocardial growth and imbalance between myocyte growth and perfusion leads to heart failure.[183] Abnormal RV perfusion reserve in diseases with pathologic afterload leads to weaker contraction, frank ischemia and chamber dilation such as that seen in the failing RV of advanced PAH.[182,184]

Recent work has further reinforced the importance of angiogenesis in cardiac disease. Animal studies have demonstrated that estrogen-dependent angiogenesis rescues the failing RV in rats with PAH.[185] Further study is necessary to test the role of vWF in controlling RV angiogenesis; however, the question about myocardial perfusion reserve in RV structure and function raised by our current findings is both interesting and may be clinically relevant in diseases characterized by increased RV afterload.

While we believe that restricted cardiac growth may be the most plausible explanation for a smaller RV along a gradient of increasing vWF, it is not the only possibility. One report does suggest that vWF stimulates platelet-mediated production of nitric oxide, which could decrease pulmonary vascular resistance, pulmonary artery pressure, and RV workload[186]; however, this report conflicts with others that suggest increased vWF is associated with decreased nitric oxide.[187]

Alternatively, because our study was cross-sectional, reverse causation could explain the findings. Diastolic dysfunction of the LV, with smaller than expected volumes, has been hypothesized to lead to diminished blood flow with subsequent systemic endothelial dysfunction and vWF elevation.[188] While our association of higher vWF with smaller RVEDV (independent of LVEDV) could suggest impaired RV relaxation, we did not measure RV diastolic function directly.

In MESA as in other studies, vWF is associated with structural and morphologic changes in the LV.[178,189] While other studies suggest increased LV mass with elevated vWF, decreased LV mass was seen in the MESA cohort.[189] In comparison with other studies MESA was notable for a lower severity of systemic hypertension and may have had less confounding by afterload. Impaired angiogenesis with increased vWF would be thought to affect both ventricles, however, while associations between vWF and RV parameters were weakened after adjustment for LV structure and function, these associations persisted. This may reflect a shared bi-ventricular mechanism but a disproportionate effect on the RV perhaps due to the presence of systemic hypertension but lack of pulmonary hypertension. Alternatively adjustment by LV parameters

may reduce residual confounding by body size.[46] Adjustment for intentional exercise, which has previously been associated separately with cardiac hypertrophy and decreased vWF, had no effect on our observed associations.[177,190] Finally, adjustment for systemic endothelial dysfunction (FMD%) and lung structure and function had no effect on the observed inverse association between vWF and RV mass and volumes, suggesting that systemic vascular dysfunction and pulmonary dysfunction may not confound our findings.

There was no association between vWF and RVEF. This may represent the absence of a relationship between vWF and contractile function. Alternatively, ejection fraction is a load-dependent measure of contractile function.[191] Therefore, RVEF may be poorly able to detect changes in contractility in the setting of very low RV afterload, which is presumed in the MESA population.

Not all patients underwent MRI or had available vWF levels, although the study sample was fairly similar to MESA participants lacking these measures. As is possible in all observational studies, residual or unmeasured confounding could account for the results. Our study was cross-sectional and, while a discussion of mechanism is relevant, causality cannot be confirmed. While agreement between MRI readers was excellent, measurement error could impact our findings and measurement of invasive pulmonary hemodynamics was not feasible in this large study of community dwelling participants free of cardiovascular disease. In addition, vWF activity assays have variable precision, which may contribute to misclassification. These assays do not, however, have a systematic bias and it is unlikely that vWF assay differences alone account for our results.[192] Finally, the absence of clinical cardiovascular disease in this cohort led to relatively small effect estimates. However, the difference in RV mass from the uppermost to lowermost quintiles of vWF was 3.8%, greater than the 2.8% increase in LV mass seen with diabetes in MESA.[55] These small changes may still reflect important pathophysiologic processes.

Chapter 3

Other inflammatory pathways and their relationship to cardiac structure, cardiac function, and clinical outcomes in individuals with pulmonary arterial hypertension

Introduction

As previously noted, right heart adaptation, function, and failure are key determinants of morbidity and mortality in common and rare diseases alike. Right heart failure is consistently identified as a key predictor and mediator of mortality in PAH.[9,14,193] While the right heart fails in most patients with rising pulmonary pressures, the right heart strengthens in a few patients.[8,155] Patients with 'adaptive' right hearts are able to withstand rising pulmonary pressures for years or even decades. [8,194]

The importance of right heart failure is not limited to PAH. Abnormalities of right heart structure and function also predict mortality in emphysema, left heart failure, and in community dwelling adults. Despite the importance of right heart function, as of now there are no therapies that act directly on the right heart to promote adaptation. In contrast, addressing neurohormonal mediators of myocardial dysfunction in left heart disease improves morbidity, mortality, and occasionally even normalizes function.[195]

Systemic inflammation is one possible mediator of myocardial dysfunction that may contribute to RV failure in PAH. High levels of inflammatory cytokines and other protein mediators of inflammation are associated with a poor prognosis in patients with left heart failure and with the presence or absence of pulmonary hypertension.[14,116] In addition, the course of PAH varies from patient to patient; those with more inflammatory sources of disease (e.g. autoimmune disease) tend to have a worse prognosis. [196] We have previously found that C-Reactive Protein (CRP), interleukin-6 (IL6) and pentraxin-3 are related to right heart morphology in participants without PAH or significant right heart strain (Figure 3).[51] These observations and preclinical work support our conceptual model that inflammation may be an important factor differentiating right heart adaptation from failure in PAH.[14,196]

We examined the relationship between right heart structure and function, circulating CRP, and circulating IL6 with outcomes in individuals with pulmonary arterial hypertension.

Methods

The Seattle Right Ventricle Translational Science (Servetus) Study is a clinic-based prospective observational cohort of participants with PAH or Chronic Thromboembolic Pulmonary Hypertension (CTEPH) at the University of Washington Medical Center. Servetus participants contributed to a blood-based biorepository, imaging biorepository (echocardiography, cardiac magnetic resonance imaging (cMRI), and ventilation-perfusion scanning), completed questionnaires (including medical history, personal histories, diet history, activity levels, sleep history), and completed mail/phone follow-up three times a year with office-based evaluation at least once yearly. Participants included men and women with PAH or CTEPH between the ages of 18 – 80 years, without overt clinical right heart failure at the time of enrollment, who were able to provide informed consent, and were English speaking.

Blood based biomarkers

The markers of interest (CRP & IL6) were measured in duplicate from serum using electro-chemiluminescence assays (mesoscale discovery (MSD) assay, Rockville, Maryland, USA. The high sensitivity V-PLEX neuroinflammation panel was run by the Wurfel Lab at the University of Washington. The test-retest correlation for CRP was 0.99 and for IL-6 was 0.99.

Echocardiogram

Echocardiography was available for the majority of SERVETUS participants within three months of the baseline examination. Outcomes of interest include RV dilation (RV basal diameter), RV function (tricuspid annular plane systolic excursion (TAPSE)), and right heart failure as estimated by the right atrial pressure. Twenty percent of scans were read in duplicate to assess inter-reader agreement (correlation for the RV basal diameter was 0.91, TAPSE was 0.99, and right atrial pressure was 0.91).

Clinical Outcomes

Three core clinical outcomes were ascertained. All participants in Servetus were followed for death using a combination of questionnaires, chart review, and notification by participant's specialty pharmacy. All patients with PAH and CTEPH receive costly medications from one of three specialty pharmacies. These pharmacies are required to notify the center when a patient dies. This was then confirmed by the research team. In addition, Servetus participants who returned questionnaires self-identified likely episodes of right heart failure by their response to the question, "Over the last four months did your weight ever increase by five or more pounds in one week?" and the follow-up question, "Approximately how many separate times did this occur?". The total number of episodes of right heart failure over two years of follow-up was recorded for all participants with available questionnaires. Finally, Servetus participants who returned questionnaires self-identified their New York Heart Association Functional Class (NYHA-FC) at baseline and after two years of follow-up.

Statistical analysis

Baseline characteristics of participants were evaluated using appropriate summary statistics and qualitatively compared between those individuals who did and did not consistently return follow-up questionnaires over the two years after the baseline assessment.

We used Cox proportional hazards to estimate the unadjusted association between blood or imaging biomarkers and the hazard of death during follow-up. In adjusted models, we included participant's age, sex, height, weight, and the etiology of their PAH or CTEPH. In additional exploratory models, we further adjusted for baseline estimates of right heart afterload (pulmonary vascular resistance and mean pulmonary arterial pressure) to evaluate whether the association was independent of the burden of pulmonary vascular disease, which might suggest that the association was driven by adaptation (or lack thereof) in the right ventricle.

Ordinal logistic regression was used to estimate the unadjusted and adjusted associations between blood or imaging biomarkers and the change in NYHA-functional class at two years. Poisson regression was used to estimate the unadjusted and adjusted associations between blood or imaging biomarkers and the frequency of right heart failure over the first two years of follow-up. Finally, linear regression was used to estimate the association between unadjusted and adjusted associated between blood and imaging biomarkers. Analyses were performed using STATA 15.1 (StataCorp, College Station, TX, USA).

Results

One hundred thirty-four participants with CTEPH or PAH were enrolled in Servetus. Due to difficulties with the blood draw or technical difficulties with the assay three participants did not have measurements for the blood-based biomarkers of interest. Of the 131 participants with available biomarkers, 89 participants consistently returned surveys for the two years after the baseline exam (67.9%). The mean age of the cohort was 52.0 years, 77.7% were women, and 89.6% were white. Participants who responded to surveys regularly over two-years of follow-up tended to be slightly older, less likely to have toxin-induced PAH, more likely to have PAH from congenital heart disease, and had less severe symptoms with less severely deranged hemodynamics (Table 1). In total, there were 22 deaths over 339 person-years (6.4 deaths per 100 person-years). This rate was higher in participants who failed to regularly respond to surveys in whom there were 12 deaths over 95 person-years (12.6 deaths per 100 person-years), and lower among those who responded to follow-up questionnaires regularly (10 deaths in 244 person-years (4.1 deaths per 100 person-years)).

Table 1. Characteristics of the SERVETUS Cohort

	Regular survey respondents (n=89)	Survey non-respondents (n=42)
Age (years)	54 ± 15	47 ± 12
Female (%)	79	76
White Race (%)	90	87
Born in the United States (%)	84 (94%)	39 (93%)
Height (cm)	167 ± 9	167 ± 10
Weight (kg)	81 ± 26	84 ± 23
Body mass index (kg/m^2)	29 ± 8	30 ± 8
Etiology of PAH (n/%)		
Toxin-induced	10 (11%)	10 (24%)
Idiopathic	32 (36%)	13 (32%)
Collagen vascular disease	19 (21%)	8 (20%)
Chronic thromboembolism	10 (11%)	4 (10%)
Congenital heart disease	16 (18%)	4 (10%)
Other	2 (2%)	3 (7%)
Medicare/Medicaid	55 (62%)	25 (60%)
Private insurance	38 (43%)	17 (41%)
Grants to help pay for meds (e.g. caring voices)	11 (12%)	5 (12%)
Medications for PAH		
PDE5 inhibitors	54 (61%)	30 (71%)
ERAs	43 (48%)	19 (45%)
Prostacyclin analogues	35 (39%)	19 (45%)
Baseline Right Heart Catheterization		
Right atrial pressure (mmHg)	8 ± 5	13 ± 13
Mean pulmonary arterial pressure (mmHg)	46 ± 12	54 ± 13
Wedge pressure (mmHg)	13 ±11	14 ± 7
Pulmonary vascular resistance (Wood units)	8 ± 5	10 ± 6
Cardiac index (L/min/m^2)	2.6 ± 0.8	2.3 ± 0.7
New York Heart Association Functional Class		
Class I/II	53 (60%)	17 (40%)
Class III/IV	36 (40%)	25 (60%)

Data presented as mean ± standard deviation or percentage as appropriate

Abbreviations: cm=centimeters, kg=kilograms, m^2=meters squared, mmHg=millimeters of mercury, PAH=pulmonary arterial hypertension, L=liters, min=minute

At baseline the average basal diameter of the RV on echocardiography was 4.6cm ± 0.9cm (n=102), The average TAPSE was 2.0 cm ± 0.5 cm (n=101) and the average right atrial pressure was 7.8 mmHg ± 5.1 mmHg. The average CRP was 6.7 mg/L ± 13.7 (range 0.1-112.7 mg/L) and IL6 was 1.7 pg/mL ± 4.2 (range 0.1 – 41.1 pg/mL).

Increasing RV basal diameter was associated with in increased risk for death. This relationship appeared to strengthen when accounting for differences in age, gender, height, weight, PAH etiology, and hemodynamics. Increased right atrial pressure (suggestive of right heart failure) was associated with an increased risk of death, whereas increasing TAPSE (suggestive of improved RV function) was associated with a lower risk of death. Neither relationship appeared to be meaningfully different with adjustment (Table 2).

Higher levels of CRP were strongly associated with the risk of death; however, accounting for pulmonary vascular resistance as a surrogate for the burden of vascular disease appeared to weaken the association. This may suggest that the relationship between CRP and death is not independent of the severity of pulmonary vascular disease or may reflect the lack of power in the models with fewer participants that were used to evaluate the importance of baseline hemodynamics. IL-6 was also strongly associated with death, and this relationship appeared to strengthen when accounting for differences in age, sex, height, weight, and etiology of PAH (Table 2).

Table 2. Cox proportional hazard regression estimating the relationship between imaging or blood-based biomarkers at the baseline exam with death during follow-up (n=131 for unadjusted and full models and 78 for models including hemodynamics)

	Hazard Ratio of Death	(95% CI)	p-value
RV Basal Diameter (per 1cm increase)			
Unadjusted	2.1	(1.4, 3.1)	0.001
Full model*	3.0	(1.6, 5.5)	<0.001
Full Model + PVR	5.5	(1.8, 16.9)	0.003
RV TAPSE (per 0.5 cm increase)			
Unadjusted	0.5	(0.3, 0.9)	0.01
Full model*	0.3	(0.1, 0.6)	0.002
Full Model + PVR	0.4	(0.2, 1.0)	0.05
Right Atrial Pressure (per 5 mmHg increase)			
Unadjusted	1.7	(1.3, 2.3)	<0.001
Full model*	2.0	(1.3, 2.9)	0.001
Full Model + PVR	2.4	(1.5, 3.9)	<0.001
C-Reactive Protein (per standard deviation (13.6mg/mL) increase)			
Unadjusted	1.4	(1.1, 1.8)	0.002
Full model*	1.5	(1.1, 2.0)	0.006
Full Model + PVR	1.3	(0.9, 1.8)	0.16
Interleukin-6 (per standard deviation (4.2 pg/mL) increase)			
Unadjusted	4.3	(2.3, 8.0)	<0.001
Full model*	5.5	(2.7, 11.2)	<0.001
Full Model + PVR	4.5	(2.1, 9.5)	<0.001

Definition of abbreviations: CI-confidence interval, RV-right ventricle, PVR-pulmonary vascular resistance

**Full model: age, sex, race/ethnicity, height and weight, etiology of pulmonary arterial hypertension*

Among participants who returned surveys, increased RV basal diameter, right atrial pressure, increased levels of IL-6 and, to a lesser extent, increased levels of CRP were associated with an increased number of heart failure episodes over two years of follow-up. TAPSE was associated with heart failure frequency, but only in adjusted models. The relationship between TAPSE and heart failure frequency was not evident until the regression was adjusted by height and weight. This suggests that TAPSE was only associated with heart failure frequency in individuals of otherwise similar height and weight.

Table 3. Poisson regression estimating the relationship between imaging and blood-based biomarkers at the baseline exam with the likely number of episodes of heart failure over two years of follow-up.

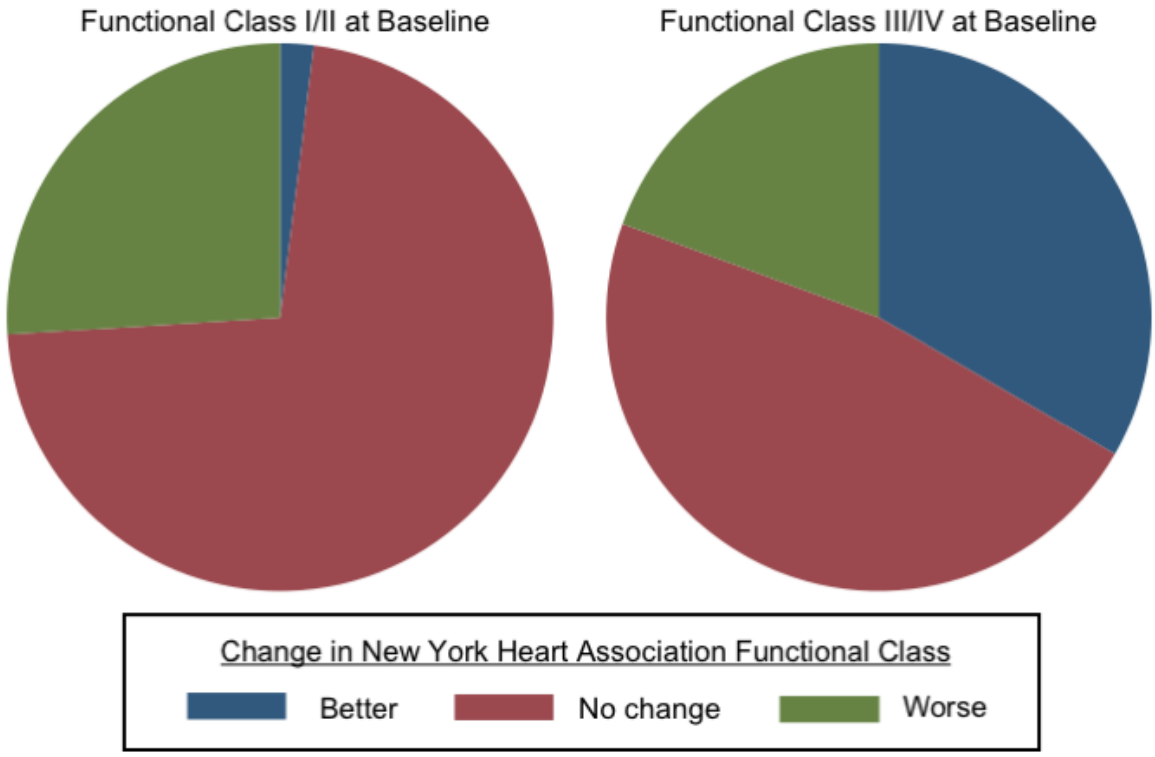
	# of episodes of heart failure	(95% CI)	p-value
RV Basal Diameter (per 1cm increase)			
Unadjusted	0.2	(0.0, 0.4)	0.03
Full model*	1.3	(0.9, 1.7)	<0.001
RV TAPSE (per 0.5cm increase)			
Unadjusted	0.0	(-0.2, 0.1)	0.65
Full model*	-0.3	(-0.6, -0.1)	0.01
Right Atrial Pressure (per 5mmHg increase)			
Unadjusted	0.3	(0.1, 0.4)	<0.001
Full model*	1.1	(0.8, 1.3)	<0.001
C-Reactive Protein (per standard deviation (13.6mg/mL) increase			
Unadjusted	0.2	(0.1, 0.3)	<0.001
Full model*	0.2	(0.1, 0.4)	0.009
Interleukin-6 (per standard deviation (4.2 pg/mL) increase)			
Unadjusted	1.0	(0.6, 1.3)	<0.001
Full model*	1.1	(0.7, 1.5)	<0.001

Definition of abbreviations: CI-confidence interval, RV-right ventricle

**Full model: age, sex, race/ethnicity, height and weight, etiology of pulmonary arterial hypertension*

For participants who returned surveys, the majority had no change in NYHA functional status at two years (53 of 91 participants; 58%). Twenty-five of 91 participants had worse self-reported NYHA functional class (27%) and 13 participants had better self-reported NYHA functional class (14%). This appeared to be qualitatively different based on baseline NYHA functional class (Figure 1).

Figure 1. Change in New York Heart Association Functional Class stratified by baseline functional class.



Increased right atrial pressure and IL-6 were associated with worsening NYHA functional class at two years and increased RV basal diameter was suggestive of an association with worsening NYHA functional class but did not reach statistical significance (Table 4). With adjustment for baseline NYHA functional class, age, gender, height, weight, and etiology of pulmonary hypertension, RV basal diameter and IL-6 were more strongly associated with change in NYHA functional class, while right atrial pressure was less strongly associated (Table 4)

Table 4. Ordinal logistic regression estimating the relationship between imaging and blood-based biomarkers with change in New York Heart Association Functional Class after two-years of follow-up

Change in NYHA functional Class		(95% CI)	p-value
RV Basal Diameter (per 1cm increase)			
Unadjusted	0.5	(0.0, 1.0)	0.07
Full model*	1.2	(0.4, 2.1)	0.003
RV TAPSE (per 0.5cm increase)			
Unadjusted	0.0	(-0.5, 0.4)	0.83
Full model*	0.2	(-0.3, 0.7)	0.51
Right Atrial Pressure (per 5mmHg increase)			
Unadjusted	0.5	(0.0, 0.9)	0.04
Full model*	0.4	(-0.3, 1.0)	0.25
C-Reactive Protein (per standard deviation (13.6mg/mL) increase			
Unadjusted	0.4	(-0.2, 1.1)	0.16
Full model*	0.9	(-0.1, 2.0)	0.07
Interleukin-6 (per standard deviation (4.2 pg/mL) increase)			
Unadjusted	1.1	(0.0, 2.1)	0.05
Full model*	1.5	(0.0, 2.9)	0.05

Definition of abbreviations: CI-confidence interval, RV-right ventricle

**Full model: age, sex, race/ethnicity, height and weight, etiology of pulmonary arterial hypertension, and New York Heart Association Functional Class*

Finally, serum levels of IL-6 appeared to be strongly associated with RV morphology, but not with echocardiographic estimates of function. CRP levels did not appear to be associated with either RV morphology or function (Table 5). Persons with porto-pulmonary hypertension (CRP 11.7 ± 16.4 mg/mL; IL-6 4.9 ± 6.2 pg/mL), collagen vascular disease (CRP 9.1 ± 18.2 mg/mL; IL-6 2.9 ± 7.8 pg/mL), and chronic thromboembolic disease (CRP 12.7 ± 29.1 mg/mL; IL-6 2.5 ± 5.3 pg/mL) had the highest levels of inflammatory cytokines. Persons with idiopathic PAH (CRP 5.7 ± 7.9 mg/mL; IL-6 1.0 ± 1.3 pg/mL), toxin-mediated PAH (CRP 4.8 ± 3.6 mg/mL; IL-6 1.0 ± 1.0

pg/mL), and congenital heart disease associated PAH (CRP 4.1 ± 4.3 mg/mL; IL-6 1.2 ± 1.8 pg/mL) had the lowest levels of inflammatory markers.

Table 5. Linear regression estimating the relationship between inflammatory biomarkers and cardiac structure and function as determined by echocardiography

		(95% CI)	p-value
Change in RV structure or function with a standard deviation (13.6mg/mL) increase in C-Reactive Protein (CRP)			
RV Basal Diameter (cm)			
Unadjusted	0.1	(-0.1, 0.2))	0.28
Full model*	0.1	(0.0, 0.3)	0.17
RV TAPSE (cm)			
Unadjusted	-0.1	(-0.1, 0.0)	0.29
Full model*	-0.1	(-0.2, 0.0)	0.15
Right Atrial Pressure (5mmHg)			
Unadjusted	0.6	(-0.3, 1.4)	0.22
Full model*	0.7	(-0.3, 1.6)	0.16
Change in RV structure or function with a standard deviation (4.2pg/mL) increase in Interleukin-6 (IL-6)			
RV Basal Diameter (cm)			
Unadjusted	0.4	(0.1, 0.7)	0.01
Full model*	0.4	(0.1, 0.7)	0.006
RV TAPSE (cm)			
Unadjusted	-0.1	(-0.3, 0.0)	0.14
Full model*	-0.2	(-0.4, 0.0)	0.13
Right Atrial Pressure (5mmHg)			
Unadjusted	2.1	(0.5, 3.7)	0.01
Full model*	2.5	(0.8, 4.2)	0.005

Definition of abbreviations: CI-confidence interval, RV-right ventricle

**Full model: age, sex, race/ethnicity, height and weight, and etiology of pulmonary arterial hypertension*

Discussion

We observed associations between imaging and inflammatory biomarkers with death and frequency of heart failure in this single institution cohort of individuals with pulmonary arterial hypertension. RV dilation, increased right atrial pressure, decreased right heart function, increased blood levels of CRP, and increased blood levels of IL6 were all associated with the risk for death or frequency of right heart failure. These associations were relatively insensitive to adjustment with the exception of CRP, which did not appear to be independent of differences in the baseline pulmonary vascular resistance.

Associations with self-reported NYHA functional class were less precise; however, RV dilation and increased levels of IL-6 also appeared to be associated with worsening NYHA functional class over time. Finally, CRP levels appeared to have little association with right ventricular structure or function; however, IL-6 levels appeared to be strongly associated with right heart dilation and increased right atrial pressure.

Our results join those of other recent reports to highlight the importance of interleukin-6 both in the development of pulmonary arterial hypertension and the RV response to increased afterload. Signaling through the IL-6 receptor on vascular smooth muscle cells appears to be important in the proliferation and accumulation of vascular smooth muscle cells in PAH. This is reinforced by the observation that the IL-6 receptor is over-expressed on vascular smooth muscle cells in patients with PAH relative to those without. In addition, mice that lack a functional IL-6 receptor appear to be protected against the development of PAH.[197] In addition, even among individuals with a similar pulmonary vascular resistance, the right heart appears to be more dilated with diminished function and cardiac output.[198]

This growing body of evidence is relevant because IL-6 receptor inhibitors already exist, are already in clinical use, and – while expensive - are well tolerated.[199,200] The contribution of pro-inflammatory mediators to disease progression and worsening RV function may also underpin the observation that a substantial number of women have worse disease control

during pregnancy and post-partum, which is also a time of increased IL-6 levels and other pro-inflammatory mediators for women.[201]

Unlike previous studies, we observed a concordance between an inflammatory biomarker, change in symptoms, frequency of heart failure episodes, RV structure, and death. In addition, levels of IL-6 were quite different among persons with different etiologies of PAH, with a nearly five-fold difference between IL-6 levels in persons with toxin-induced PAH and porto-pulmonary hypertension. This underlying difference suggests that there may be identifiable clinical phenotypes that would be more or less amenable to treatment with immune modulating drugs. In particular, consideration of an anti-IL-6 receptor antibody in PAH related to collagen vascular disease would be a reasonable start. This opinion is based on the relatively high levels of IL-6 in this cohort, and based on the fact that tocilizumab is already being used in a systemic sclerosis population with good safety and a possible benefit on skin findings of scleroderma.[200]

This study certainly has limitations. In particular, this was an observational cohort and the direction of the relationships among the pathways of interest are not well understood. For example, It is possible that the relationship between IL-6 levels and outcomes is confounded by other factors associated with both inflammation and outcomes in PAH. In addition, because the majority of participants already had disease at the baseline exam, the inference is further obfuscated by the possibility that observed relationships represent reverse causation, whereby adaptive, mal-adaptive and by-stander pathways can all increase in the setting of active and/or advanced disease.[202] Finally, there were a relatively small number of participants in analyses, and a large number of analyses were performed.

Conclusion

We observed a phenotype of RV dilation, increased right atrial pressure, and increasing inflammatory biomarkers that was associated with poor outcomes over follow-up. In particular, blood levels of IL-6 were strongly and consistently predictive of poor outcomes, an association which may be mediated by pulmonary vascular disease and/or right heart failure. Medications targeting the IL-6 pathway might well be effective in some patients with right heart failure.

Conclusion

In conclusion, this thesis represents a substantial review of much of the work that I have completed over the last 7 years related to RV structure and function, incident heart failure, and potential targets for resiliency for the right heart. The histamine work has progressed to a randomized clinical trial, while the work on the role of inflammatory biomarkers is just beginning. It is an exciting time to be involved in pulmonary vascular research as the entire knowledge-base of its pathophysiology is rapidly expanding, with opportunities to make substantial progress synthesizing the vast amount of new information coming forth from this vibrant research community.

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It is rare in scientific writing that I get a chance to pause and thank those around me. This dissertation is not an end, but is a wonderful waypoint along the road and I feel lucky and grateful to have been able to walk this road with an amazing group of individuals (AMDG).

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