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BIOMARKERS OF INFLAMMATION IN HEART FAILURE PATIENTS WITH REDUCED AND PRESERVED EJECTION FRACTIONS: MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

By

Michelle Lynne Stone

A thesis submitted to the Department of Clinical and Applied Movement Sciences in partial fulfillment of the requirements for the degree of Master of Science in Health, Exercise Science and Chronic Disease

UNIVERSITY OF NORTH FLORIDA

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Dedication and Acknowledgements

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Nomenclature

cm	centimeter
MET·min/wk	MET minutes per week
mg/dL	milligrams per deciliter
mg/L	milligrams per liter
mmHg	millimeters of mercury
pg/mL	picogram per liter

List of Abbreviations

AHA	American Heart Association
BP	blood pressure
CI	confidence interval
CVD	cardiovascular disease
CHD	coronary heart disease
CHF	congestive heart failure
DHF	diastolic heart failure
EF	ejection fraction
FHS	Framingham Heart Study
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HDL	high density lipoprotein
HTN	hypertension
HR	hazard ratio
LDL	low density lipoprotein
LV	left ventricle/left ventricular
LVEF	left ventricular ejection fraction
MESA	Multi-Ethnic Study of Atherosclerosis
MET	metabolic equivalent
MI	myocardial infarction
PA	physical activity

SAS	statistical analysis software
SHF	systolic heart failure
WC	waist circumference

Abstract

Purpose: Examine the relationships between high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α receptor-1 (sTNF-R1) and the cumulative risk of heart failure with reduced (HFrEF) and preserved (HFpEF) ejection fractions in a diverse, population-based sample.

Methods: Study sample included 6,814 adult (45-84 years of age) men and women who participated in the Multi-Ethnic Study of Atherosclerosis and were free of cardiovascular disease at baseline. Cox regression was used to calculate the hazard ratios (HR) associated with elevated baseline hs-CRP (> 3-10 mg/L), IL-6 (> 75th percentile) and sTNF-R1 (> 75th percentile) and risk of overall HF, HFrEF (ejection fraction [EF] < 50%), and HFpEF (EF ≥ 50%).

Results: During ~11.2 years of follow-up there were 178 incident HF diagnoses. Elevated hs-CRP, IL-6 and sTNF-R1 were associated with a significant increased risk of HF overall (HR 1.76; 95% Confidence interval [CI] 1.22-2.52, HR 1.57; 95% 1.07-2.30, and HR 1.91; 95% CI 1.08-3.38, respectively). Elevated hs-CRP was a significant predictor in both HFrEF and HFpEF (HR 2.05; 95% CI 1.26-3.35, and HR 1.89; 95% CI 1.09-3.28, respectively). Baseline IL-6 concentrations were significantly associated with increased risk of HFrEF in nonsmokers only (HR 2.33; 95% CI 1.04-5.23) and of HFpEF in African Americans only (HR 5.89; 95% CI 1.52-22.80).

Conclusion: In a diverse sample of U.S. adults, elevated hs-CRP, IL-6 and sTNF-R1 were significant predictors of HF. Furthermore, both hs-CRP and IL-6 were significant predictors in HFrEF and HFpEF.

Chapter One: Introduction

Heart failure (HF) is a condition characterized by an inability of the heart to supply sufficient blood to the body to meet metabolic demands or accommodate systemic venous return (1). This disease manifests either due to failure of the left ventricle (LV) to fill with enough blood or to contract with enough force or, occasionally, a combination of the two (2, 3). The inability to contract with sufficient force often results in a reduced percentage of blood ejected from the LV, also known as the ejection fraction (EF). These patients are considered to have heart failure with reduced ejection fraction (HFrEF). Those with an inability to properly fill the LV can often still eject a volume of blood that is proportional to their end diastolic volume, resulting in a normal, or preserved, EF. Accordingly, these patients are considered to have heart failure with preserved ejection fraction (HFpEF). Although these subtypes share several similarities, recent literature has demonstrated distinct differences in the epidemiology, etiology, treatment, and prognosis (4, 5) of HFrEF and HFpEF. Chronic inflammation, characterized using several different inflammatory biomarkers, has been positively associated with HF incidence, severity, and prognosis. These biomarkers include C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α receptor-1 (sTNF-R1) (6–10).

This chapter includes relevant background information pertaining to HF, CRP, IL-6, and sTNF-R1. This is followed by a focused review of the existing literature regarding the relationship between these variables. The chapter concludes with the purpose and significance of the research, a project description, and limitations inherent to the study design.

BACKGROUND

HEART FAILURE

The prevalence of HF in the adult population ranges from 1 to 3% (5, 11–13) but increases to upwards of 10% in older populations (13–15). The prevalence of HFpEF among those with HF varies widely depending on several factors including the diagnostic criteria, clinical setting, age and sex of the population, and year of publication (2). Nonetheless, most investigations have found the prevalence of HFpEF in this population is about 50% (16–20).

The age- and sex-adjusted incidence of HF declined 37% from 2000 to 2010 with a much greater rate reduction seen in those with HFrEF (-45%) than HFpEF (-28%) (*P* for interaction = =0.08) (21). However, the prevalence of HF is expected to increase 23% equating to a 46% increase in the number of Americans living with HF from 2012 to 2030 (22). The increasing prevalence is largely due to the growth of the aging population as well as improvements in life-prolonging HF therapy (2, 11, 23). Despite these advancements, mortality rates remain high at about 19.9 per 100,000 (24) with little or no improvement over the past couple of decades and a 5-year survival rate of ~50% (21, 25, 26).

Though it is believed the first case of HF was identified roughly 3,500 years ago, it was not until the 1950's that the idea of cardiac contractility came about and was later believed to account for changes observed in HF (27). Research then focused on understanding reduced contractility and developing ionotropic medications that would increase EF in these patients. Until the late 1980-1990's, LV systolic dysfunction was considered a prerequisite for HF however, repeated observations of HF without LV systolic dysfunction lead to the recognition of heart failure with LV diastolic dysfunction (5, 27–29). The two subtypes were originally termed systolic and diastolic HF however, the preferred terminology today is HFrEF and HFpEF,

respectively, as many HF patients demonstrate some degree of both systolic and diastolic dysfunction (2, 3) but can still be distinguished by left ventricular ejection fraction (LVEF) (3, 4).

Despite the recognition of two distinct HF subtypes over two decades ago, a harmonious definition for HFrEF and HFpEF has yet to be established (2, 3, 30). In the most recent guideline for the management of HF (3) set out by the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA), HF was defined as, "a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood." Impairments that may lead to HF include, but are not limited to, abnormalities of the myocardium, endocardium, pericardium, heart valves, great vessels, or some metabolic abnormalities. However, most symptomatic patients have LV myocardial dysfunction. The ACCF/AHA also emphasized that LV dysfunction and cardiomyopathy are not interchangeable with HF but instead they should be used to describe possible reasons for the development of systolic or diastolic LV dysfunction. In the literature, HF subtypes have been classified by various LVEF cutpoints however, the most recent definition from the ACCF/AHA classifies HFrEF as an EF $\leq$ 40% and HFpEF as an EF $\geq$ 50%. Ejection fraction values ranging from 41-49% are considered heart failure with mid-range ejection fraction (HFmrEF).

Though useful for defining HFrEF and HFpEF, more is required for a diagnosis than EF. Several epidemiological studies have developed their own diagnostic criteria for diagnosing HF that have continued to be used throughout the years (31–34). These criteria include a combination of determinants include signs and symptoms, medical history review, radiographic evidence, and response to therapy however, they do not differentiate between HFrEF and HFpEF. In response, studies that have aimed to differentiate between HFrEF and HFpEF have

modified the previously existing criteria to include an EF cut point (29). Several serological markers, including biomarkers of inflammation, have been implicated to strengthen the HF diagnosis however; there is a need to investigate novel biomarkers for the evaluation and management of patients with HF, particularly in HFpEF (35–37).

INFLAMMATORY BIOMARKERS

C-Reactive Protein

C-reactive protein (CRP) is an acute phase plasma protein produced by the liver in response to inflammation (38). Activation of CRP in hepatocytes is primarily regulated at the transcriptional level by interleukin-6 (IL-6) but interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) can also regulate CRP to some degree (38–40). There is also evidence of extrahepatic production of CRP by lymphocytes, neurons, renal cells and in the atherosclerotic lesion, specifically by smooth muscle and macrophages (39, 41).

In adults with no prior history of cardiovascular disease, a single, non-fasting measure of CRP can be a strong predictor of future vascular events even after controlling for traditional risk factors including age, smoking, cholesterol, blood pressure, and diabetes (42). C-reactive protein has been shown to contribute to atherogenesis and predict incident myocardial infarction, stroke, peripheral artery disease, and sudden death (39, 42). “High-sensitivity” assays are recommended for the measurement of CRP as these are designed to detect CRP concentrations across a low-normal range and are widely available (42).

In a 2003 statement (43), the Centers for Disease Control and the AHA recommended using a high-sensitivity C-reactive protein (hs-CRP) cut point of > 3 mg/L to define high risk in the adult population. Being a biomarker of inflammation, CRP concentrations can be elevated

over 100-fold in the presence of major infections, trauma, or acute hospitalizations. For this reason, it is also recommended that concentrations > 10 mg/L should be discarded.

Interleukin-6

Interleukin-6 (IL-6) is a proinflammatory cytokine secreted by various cells including activated macrophages and lymphocytes (40). Most cytokines function at the paracrine/autocrine level however, IL-6 is unique in that it is predominately a circulatory molecule therefore its major actions take place away from its site of origin. Secretion and expression of IL-6 is induced by IL-1 and TNF-α (44) and IL-6 can, in turn, regulate the activity of IL-1 by directly inducing the release of its receptor antagonist and TNF-α. Circulating concentrations of IL-6 increase with obesity and in the presence of systemic infection or inflammation (40, 45). In healthy individuals, as much as one-third of total circulating IL-6 concentrations are estimated to originate from adipose tissue.

Interleukin-6 is sometimes referred to as a "remodeling" biomarker as it can directly affect cell-to-cell communication between myocytes and fibroblasts, and changes in concentration are associated with changes in cardiac extracellular matrix and function (37). In a prospective study of older adults without baseline cardiovascular disease (10), IL-6 was a significant predictor of incident coronary heart disease, stroke, and congestive HF. Immunosorbent assays are often used to measure IL-6 levels and high risk is often determined by the sample-specific upper tertile (9, 10, 46).

Soluble Tumor Necrosis Factor-α Receptor-1

Tumor necrosis factor-α is the smaller and more abundant isoform of tumor necrosis factor. It is a proinflammatory cytokine produced primarily by macrophages in response to inflammation however, adipocytes have also been shown to express TNF-α (47). Two distinct

surface receptors, TNFR-1 and TNFR-2, mediate the effects of TNF and exist in either membrane-bound or soluble forms (48). Though the extracellular domains of these receptors are conserved, the cytoplasmic portions are not, suggesting differing downstream processes. Both receptors have been found in human myocytes with TNFR-1 being the predominate subtype in most cells, including the heart (48, 49). Binding of TNF-α to TNFR-1 induces an inflammatory response in the myocytes. Additionally, the extracellular portion of the receptor can then be cleaved by a proteolytic enzyme, releasing soluble tumor necrosis factor-α recptor-1 (sTNF-R1) which can then diffuse into circulation (36). Elevated sTNF-R1 has been significantly associated with an increased risk of HF across whites and blacks, and males and females (50).

FOCUSED LITERATURE REVIEW

Although existing literature has demonstrated an increased risk of HF in those with elevated hs-CRP (51, 52), IL-6 (9, 51) and sTNF-R1 (50), there are fewer investigations that have determined these relationships in HFrEF and HFpEF. Furthermore, there is limited data examining these relationships in multi-ethnic, diverse samples.

PURPOSE AND SIGNIFICANCE

The purpose of this study was to examine the relationship between elevated hs-CRP, IL-6, and sTNF-R1 and the cumulative risk of HFrEF and HFpEF in a diverse sample of U.S. adults 45-84 years of age. The specific research question addressed was:

1. Is there an association between elevated hs-CRP, IL-6, or sTNF-R1 and the cumulative risk of HFrEF or HFpEF?

To the extent of our knowledge, this is the first study to examine the relationship between elevated hs-CRP, IL-6 and sTNF-R1 and incidence HFrEF and HFpEF in a diverse sample of U.S. adults 45-84 years of age who participated in the Multi-Ethnic Study of Atherosclerosis (MESA).

PROJECT DESCRIPTION

The MESA (53) is a community-based, multi-center, prospective cohort study including 6,814 men and women 45-84 years of age who were free of clinical cardiovascular disease at baseline. Multivariable hazard ratios were calculated using the proportional hazards regression procedure to determine the cumulative risk of HFrEF and HFpEF according to baseline hs-CRP, IL-6 and sTNF-R1. Limitations include:

1. Biomarkers of inflammation were measured at a single time point; therefore, it is unclear if elevated concentrations were indicative of acute or chronic inflammation.
2. Inherent in the design of the MESA, the diverse, multi-ethnic sample was not representative of the U.S. population, affecting the generalizability of the results.
3. The sample size of those with HF was relatively small, especially when categorized by HFrEF and HFpEF. Additionally, sample sizes were even smaller for variables such as sTNF-R1 in which concentrations were only analyzed in a subset of the sample.
4. Self-reported data was used for some of the covariates which is subject to recall bias and self-report bias.
5. There is a potential for residual confounding in which there may be additional confounding factors that were not included.

6. Incident HF was defined as time to first HF event, therefore subsequent HF events and potential changes in EF over time were not included in the analysis.
7. Individuals without sufficient EF data at the time of their first HF event were not included in the analyses.

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Chapter Two: Review of Literature

Heart failure (HF) is a complex clinical syndrome characterized by an inability of the heart to deliver enough blood to meet metabolic demands or doing so only at the cost of increased filling pressures (1, 2). This results from injury or stress to the myocardium which often occurs due to ischemic heart disease, hypertension, chronic obstructive pulmonary disease, rheumatic heart disease and diabetes (3, 4). When the heart fails to compensate for injury, signs and symptoms of HF that develop include dyspnea, fatigue, and fluid retention leading to pulmonary congestion and/or peripheral edema (2, 5). Though commonly associated with congestion, exercise tolerance is often limited in HF patients with and without evidence of fluid retention (2). Underlying abnormalities of the myocardium are common in HF and often result in left ventricular (LV) systolic and/or diastolic dysfunction, however, abnormalities of the pericardium, endocardium, heart rhythm, and conduction are also observed (5, 6). One of the most common noninvasive techniques used to determine the presence of LV systolic or diastolic dysfunction is by Doppler echocardiography (7). Also, this method is often used to determine left ventricular ejection fraction (LVEF).

Ejection fraction (EF) refers to the ratio of stroke volume to ventricular-end diastolic volume (8). This measurement is often used as a quantitative measurement of left ventricular function and is positively associated with survival in HF patients (9). Although right ventricular function is also of concern when evaluating HF patients, isolated right-sided HF is uncommon (10). Furthermore, the most common manifestation of right-sided HF is due to left-sided HF and it is recommended that these patients be managed as left-sided HF patients. In the most recent guidelines for the management of HF (2), the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA), define subtypes of left-sided HF by LVEF. These subtypes include heart failure with reduced ejection fraction (HFrEF) (EF $\leq 40\%$), heart failure

with preserved ejection fraction (HFpEF) (EF ≥ 50%) and heart failure with mid-range ejection fraction (HFmrEF) (EF 41-49%).

This chapter includes a focused review on HF terminology as well as the two most prevalent HF subtypes, HFrEF and HFpEF. Existing literature that has investigated the relationship between high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) or soluble tumor necrosis factor-α receptor-1 (sTNF-R1) and HF overall, HFrEF or HFpEF are also included. It concludes with a summary of the literature and the explanation of the need for additional research.

TERMINOLOGY

Until the 1980-1990's, other than a few rare cases, HF was believed to occur exclusively in those with systolic dysfunction (11, 12). In fact, early drug trials in HF that sought to establish a medication that would improve adverse outcomes in this population either included only those with a reduced EF or did not differentiate by EF (5, 12). Over time, the repeated observation that there were individuals with signs and symptoms of HF but without overt reduced systolic dysfunction lead to the discovery of HF with diastolic dysfunction (1, 12). These two subtypes were accordingly named systolic heart failure (SHF) and diastolic heart failure (DHF). Although these terms seemed fitting at the time, it was soon discovered that LV systolic and LV diastolic dysfunction are not mutually exclusive with many HF patients exhibit some degree of both (2, 5, 13). For this reason, the preferred terminology today is heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF).

Terminology referring to HF overall has also changed over the years. Because pulmonary congestion has been long recognized as a cardinal sign of HF, the term congestive heart failure

(CHF) is frequently used interchangeable with HF. Although CHF may accurately describe some HF patients, it is not always accurate as some patients present without signs or symptoms of volume overload (2). Furthermore, CHF may be misleading because this acronym is also used to refer to chronic heart failure rather than congestive heart failure (14). For these reasons, "heart failure" is the preferred terminology over "congestive heart failure" (2).

It is also of note that the 2013 ACCF/AHA Guideline for the Management of HF (2) emphasized that cardiomyopathy and LV dysfunction are not synonymous with HF. Instead, these terms should be used to describe possible reasons for the development of structural and functional abnormalities in HF.

DIAGNOSIS

Consistent with HF being termed a clinical 'syndrome' in the 1990's, no single sign, symptom, or clinical history item alone is used to diagnose HF but rather a combination of several criteria is required (2, 15). Currently, a national or international consensus on a set of diagnostic criteria for HF does not exist (2, 16). Instead, HF is often a diagnosis of exclusion following a close medical history review and physical examination (2). In light of the fact that there is not a single harmonious definition, many epidemiological, clinical, and community-based studies have created their own criteria or used criteria produced by various organizations to diagnose HF. Commonly used criteria include the Framingham criteria (17), Gothenburg criteria (18), Boston criteria (19) , and the Cardiovascular Health Study (CHS) (20) criteria, all of which rely on a combination of medical history review, signs and symptoms, radiographic evidence, and response to therapy. Overall, these criteria produce similar estimates, (21) however, they were not designed to differentiate by HFrEF and HFpEF. The Framingham

criteria is one of the most widely used and validated of them all. It has been shown to be very sensitive (92%) and moderately specific (79%) for the diagnosis of HF (22). Specifically, absence of the Framingham criteria can rule out the presence of HF whereas the presence of the criteria should be used in combination with radiographic evidence, such as an echocardiography, to confirm the diagnosis. The Framingham criteria are listed in Table 1 below. A definite diagnosis requires a minimum of two major, or one major and two minor criteria present concurrently. Additionally, minor criteria cannot be attributable to any other condition.

Table 1. Framingham Clinical Diagnostic Criteria for Congestive Heart Failure

Major Criteria	Minor Criteria
Paroxysmal nocturnal dyspnea or orthopnea	Ankle edema
Neck-vein distention	Night cough
Rales	Dyspnea on exertion
Cardiomegaly	Hepatomegaly
Acute pulmonary edema	Pleural effusion
S_3 gallop	Vital capacity decrease 1/3 from maximum
Increased venous pressure ≥ 16 cm of water	Tachycardia (rate of ≥ 120 bpm)
Circulation time ≥ 25 seconds	
Hepatojugular reflux	
Major or Minor Criterion	
Weight loss ≥ 4.5 kg in 5 days in response to treatment*	
*Serves as a major criterion if it occurred during therapeutic intervention for CHF; if due to other factors it is a minor criterion.	

Note. Adapted from "The Natural History of Congestive Heart Failure: the Framingham Study" by McKee et al. *New England Journal of Medicine.* 1971;285(26), 1441-1446.

Diagnosis by HFrEF and HFpEF is complicated by the heterogeneity of the disease processes and can be particularly difficult for HFpEF (2, 5). Maestre et al. (22) cross-matched

HF confirmed by echocardiographic diagnostic criteria of LV dysfunction with the results obtained using the Framingham clinical diagnostic criteria for HF to see how the latter performed in ruling out SHF (EF < 45%) and DHF (EF > 45%). The analysis revealed that the Framingham criteria can conclusively rule out the presence of SHF but not DHF (likelihood ratio for negative test result 0.04 vs. 0.10, respectively). Other than in isolated events, systolic and diastolic LV dysfunction alone cannot accurately distinguish between HF subtypes as many individuals display characteristics of both (2, 5, 13). However, when categorized by LVEF, distinct differences in etiology, risk profiles, and response to treatment are revealed (23). Additionally, most clinical trials and guidelines have customarily used LVEF for categorizing HFrEF and HFpEF though some might argue it is not the optimal parameter for evaluating LV systolic function (24, 25).

Various EF cutpoints have been used to define HFrEF and HFpEF however, the 2013 ACCF/AHA guidelines (2) for HF management defines HFrEF by an EF ≤ 40% and HFpEF by an EF ≥ 50%. Additionally, a third subtype that is often excluded altogether or grouped into one of the other two classes also exists. This subclass is considered heart failure with mid-range ejection fraction (HFmrEF) and it is classified as an EF ranging from 41% to 49%. Participants with HFmrEF have been reported to most likely have primarily mild systolic dysfunction but also have features of diastolic dysfunction (5). Aside from classifying EF, the ACCF/AHA Guidelines (2) also reported several criteria that have been proposed to define HFpEF including:

1) Clinical signs or symptoms of HF
2) Evidence of preserved or normal LVEF

3) Evidence of abnormal left ventricular diastolic dysfunction (LVDD) that can be determined by Doppler echocardiography or cardiac catheterization

However, these proposed criteria were adapted from a 2000 study (26) in which Vasan et al. underscored the importance of distinguishing between the HF subtypes and called for the development of uniform criteria to diagnose HFpEF. Other than the brief mention of these proposed criteria, the guideline itself did not recommend any specific diagnostic criteria for HFpEF. In place, large epidemiological studies looking to define HFpEF have instead adapted previous definitions to include a LVEF cut point. Some of these key studies have been included in Table 2 below.

Table 2. Definitions of HFpEF Used in Epidemiological Studies of HFpEF				
Study	Cohort	Year	LVEF	HFpEF Diagnosis
Kupari (27)	Helsinki Ageing Study	1997	FS ≥ 0.25	At least 3 of the following (1) history of breathlessness on ordinary effort; (2) audible ventricular gallop sound or HR 90 bpm at rest; (3) pulmonary venous congestion on CXR (consensus of 2 observers) or abnormal neck vein distention or palpable hepatomegaly; and (4) cardiothoracic ratio > 0.55 on CVR.
				FS from protocol echocardiogram at study visit
Senni (28)	Olmsted County	1998	≥ 50%	Modified Framingham criteria. LVEF from abstraction of medical record from within 3 wk of HF diagnosis.
Vasan (29)	FHS	1999	≥ 50%	Framingham criteria
				LVEF from protocol echocardiogram at FHS study visit
Devereux (30)	SHS	2000	> 54%	Modified Framingham criteria
				LVEF from protocol echocardiogram at SHS study visit
Kitzman (31)	CHS	2001	Qualitative as normal;	Expert panel adjudication based on review of pertinent data on hospitalization or outpatient visits for CHF, including history, physical examination, chest x-ray reports, and medication administration. Self-report of a physician diagnosis of CHF was confirmed by medical record documentation.
Gottdiener (32)	CHS	2002		Qualitative assessment of systolic function from protocol echocardiogram at CHD study visit
Bursi (33)	Olmsted County	2006	≥ 50%	Framingham criteria Prospective study-specific echocardiogram
Owan (34)	Mayo Clinic	2006	≥ 50%	ICD/DRG discharge codes (validated against Framingham criteria in a subset)
				LVEF from clinical echocardiogram performed within 30 days of HF hospitalization
Bhatia (35)	EFFECT study	2006	> 50%	Framingham criteria LVEF abstracted from HF hospitalization
Lam (36, 37)	FHS	2011	> 45%	Framingham criteria
				LVEF assessed within 1 y of hospitalization (without intervening event)
Gerber (38)	Olmsted County	2015	> 50%	ICD codes from hospital discharges or outpatient visits (validated against Framingham criteria in a subset);
				LVEF assessed at Mayo clinical by echocardiography w/in 90 days of HF diagnosis
CHF indicates chronic heart failure; CHS, Cardiovascular Health Study; CXR, chest X-ray; DRG, diagnosis-related group; EFFECT, Enhanced Feedback for Effective Cardiac Treatment; FHS, Framingham Heart Study; FS, fractional shorting; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HR, hazards ratio; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; and SHS, Strong Heart Study.				

Note. Adapted from, "Heart Failure with Preserved Ejection Fraction in Perspective" by Pfeffer et al. *Circulation research.* 2019;124(11), 1598-1617.

PATHOPHYSIOLOGY

An estimated 17 primary etiologies of HF exist and over two-thirds can be attributed to just four underlying conditions: ischemic heart disease, chronic obstructive pulmonary disease, hypertensive heart disease, and rheumatic heart disease (39). In many cases the exact etiology of HF is unknown or related to a combination of different etiologies. Regardless, HF often manifests secondary to structural and/or functional abnormalities of the myocardium which most often result in systolic and/or diastolic dysfunction (5, 6). However, HF can also result from abnormalities of the pericardium, myocardium, endocardium, heart rhythm, or conduction.

Cardiac remodeling describes a progressive series of changes in the shape, size, and function of the heart (40). It is one of the most important pathophysiological processes in the development and progression of HF (41). Cardiac remodeling is often initiated by damage to the myocardium or increases in wall stress and is characterized by changes in the cardiomyocytes themselves and in the makeup of the extracellular matrix (ECM) (40). The ECM is important for maintaining the structural and functional integrity of the heart, predominately via collagen fibrils. Molecules involved in the composition or activity of the extracellular matrix that are released into circulation can be measured to detect remodeling. For example, serum concentrations of collagen fragments are positively correlated with remodeling and the development of fibrosis in the heart.

Heart failure is often considered a disease of the elderly, particularly in HFpEF (42–44). Left ventricular wall stress can occur in response to age-related modifications of the cardiovascular system such as increased afterload, diminished chronotropic and inotropic responses, increased intracardiac pressures with ventricular filling, and impaired vasodilation (45, 46). Cardiac remodeling response to stress can result in hypertrophy of the cardiomyocytes

(47). Hypertrophied cardiomyocytes demand more oxygen and energy, and failure to meet these needs can result in a hypoxic environment in which excess free radicals are produced (48). Interestingly, deficits in myocardial oxygen use in HFpEF has been correlated with hemodynamic severity (3), however, the cellular mechanisms in HFpEF specifically remain unclear (1). In response, cardiomyocytes release pro-inflammatory cytokines and chemokines in order to attract macrophages to the area (49). Macrophages are rich in matrix metalloproteinases (MMPs) which are key regulators in ECM turnover (50). Although tissue inhibitors of metalloproteinases (TIMPs) are responsible for inhibition of these proteins, with aging, levels of some TIMPs and MMPs isoforms are increased and correlate with variables of diastolic dysfunction (51). The isoform MMP-9, can process several cytokines including IL-6 and TNF-α and is strongly associated with increased LV stiffness and end diastolic dimension in the aging heart (50).

This describes one probable pathophysiological explanation behind cardiac remodeling and the development of HF overall in the aging heart. Although several other etiologies and resultant compensatory mechanisms exist, elucidating this disease process in older populations is important given the significant increase in prevalence and incidence in the elderly (44). Other than LV hypertrophy and stiffness, other potential compensatory mechanisms include increased cardiac output via the Frank-Starling mechanism and increased mean arterial pressure via neurohormonal systems (4).

HFrEF

As previously reported, left ventricular cardiac remodeling is one of the most important pathophysiological processes in HF (41). Four patterns of LV remodeling that have been identified include normal geometry, concentric remodeling, concentric hypertrophy and eccentric

hypertrophy. In HFrEF, eccentric hypertrophy is the most common type of LV remodeling, however, a subset of those with HFrEF have concentric hypertrophy. The classic history of HFrEF begins with the initial damage to the myocardium, which initiates compensatory mechanisms including LV hypertrophy. Neurohormonal activation is also increased in attempt to stabilize cardiac output and contractility leading to further LV systolic dysfunction.

In a large multinational longitudinal study, Nauta et al. (41) examined differences in the pathophysiology, clinical characteristics, and response to treatment in HFrEF patients according to LV geometry. To the surprise of the investigators, a sizable portion of those with HFrEF exhibited reduced LVEF with concentric remodeling rather than eccentric remodeling. Additionally, many of those with HFrEF and concentric hypertrophy had a profile similar to HFpEF patients as they were predominately older, hypertensive women. In a sub-analysis, the investigators examined the biomarker profiles according to LV remodeling in which HFrEF patients with concentric hypertrophy were characterized by markers of oxidative stress and inflammation.

HFpEF

Patients with HFpEF comprise about half of all patients with HF (27, 29, 31, 33, 52), yet they frequency experience delayed diagnosis and have limited treatment options (5, 53). These patients are more likely to be elderly women with small, hypertrophied ventricles (43, 54) and several cardiovascular and non-cardiovascular comorbidities including obesity, hypertension, coronary artery disease, and metabolic syndrome (1, 54). The high prevalence of comorbid conditions in HFpEF may explain the cardiac changes observed in this subtype (1).

Conversely, HFpEF is characterized by the presence of left ventricular diastolic dysfunction (LVDD) (55). Evidence of LVDD is considered a preclinical condition defined as

the inability of the LV to fill to an adequate end-diastolic volume at an acceptable pressure (56). Kane et al. (13) reported about one in four adults with moderate-severe diastolic dysfunction may develop HF. Although LVDD can occur in HFrEF just as LV systolic dysfunction can occur in HFpEF, LVDD has been reported to be the primary mechanism in HFpEF and the cause of the most unifying hemodynamic finding in HFpEF, elevated LV filling pressures (1, 55). Furthermore, diastolic dysfunction has been shown to be an independent and common predictor of HFpEF and can help strengthen the diagnosis in the presence of other criteria including signs and symptoms of HF which are often nonspecific (54).

Despite the strong associations with LVDD in HFpEF it is important to also consider these patients may exhibit several other abnormalities including, but not limited to, LV systolic dysfunction, left atrial dysfunction, or long-standing pulmonary hypertension leading to right ventricular dysfunction (1, 12). While there has been some success elucidating several organ-level pathophysiological processes in HFpEF, the cellular mechanisms behind many of the cardiac changes observed in HFpEF are not well understood.

SUMMARY

In summary, HFrEF and HFpEF are two distinct HF subtypes. Left ventricular cardiac remodeling resulting from injury or stress, including age-related changes, is an important consideration in the development of HF. Those with HFrEF are typically characterized by eccentric LV hypertrophy whereas those with HFpEF typically exhibit concentric LV hypertrophy. Nonetheless, it is important to note that neither eccentric or concentric remodeling are unique to just HFrEF or HFpEF.

RISK FACTORS

Traditional HF risk factors are common among the US population. Using data from the 2007-2010 National Health and Nutrition Examination Survey, Kovell et al. (57) estimated one-third of the US adult population has at least one HF risk factor. Common established risk factors for HF overall include, but are not limited to; older age, male sex, hypertension, obesity, LV hypertrophy, myocardial infarction, diabetes, smoking, metabolic syndrome, coronary artery disease, race/ethnicity, and immune activation (2, 21, 23, 43, 56).

Using data from the Framingham Heart Study (FHS) original and offspring cohorts, Cardiovascular Health Study (CHS), and the Prevention of Renal and Vascular End-stage Disease study (PREVEND), Ho et al. (23) examined risk factors that were significant for HFrEF and HFpEF (Table 3). Data from these three longitudinal cohort studies were also used to examine significant differential effects of clinical covariates on HFpEF versus HFrEF (Table 4). Male sex was a significant risk factor in HFrEF only whereas age was a significantly stronger risk factor for HFpEF. Left bundle branch block and previous myocardial infarction were associated with significantly greater risk in HFrEF compared to HFpEF. Smoking status, LV hypertrophy, and left bundle branch block were also stronger risk factors in HFrEF than HFpEF.

Table 3. Final Risk Prediction Models for HFpEF and HFrEF		
Risk factors	sHR* (95% CI)	*P* value
HFpEF		
Age per, 10 years	1.90 (1.74-2.07)	< 0.0001
Male sex	0.93 (0.78-1.11)	0.43
Systolic BP, per 20mmHg	1.14 (1.05-1.24)	0.003
Body mass index, per 4kg/m2	1.28 (1.21-1.37)	< 0.0001
Antihypertensive treatment	1.42 (1.18-1.71)	0.0002
Previous myocardial infarction	1.48 (1.12-1.96)	0.006
HFrEF		
Age, per 10 years	1.66 (1.52-1.80)	< 0.0001
Male sex	1.84 (1.55-2.19)	< 0.0001
Systolic BP, per 20mmHg	1.20 (1.10-1.30)	< 0.0001
Body mass index, per 4kg/m2	1.19 (1.11-1.28)	< 0.0001
Antihypertensive treatment	1.35 (1.13-1.63)	0.001
Diabetes mellitus	1.83 (1.48-2.26)	< 0.0001
Current Smoker	1.41 (1.14-1.75)	0.0015
Previous myocardial infarction	2.60 (2.08-3.25)	< 0.0001
ECG LV hypertrophy	2.12 (1.55-2.90)	< 0.0001
Left bundle branch block	3.17 (2.11-4.78)	< 0.0001

BP, blood pressure; CI, confidence interval; HF, heart failure; HFpEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; LV, left ventricular; ECG; electrocardiogram; sHR, subdistribution hazard ratio.

*Hazard ratio is expressed per increase in continuous variables as specified in the table and for presence vs absence of dichotomous variables.

Note. Adapted from, "Predicting Heart Failure with Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Subtypes" by Ho et al. 2016. *Circulation: Heart Failure*, *9*(6), e003116.

Table 4. Differential Effects of Risk Factors on HFpEF vs HFrEF			
	HFpEF sHR* (95% CI)	HFrEF sHR* (95% CI)	*p* for equality
Age, per 10 years	1.91 (1.78–2.06)	1.69 (1.59–1.81)	0.02
Male sex	0.91 (0.79–1.05)	1.87 (1.63–2.16)	< 0.0001
Systolic BP, per 20mmHg	1.13 (1.05–1.21)	1.20 (1.12–1.28)	0.24
Body mass index, per 4 kg/m2	1.28 (1.22–1.36)	1.18 (1.11–1.25)	0.05
Antihypertensive treatment	1.41 (1.21–1.65)	1.33 (1.14–1.54)	0.59
Diabetes mellitus	1.42 (1.17–1.72)	1.58 (1.32–1.90)	0.44
Current Smoker	1.04 (0.85–1.28)	1.44 (1.21–1.72)	0.02
Previous myocardial infarction	1.30 (1.02–1.67)	2.70 (2.25–3.24)	< 0.0001
ECG LV hypertrophy	1.16 (0.84–1.60)	2.08 (1.60–2.69)	0.009
Left bundle branch block	1.30 (0.81–2.09)	3.65 (2.62–5.09)	0.0008
*Hazard ratio is for the presence versus absence of dichotomous predictors, and per increase in continuous predictors as specified in the table with all covariates shown in the model simultaneously. BP indicates blood pressure; CI, confidence interval; HF, heart failure; HFpEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; and sHR, subdistribution hazard ratio.			

Note. Adapted from, "Predicting Heart Failure with Preserved and Reduced Ejection Fraction: the International Collaboration on Heart Failure Subtypes" by Ho et al. 2016. *Circ Heart Fail*, *9*(6), e003116.

Many of the original large longitudinal cohort studies in HF have historically included almost exclusively white participants including the FHS (100% whites), PREVEND (96% whites), and CHS (85% whites) (23). More recent investigations that have examined HF risk across different race/ethnicities have revealed significant risk differences (44, 58, 59). Therefore, it is important to include a brief synopsis of some of the investigations that have examined HF in more diverse samples.

The Health, Age, and Body Composition, or Health ABC Study, examined the epidemiology of incident HF by race and gender in an elderly cohort of 2934 participants (58.6% white, 41.4% black) without HF at baseline. The median follow-up time was 7.1 years in which 8.8% of the population developed HF. They found that men and blacks were more likely to

develop HF, however, there were not statistically significant sex-based differences observed. Blacks had a higher overall proportion of HF attributable to modifiable risk factors compared to whites (67.8% vs. 48.9%) with a > 5% higher population attributable risk in 6 of 8 risk factors assessed (coronary heart disease, uncontrolled blood pressure, LV hypertrophy, reduced glomerular filtration rate, smoking, and increased heart rate). Differences in survival rates after HF were not statistically significant, however, rehospitalization rates were significantly higher in blacks.

Bahrami et al. (58) examined differences in incident CHF in 6814 men and women (45-84 years of age) who participated in the Multi-Ethnic Study of Atherosclerosis. This cohort included whites (38.5%), African Americans (27.8%), Hispanics (21.9%) and Chinese Americans (11.8%) who were free from cardiovascular disease at baseline. Although they did not stratify by LVEF, it was noted that 63% of the CHF participants had preserved LV function (LVEF $\geq$ 40%) After a median follow-up of 4.0 years, 79 participants developed CHF. Cox proportional hazards models were used to obtain hazard ratios (HRs). African American participants had the highest incidence rate of CHF, followed by Hispanic, white, and Chinese Americans. After controlling for age, sex, hypertension, obesity, serum cholesterol level, and smoking status, analysis revealed African Americans had a significantly greater risk of CHF compared to whites (Hazard ratio [HR] 2.00; 95% Confidence interval [CI] 1.11-3.61). However, this relationship was attenuated and no longer statistically significant after controlling for baseline LVEF determined by magnetic resonance imaging. There was not a significant association seen in the other race/ethnicities.

HEART FAILURE AND INFLAMMATION

In 2001, the National Institutes of Health's Biomarkers Definitions Working Group defined biological markers, or biomarkers, as, "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (60). Biomarkers can be used to help diagnose an abnormal condition, to stage the extent of a disease, to indicate disease prognosis, or to determine response to intervention.

In a 2012 review, van Kimmenade et al. (61), examined the emerging roll of biomarkers in HF after the clinical introduction of testing B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) in the early 2000's. Combining recommendations from various investigators and organizations, van Kimmenade et al. defined four conditions that should be met in order for the investigation of a biomarker to be useful. These conditions are as follows:

1) The methods by which a novel biomarker is judged (compared to or in combination with other biomarkers) should be thorough and evaluated across a wide range of patients that are typical of the diagnosis for which the biomarker will be applied. Additionally, the statistical methods used to evaluate the biomarker should be contemporary, rigorous, standardized and fair.
2) Measurement of a novel HF biomarker should be easily achieved within a short period of time and provide acceptable accuracy, and assays for its measurement should have defined biological variation and low analytical imprecision.
3) The biomarker should primarily reflect important (patho)physiological process(es) involved in HF presence and progression; use of a biomarker that is reflective of heart disease but originates outside of the myocardium is acceptable as long as such a

biomarker provides independently useful information involved in the diagnosis, prognosis, progression, or therapy of HF syndromes.

4) The biomarker must provide clinically useful information for caregivers (physicians, nurses, and others) and patients to facilitate more swift and reliable establishment/rejection of a diagnosis and more accurate estimation of prognosis and to inform more successful therapeutic strategies. The information from such a biomarker should not recapitulate clinical information already available at bedside and must be additional to information provided by other biomarkers.

According to the authors, although many are close, BNP and NT-proBNP are the only biomarkers thus far that have met the above criteria. This is consistent with the 2017 Focused Update (62) of the ACCF/AHA guidelines, which recommend measuring natriuretic peptide biomarkers for the prevention, diagnosis and prognosis of HF. In addition to BNP and NT-proBNP, the 2013 ACCF/AHA guidelines recommended using cardiac troponin T or I, biomarkers of myocardial injury, for evaluating HF prognosis but not for predicting new onset HF. Although other common cardiovascular biomarkers have been implicated in HF, current evidence is not enough to make these recommendations. Furthermore, considering the difficulties diagnosing HF, particularly in HFpEF, a multi-marker strategy may be useful in establishing risk and diagnosing HF in combination with other diagnostic criteria.

So far, the natriuretic peptides, specifically BNP, have been shown to have the best predictive value in HF compared to other biomarkers (40). Although BNP is not without some limitations, one being that it can be associated with a variety of cardiac and noncardiac causes (2). Furthermore, the predictive value of BNP in HFpEF is not as well established as in HFrEF or

HF overall. A 2012 analysis (63) examined BNP values in 159 HFpEF (EF > 50%) patients and found that about one-third had BNP levels below typical diagnostic thresholds. Gladden et al. (54) attributed the lower BNP values observed in HFpEF to obesity, elevated filling pressures in the early disease process, and lower wall stress secondary to concentric remodeling (64). However, other reports have distinguished that while BNP values in HFpEF are lower than in HFrEF, they are still elevated (40). According to an AHA Scientific Statement (40), there is a need for appropriate biomarkers that can properly diagnose HFpEF and provide pathophysiologically relevant classification. Since HF represents a complex, heterogeneous syndrome, biomarkers in HF are most commonly classified by the disease process they are involved in (61) . Candidate biomarkers involved in inflammation in HF include CRP, TNF-α, sTNF-R1, and IL-6. Additionally, IL-6 can also be classified under the extracellular-matrix remodeling disease process.

A prospective analysis from the Health ABC study (65), examined the associations between CRP, IL-6 and TNF-α and incident HF overall, HFrEF, and HFpEF. The sample included 2610 older adults without HF at baseline. Following adjustment for baseline characteristics and medication, IL-6, TNF-α, and CRP were significant predictors of incident HF overall in individuals without baseline atherosclerotic disease (n = 1945) (Hazard ratio [HR] 1.40; 95% Confidence interval [CI] 1.18-1.66; $p < 0.001$, HR 1.40; 95% CI 1.06-1.84; $p = 0.018$, and HR 1.19; 95% CI 1.05-1.36; $p = 0.009$, respectively). In those with atherosclerotic disease at baseline (n = 665), including coronary heart disease, cerebrovascular disease, or peripheral artery disease, IL-6 and TNF-α were significant predictors of HF risk (HR 1.23; 95% CI 1.02-1.48; $p = 0.032$, and HR 1.73; 95% CI 1.26-2.38; $p = 0.001$, respectively), however, CRP was not. This relationship was also examined by HFrEF (EF ≤ 45%) and HFpEF (EF > 45%). In HFrEF, risk

associated with elevated IL-6 was borderline significant (HR 1.21; 95% CI 0.99-1.48; $p = 0.067$) whereas TNF-α and CRP were not significant. In HFpEF, IL-6 and TNF-α were strongly associated with risk (HR 1.49; 95% CI 1.19-1.86; $p < 0.001$, and HR 1.81; 95% CI 1.23-2.68, $p = 0.003$, respectively) however, CRP was not. The weak association with CRP in this study is contradicting to other studies that have reported CRP as strong independent predictor of HF (66–69). The authors attributed this finding to the rigorous controlling for confounders in their study. In some studies, the predictive value of CRP was attenuated in patients with other traditional cardiovascular risk factors present (70–72) however, this phenomenon does not seem to represent the majority.

Bozkurt et al. (73) examined the emerging role of proinflammatory cytokines, CRP, and erythrocyte sedimentation rate in patients with HF. Table 5 includes a number of investigations that have reported concentrations of various cytokine and cytokine receptors in HF. Out of all of the cytokines and cytokine receptors, TNF-α appears to be the most well-studied with a majority of studies reporting elevated concentrations in HF patients. According to the table, the second most well investigated cytokine is IL-6, which is also elevated in a majority of the studies. It is also evident that although fewer studies have investigated concentrations of sTNF-R1 in HF, those that have all demonstrate these concentrations are also elevated. Additionally, Table 6 shows CRP concentrations are also elevated in HF patients.

Table 5. Peripheral Levels of Cytokines and Cytokine Receptors in Heart Failure

	Cytokines					Cytokine receptors				
	TNF-α	IL-1	IL-2	IL-6	IFN-γ	sTNFR1	STNFR2	IL- 1RA	ST-2	IL-6R
Levine et al. [1]	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
McMurray et al. [32]	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dutka et al. [11]	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
Wiederman et al. [18]	+	−	nd	+	−	nd	nd	nd	nd	nd
Katz et al. [12]	+	−	+	nd	nd	nd	nd	nd	nd	nd
Matsumori et al. [10]	+	−	−	−	−	nd	nd	nd	nd	nd
Ferrari et al. [19]	+	nd	nd	nd	nd	+	+	nd	nd	nd
Torre-Amione et al. [6]	+	nd	nd	nd	nd	+	+	nd	nd	nd
Torre-Amione et al. [5]	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Milani et al. [82]	+	nd	nd	nd	nd	+	nd	+	nd	nd
Munger et al. [20]	-	-	nd	+	nd	nd	nd	nd	nd	Nd
Testa et al. [30]	+	+	−	+	nd	nd	+	+	nd	+
Anker et al. [83]	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
MacGowan et al. [21]	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Mohler et al. [41]	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Nishigaki et al. [84]	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Anker et al. [17]	+	nd	nd	nd	nd	+	+	nd	nd	nd
Tsutamoto et al. [24]	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Aukrust et al. [13]	+	nd	nd	+	nd	+	+	nd	nd	−
Dibbs et al. [25]	+	nd	nd	+	nd	nd	nd	nd	nd	−
Rauchhaus et al. [26]	+	nd	nd	+	nd	+	+	nd	nd	nd
Deswal et al. [34]	+	nd	nd	+	nd	+	+	nd	nd	nd
Weinberg et al. [14]	nd	nd	nd	nd	nd	nd	nd	nd	+	nd

nd: Not done, +: levels elevated, -: levels not elevated

TNF-α, Tumor necrosis factor alpha, IL-1 Interleukin-1, IL-2 Interleukin-2, IL-6 Interleukin-6, IFN-c interferon gamma, sTNFR1 soluble TNF receptor R1, sTNFR2 soluble TNF receptor R2, IL-1RA IL-1 receptor antagonist, IL-6R IL-6 receptor, sST2 soluble ST2-member of IL-1 receptor family

Adapted from, "Biomarkers of inflammation in heart failure." by Bozkurt et al. 2010. *Heart failure reviews*, *15*(4), 331-341.

Table 6 demonstrates the associations of various biomarkers in HF and the strength of these associations (73). Concentrations of TNF-α, IL-6, sTNF-R1, and CRP are shown to correlate with disease severity, prognosis and HF outcomes. Additionally, three of these four biomarkers with available data (TNF-α, IL-6, and CRP) also demonstrate predictive value in the development of HF in asymptomatic patients. There was no data for this association in sTNF-R1.

Table 6. Role of Inflammatory Biomarkers in Heart Failure

	TNF-α	IL-6	IL-18	sTNF-R1	sTNF-R2	IL-1 RA	sST2	IL-10	Chemokines (MCP-1)	CRP	ESR
Levels are elevated in HF	+++	++	+	++	++	+	+	+	+	++	++
Supporting References	[1, 5, 6, 10–13, 17–21, 24–26, 30, 32, 34, 35, 41, 82–84]	[22, 27, 40]	[23]	[19, 37, 38]	[22, 38]	[22]	[14, 16]	[22]	[3]	[71, 73, 74]	[76, 79]
Levels correlate with disease severity	+++	+++	n/d	++	++	+	n/d	n/d	+	++	n/d
Supporting references	[1, 5, 11, 13, 27, 30–32, 34]	[5, 20, 24, 27, 28, 30, 34, 40]		[28, 30, 34, 39]	[28, 30, 34, 39]	[30]			[3]	[71, 74]	
Levels correlate with prognosis and HF outcomes	+++	+++	n/d	++	++	n/d	+	n/d	n/d	++	+
Supporting references	[19, 24, 26, 34, 35, 41–44]	[24, 41–44]		[26, 34]	[26, 34]		[14, 16, 85]			[43, 73–75]	[76, 79]
Levels predict development of HF in asymptomatic patients	++	++	n/d	n/d	n/d	n/d		n/d	n/d	++	n/d
Supporting references	[27]	[27]								[27]	
Levels change with HF therapy	+++	+++	n/d	++	++	n/d		++	n/d	+	n/d
Supporting references	[47, 48, 51, 52, 54, 55, 57, 58, 86]	[42, 47, 50, 56]		[22, 58]	[22, 51, 54, 57, 58]			[22, 52, 54]		[64, 86]	

TNF-a Tumor necrosis factor alpha, IL-6 Interleukin-6, IL-18 Interleukin-18, sTNFR1 soluble TNF receptor R1, sTNFR2 soluble TNF receptor R2, IL-1RA IL-1 receptor antagonist, sST2 soluble ST2-member of IL-1 receptor family, IL-10 Interleukin-10, MCP-1 Monocyte chemoattractant protein-1, CRP C-reactive protein, ESR erythrocyte sedimentation rate, HF heart failure, n/d no data available

+++ Supported by large number of studies and more than one large-scale clinical trial

++ supported by several number of studies and/or small-scale clinical trials and/or one large-scale clinical trial

+ supported by one small study or one small clinical trial

[a] One study suggested that elevated levels were associated with increased mortality [76], whereas the other study suggested that elevated levels were associated with better prognosis [79]

Adapted from, "Biomarkers of inflammation in heart failure." by Bozkurt et al. 2010. *Heart failure reviews*, *15*(4), 331-341.

SUMMARY

In summary, the evidence suggests various cytokines, cytokine receptors, and biomarkers of inflammation are elevated in HF patients and positively associated with HF risk. Currently, there is insufficient evidence supporting the use of these biomarkers in the diagnosis and prognosis of HF. Considering the challenges that are associated with diagnosing HF, there is an emerging need to determine the efficacy of using common biomarkers of other disease processes

in HF. It is likely that a combination of biomarkers may provide the greatest risk stratification rather than one single biomarker. Additionally, given the distinct differences in etiology and risk factors of HFrEF and HFpEF, there is also a need to investigate this relationship by HF subtype.

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Chapter Three: Methodology

The purpose of this prospective study was to examine the associations between elevated high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and soluble tumor necrosis factor-α receptor-1 (sTNF-R1) and the risk of heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFrEF). This section provides the details of the methodology used to address the research question.

DATA COLLECTION

The Multi-Ethnic Study of Atherosclerosis (MESA) is an ongoing prospective study funded by the National Heart, Lung, and Blood Institute of the National Institutes of Health. The primary objectives of the MESA are to determine the characteristics related to the progression of subclinical cardiovascular disease (CVD) and also from subclinical to clinical CVD. Details on the MESA have been published elsewhere (1). In brief, the sample included 6,814 men and women (45-84 years of age) who were free from clinical cardiovascular disease at baseline. Participants identified as Caucasian (38%), African American (928%), Hispanic (22%), or Chinese American (12%) and were recruited from six university-affiliated field centers in the United States:

1) University of California, Los Angeles, CA
2) University of Minnesota, St. Paul, MN
3) Northwestern University, Chicago, IL
4) Wake Forest University, Winston-Salem, NC
5) John Hopkins University, Baltimore, MD
6) Columbia University, New York, NY

Since the MESA is concerned with the natural history of subclinical and clinical CVD, participants with any known clinical disease were excluded from the sample. Additionally, participants with any incompatibility with the long-term design of the study or other components of the MESA exam were also excluded. Eligibility status was determined based off self-reported information.

The exclusion criteria were:

1. Age younger than 45 or older than 84 years
2. Physician-diagnosed:
    a. Heart attack
    b. Angina or taking nitroglycerin
    c. Stroke or TIA
    d. Heart failure
3. Current atrial fibrillation
4. Having undergone procedures related to CVD
5. Active treatment for cancer
6. Pregnancy
7. Any serious medical condition which would prevent long-term participation
8. Weight > 300 pounds
9. Cognitive inability as judged by the interviewer
10. Living in a nursing home or on the waiting list for a nursing home
11. Plans to leave the community within five years
12. Language barrier (speaks other than English, Spanish, Cantonese or Mandarin)
13. Chest CT scan in the past year

The baseline exam took place from July 2000-2002 and an additional four exams have been completed since 2012. Since Exam 1, participants have been contacted every 9-12 months to determine if any events of interest had occurred.

The current study was reviewed and approved by the Institutional Review Board of the University of North Florida (Appendix A). Data from the MESA was requested and obtained from the National Institutes of Health/National Heart, Lung, and Blood Institute: Biologic Specimen and Data Repository Information Coordinating Center (2).

PRIMARY DEPENDENT VARIABLE

The primary dependent variable was time to congestive heart failure (TTCHF). The variable name in the MESA is congestive heart failure (CHF) but this will be referred to as "HF" instead as it is the preferred terminology. Heart failure was an adjudicated event classified as either definite, probable, or absent. Potential HF events were primarily identified by post-baseline follow-up calls but other means of identifying events included participant notification, MESA clinic visits, National Death Index, and public obituaries. Medical records were obtained and reviewed by a panel of physicians to determine if the event met MESA criteria. Probable HF required evidence of HF symptoms, physician diagnosis of HF, and patient receiving medical treatment for HF. Definite HF required one or more additional criteria:

1) Pulmonary edema/congestion by chest x-ray
2) Dilated ventricle or poor left ventricular function by echocardiography or ventriculography
3) Evidence of left ventricular diastolic dysfunction

Participants who did not meet any criteria or with just a physician diagnosis of HF without any other evidence were considered to not have HF. If the medical report specified a specific ejection fraction (EF), the measured value was recorded. For reports that specified EF value as a range, the midpoint value rounded down to the nearest whole number was recorded. For some reports, EF was classified as either 'low' or 'normal.' Both 'probable' and 'definite' HF events were included in this analysis. Additionally, for the subtype analysis, those with an EF < 50% or classified as "low" at the time of diagnosis were considered HFrEF and those with an EF ≥ 50% or classified as "normal" were considered HFpEF.

PRIMARY INDEPENDENT VARIABLES

The primary independent variables in the study included hs-CRP, IL-6, and sTNF-R1. Baseline serum samples were drawn after at least 12 hours of fasting, processed 15-30 minutes following venipuncture, and stored at either the University of Vermont or the University of Minnesota (3). All biomarkers were analyzed in the Laboratory for Clinical Biochemistry Research at the University of Vermont (Burlington, VT).

High-sensitivity C-reactive protein was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). This instrument utilizes a particle enhanced immunonephelometric assay to determine hs-CRP concentration. The assay range is 0.175-1100mg/L. Concentrations > 10 mg/L were excluded as this is more indicative of acute infection or trauma (4). The hs-CRP variable was then dichotomized as either elevated (> 3-10 mg/L) or normal (≤ 3 mg/L). This range of hs-CRP concentration is classified as high risk according to the 2003 statement for healthcare professionals from the Centers for Disease Control and

Prevention and the American Heart Association (4). Following dichotomization, the variable was log-transformed.

Interleukin-6 concentration was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). The lower detection limit was < 0.095 pg/mL with a detection range of 0.156-10.0 pg/mL. Participants were grouped by gender and 10-year age bands for categorization. The calculated outliers for each age and gender group were excluded and the remaining values were log-transformed. The 75th percentile of the log-transformed variable for each group was considered elevated and values below the 75th percentile were considered normal. All elevated groups were combined into one and all non-elevated groups were also combined resulting in a single dichotomized variable specific for age and gender.

The sTNF-R1 variable was measured by ultra-sensitive ELISA (Quantikine HS Human sTNF R1 Immunoassay; R&D Systems, Minneapolis, MN). The lower detection level was 1-3 pg/mL with a detection range of 7.8-500 pg/mL. The variable was then dichotomized using the same methods as previously described in IL-6. Participants were grouped by gender and 10-year age bands for categorization. The calculated outliers for each age and gender group were excluded and the remaining values were log-transformed. The 75th percentile of the log-transformed variable for each group was considered elevated and values below the 75th percentile were considered normal. All elevated groups were combined into one and all non-elevated groups were also combined resulting in a single dichotomized variable specific for age and gender.

OTHER INDEPENDENT VARIABLES

The potential confounding variables that were controlled for in this study were all measured at Exam 1, the baseline exam, and they included the following:

AGE

Age was self-reported on the personal history form. This was included in the analysis as a continuous variable. Additionally, age was categorized in bands of 10 for the descriptive results to demonstrate the age distribution by heart failure status in the sample.

SEX

Sex was self-reported on the personal history form as either male or female.

RACE/ETHNICITY

Race was self-reported as either Caucasian, African American, Hispanic, or Chinese.

SMOKING

Smoking status was self-reported on the personal history form and was a created variable categorized as "Current, Former, or Never." Those who answered "yes" to the question, "Have you smoked cigarettes in the past 30 days?" were considered current smokers. Those who answered "no" to having smoked cigarettes in the past 30 days but "yes" to the question, "Have you smoked at least 100 cigarettes in your lifetime?" were considered former smokers. Those who answered "no" to having smoked at least 100 cigarettes in their lifetime were considered never smokers.

PHYSICAL ACTIVITY

Physical activity (PA) was self-reported on the PA form. This was also a created variable, rather than a variable that is directly obtained as part of the MESA exam. Total intentional

exercise was assessed based off the self-reported number of days per week and hours/minutes per day spent participating in the following activities:

1) Walking for exercise, pleasure, social reasons, walking during work breaks, or walking the dog is classified as intentional walking
2) Dancing in church, ceremonies, or for pleasure
3) Team sports such as softball, volleyball, basketball or soccer
4) Dual sports such as tennis, racketball, or paddleball
5) Individual activities such as golf, bowling, yoga, or T'ai Chi
6) Moderate effort conditioning activities such as low impact aerobics, recreational (slow) bicycling, rowing on a rowing machine or in a lake, swimming in a pool or lake, or using weight-lifting or conditioning machines at a health club.
7) Heavy effort conditioning activities such as high impact aerobics (e.g., Tai-bo, kick boxing, judo, karate), competitive or maximum effort running, bicycling, swimming, and work on health club machines.

A continuous MET·min/wk variable was calculate based off participant responses. A dichotomized PA variable was then created according to the 2018 Department of Health and Human Services Physical Activity Guidelines for Americans (5). Those who reported ≥ 500 MET·min/wk were considered sufficiently active and those < 500 MET·min/wk were considered insufficiently active.

WAIST CIRCUMFERENCE

Waist circumference measurements ≥ 102 cm or ≥ 88 cm for men and women, respectively, were considered at risk (6).

BLOOD PRESSURE

The last two of three blood pressure (BP) measurements were averaged for both systolic blood pressure (SBP) and diastolic blood pressure (DBP). These variables were then categorized according to the most recent blood pressure recommendations (7) as: normal SBP < 120 mmHg and DBP < 80 mmHg: elevated SBP 120-129 mmHg and DBP < 80 mmHg: or hypertensive SBP ≥ 130 mmHg or ≥ 80 mmHg. Additionally, participants taking any anti-hypertensive medications were classified as hypertensive as well.

LOW-DENSITY LIPOPROTEIN CHOLESTEROL

The low-density lipoprotein cholesterol (LDL-C) variable was a created variable in the MESA and was categorized according to the National Cholesterol Education Program Guidelines (NCEP) (8). Using these categorizations, LDL-C was dichotomized as < 130 mg/dL or ≥ 130 mg/dL. Additionally, lipid-lowering medication use was self-reported on the medications form and confirmed during the medication interview. Participants taking lipid-lowering medications were also classified as having elevated LDL-C.

HIGH-DENSITY LIPOPROTEIN CHOLESTEROL

The continuous high-density lipoprotein cholesterol (HDL-C) variable was used to create a gender-stratified dichotomous variable according to the 2001 NCEP Guidelines (8), in which concentrations < 40 mg/dL for females or < 50 mg/dL for males were considered low. Additionally, participants taking lipid-lowering medications were also classified as having low HDL-C.

TRIGLYCERIDES

Triglycerides were categorized in the MESA according to the 2001 NCEP Guidelines (8). Using these categorizations, triglyceride levels were dichotomized as < 150 mg/dL or ≥ 150

mg/dL. Additionally, participants taking lipid-lowering medications were also classified as having elevated triglycerides.

FAMILY HISTORY OF MYOCARDIAL INFARCTION

Family history of myocardial infarction was a created variable dichotomized (Y/N) by the self-reported history of myocardial infarction in a parents, siblings, or children.

STATINS

Statin use was reported on the medications form and confirmed during the medication interview. Using this information, the MESA created a dichotomized (Y/N) statin use variable.

ORAL STEROIDS

Oral steroid use was reported on the medications form and confirmed during the medication interview. Using this information, the MESA created a dichotomized (Y/N) oral steroid use variable.

ASPIRIN

Aspirin use was reported on the medications form and confirmed during the medication interview. Using this information, the MESA created a dichotomized (Y/N) aspirin use variable.

NSAIDS (EXCLUDING ASPIRIN)

Use of NSAIDS, excluding aspirin, was reported on the medications form and confirmed during the medication interview. Using this information, the MESA created a dichotomized (Y/N) NSAID use variable.

STATISTICAL ANALYSIS

Statistical Analysis Software 9.4 (9) was used for data management in which complex variable recodes, coding verification, and statistical analyses were performed. Descriptive

characteristics were obtained using the means (PROC MEANS) and frequency (PROC FREQ) procedures for continuous and categorical variables, respectively. The univariate procedure (PROC UNIVARIATE) was used to calculate outliers and to determine the 75th percentile of IL-6 and sTNF-R1. SAS was also used to log-transform hs-CRP, IL-6 and sTNF-R1 to make the distributions normal or near normal.

Separate proportional hazards regression procedures (PROC PHREG) were used to calculate multivariable adjusted hazard ratios (HR) to determine risk of HF overall, HFrEF and HFpEF according to baseline hs-CRP, IL-6 and sTNF-R1 concentrations. Participants without the necessary EF data were excluded from the subtype analysis. After creating unadjusted and age-adjusted models, three additional models were made. Model 1 adjusted for demographics and behavioral covariates including age, gender, race/ethnicity, smoking, and PA. Model 2 adjusted for the variables in Model 1 plus WC, BP, HDL-C, LDL-C, triglycerides, and self-reported family history of MI. Model 3, the fully adjusted model, included Model 2 covariates plus anti-inflammatory medication use including statins, oral steroids, aspirin, and NSAIDS (excluding aspirin).

In a separate analysis using proportional hazards regression, covariates included in the fully adjusted model were chosen using a stepwise backward elimination process. For each model that was tested, potential covariates that did not contribute significantly based on $p = 0.05$ were removed and excluded from subsequent analyses. A final parsimonious model was included to help elucidate the relationship between the independent and dependent variables.

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Chapter Four: Manuscript

BIOMARKERS OF INFLAMMATION IN HEART FAILURE PATIENTS WITH REDUCED AND PRESERVED EJECTION FRACTIONS: MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

ABSTRACT

Purpose: Examine the relationships between high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor-α receptor-1 (sTNF-R1) and the cumulative risk of heart failure with reduced (HFrEF) and preserved (HFpEF) ejection fractions in a diverse, population-based sample.

Methods: Study sample included 6,814 adult (45-84 years of age) men and women who participated in the Multi-Ethnic Study of Atherosclerosis and were free of cardiovascular disease at baseline. Cox regression was used to calculate the hazard ratios (HR) associated with elevated baseline hs-CRP (> 3-10 mg/L), IL-6 (> 75th percentile) and sTNF-R1 (> 75th percentile) and risk of overall HF, HFrEF (ejection fraction [EF] < 50%), and HFpEF (EF ≥ 50%).

Results: During ~11.2 years of follow-up there were 178 incident HF diagnoses. Elevated hs-CRP, IL-6 and sTNF-R1 were associated with a significant increased risk of HF overall (HR 1.76; 95% Confidence interval [CI] 1.22-2.52, HR 1.57; 95% 1.07-2.30, and HR 1.91; 95% CI 1.08-3.38, respectively). Elevated hs-CRP was a significant predictor in both HFrEF and HFpEF (HR 2.05; 95% CI 1.26-3.35, and HR 1.89; 95% CI 1.09-3.28, respectively). Baseline IL-6 concentrations were significantly associated with increased risk of HFrEF in nonsmokers only (HR 2.33; 95% CI 1.04-5.23) and of HFpEF in African Americans only (HR 5.89; 95% CI 1.52-22.80).

Conclusion: In a diverse sample of U.S. adults, elevated hs-CRP, IL-6 and sTNF-R1 were significant predictors of HF. Furthermore, both hs-CRP and IL-6 were significant predictors in HFrEF and HFpEF.

INTRODUCTION

Heart failure (HF) is a complex clinical syndrome in which the heart is unable to deliver the requisite amount of blood to meet metabolic demands or does so only at the cost of increased filling pressures (1, 2) . The prevalence of HF in the general adult population ranges from 1-3% (3–6) and increases starkly to over 10% in older populations (6–8). According to estimates from the 2011-2014 National Health and Nutrition Examination Survey, approximately 6.5 million Americans are living with HF and this is projected to increase to over 8 million by 2030 (9, 10).

Heart failure most often manifests due to structural or functional abnormalities of the left ventricle (LV) and resultant systolic or diastolic dysfunction, or a combination of the two (1, 11). In early investigations, HF was almost exclusively thought to be associated with reduced systolic function however, repeated observations of HF without systolic dysfunction in the late 20th century lead to the recognition of HF with diastolic dysfunction (2, 3, 12). These two HF subtypes were accordingly termed systolic and diastolic HF, though the preferred terminology today is heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). The change in terminology is largely due to the observation that many HF patients demonstrate some degree of both systolic and diastolic dysfunction (11, 13) but when classified by left ventricular ejection fraction (LVEF), HFrEF and HFpEF exhibit marked differences in etiology, risk profiles, and response to treatment (14–18). The need to differentiate between these distinct subtypes is emphasized by evidence indicating that HFpEF constitutes ~50% of all HF cases (19–23). Furthermore, due to the temporal sequence of discovery, early drug trials only included those with HFrEF and medications implemented to reduce mortality in HFrEF have not been shown to be efficacious in HFpEF (3, 11).

Unfortunately, making this distinction can be difficult because, currently, there is not a nationally or internationally agreed upon definition, classification system, or gold standard for the clinical diagnosis of HFpEF (11, 13, 14). Despite this lack of consensus, numerous diagnostic classifications, produced by various organizations, do exist. The 2013 American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) Guideline (11) for the Management of Heart Failure describes the proposed criteria to define HFpEF including; clinical signs or symptoms of HF, evidence of preserved or normal LVEF, and evidence of abnormal LV diastolic dysfunction determined by Doppler echocardiography or cardiac catheterization. Nonetheless, diagnosis of HFpEF remains difficult and primarily a diagnosis of exclusion (24, 25) A 2017 Scientific Statement from the AHA (26) highlighted the need for novel biomarkers to add meaningful diagnostic information and provide a classification relevant to the pathophysiology of HFpEF. According the 2017 Focused Update (27) of the aforementioned ACCF/AHA guidelines, there is only sufficient evidence on the efficacy of a few biomarkers, namely B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide, cardiac troponin, and some biomarkers of myocardial fibrosis, to recommend their use in the prevention, diagnosis, and prognosis of HF. Several other biomarkers in HF have been implicated but there remains a paucity of data defining their value and the need for further research, including that on biomarkers of inflammation.

Biomarkers including C-reactive protein (CRP) and pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) have been found to be elevated in those with HF (28–31) and associated with HF onset (32–35) and prognosis (30, 36–39). One of the two soluble TNF-α receptors, soluble tumor necrosis factor-α receptor-1 (sTNF-R1), is also used as a marker of TNF-α activity and has been shown to be elevated in HF patients and associated

with disease severity (40). However, few have investigated the relationship of each of these biomarkers by HF subtype (29, 37, 41, 42). The aim of this study was to investigate the association between elevated high-sensitivity CRP (hs-CRP), IL-6, and sTNF-R1 and incident HFrEF and HFpEF in a diverse sample of U.S. adults who participated in the Multi-Ethnic Study of Atherosclerosis (MESA).

METHODS

This study analyzed data from the MESA (43), a continuous, multicenter prospective cohort study sponsored by the National Heart Lung and Blood Institute of the National Institutes of Health. The primary objectives of the MESA are to 1) determine characteristics related to the progression of subclinical CVD and 2) to determine the characteristics related to the progression of subclinical to clinically overt CVD in a diverse, population-based sample. Details on the study have been published elsewhere (43). In brief, participants were recruited from six regions in the United States (US) and the final sample included 6,814 Caucasian, African American, Hispanic, and Chinese men and women (45-84 years of age) who were free of any known CVD at baseline. The first exam took place over 24 months from 2000-2002 and four additional exams have been completed since 2012. Participants were contacted for a follow-up interview every 9-12 months to determine if any new CVD conditions, hospitalizations, treatments, or changes in life habits had occurred. Additionally, information on any CVD events that occurred during follow-up was collected from participant interviews, medical records, autopsy reports, death certificates, and, in the case of out-of-hospital deaths, interviews with or questionnaires administered to physicians, relatives, or friends. Data from the MESA was requested and obtained from the National Institutes of Health/National Heart, Lung, and Blood Institute: Biologic Specimen and Data

Repository Information Coordinating Center (44). The use of MESA data was approved by the Institutional Review Board of the University of North Florida.

Dependent Variable

The primary dependent variable was time to HF. Heart failure is an adjudicated event in which interim medical records and deaths records are abstracted and reviewed by at least two physicians. Eligible HF events are classified as either definite, probable, or absent. Since asymptomatic disease is not an endpoint of the MESA, HF classification requires the presence of HF symptoms. In addition to symptoms, classification of probable HF also requires a physician diagnosis of HF and evidence of the patient receiving medical treatment for HF. Classification of definite HF requires the same criteria as probable HF and one or more additional criterion such as pulmonary edema/congestion; dilated ventricle or poor LV function; or evidence of LV diastolic dysfunction. Participants with a diagnosis of HF but no other evidence are considered to not have HF. For those with verified HF events, available EF information is obtained and recorded either by the quantitative value or as 'Normal' or 'Low.' In the present study, incident HF overall included those determined to have either probable or definite HF. Participants with an EF ≤ 50% or with an EF classification of 'Low' at the time of diagnosis were classified as HFrEF, and those with an EF ≥ 50% or an EF classification of 'Normal' were classified as HFpEF.

Independent Variables

The inflammatory biomarkers of interest included hs-CRP, IL-6 and sTNF-R1. Blood samples were drawn after at least 12-hours of fasting, processed 15-30 minutes following venipuncture, and stored at either the University of Vermont or the University of Minnesota (45). All biomarkers were measured in the Laboratory for Clinical Biochemistry Research at

University of Vermont (Burlington, VT). High sensitivity CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). This instrument utilizes a particle enhanced immunonephelometric assay to determine hs-CRP concentration. The hs-CRP variable was dichotomized as elevated (> 3-10 mg/L) or normal (< 3 mg/L) (46). Both IL-6 and sTNF-R1 concentrations were measured using an ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay and Quantikine Human STNF R1 Immunoassay; R&D Systems, Minneapolis, MN). For both IL-6 and sTNF-R1, participants were grouped by age bands of 10 (45-55, 56-65, 66-75, > 75) and gender (M/F) and dichotomized at the 75th percentile as either elevated or normal for each group. All elevated groups were combined into one group and all normal groups were also combined.

Other Independent Variables

Age, sex, race/ethnicity, physical activity (PA) and smoking status were self-reported at baseline on the personal history form. Age was categorized into four groups; 45-55, 56-65, 66-75, and > 75 years of age for the descriptive results but was used as a continuous variable in the analysis. The PA variable was dichotomized using the U.S. Department of Health and Human Services (DHHS) PA guidelines (47); values ≥ 500 MET·min/wk were considered sufficient and those < 500 MET·min/wk were considered insufficient. Smoking status was a calculated variable in MESA in which participants were categorized as never smokers, former smokers or current smokers. Family history of myocardial infarction (MI) was self-reported on the medical history form and included any history of MI in parents, siblings, or children. Waist circumference (WC) measurements ≥ 102 cm or ≥ 88 cm for men and women, respectively, were considered at risk. Blood pressure (BP) was categorized into three groups: systolic blood pressure (SBP) < 120 mmHg and diastolic blood pressure (DBP) < 80 mmHg was considered normal: SBP 120-129

mmHg and DBP < 80 mmHg elevated: and SBP > 130 mmHg or DBP ≥ 80 mmHg hypertensive. Participants who reported anti-hypertensive medication use were also considered hypertensive. Using the National Cholesterol Education Program Guidelines (48), triglyceride values ≥ 150 mg/dL and low-density lipoprotein values ≥ 130 mg/dL were considered elevated. High-density lipoprotein levels < 40 mg/dL for females or < 50 mg/dL for males were considered low. Participants who reported lipid-lowering medication use were also considered to have elevated lipid levels. All self-reported medication use was confirmed during a medication use interview. Anti-inflammatory medications included; oral steroids (Y/N), statins (Y/N), aspirin (Y/N), and non-steroidal anti-inflammatory agents (excluding aspirin) (Y/N).

STATISTICAL ANALYSIS

Data was managed using SAS version 9.4 (SAS Institute, Cary, NC) (49). SAS was used for variable recoding, coding verification, and statistical analyses. Descriptive characteristics were obtained using the means and frequency procedures for continuous and categorical variables, respectively. The univariate procedure was used to calculate outliers and to determine the 75th percentile of IL-6 and sTNF-R1. SAS was used to log-transform hs-CRP following dichotomization and IL-6 and sTNF-R1 prior to classification. Separate proportional hazards regression models were used to calculate multivariable adjusted hazard ratios (HRs) to determine risk of HF overall, HFrEF, and HFpEF in those with elevated hs-CRP, IL-6 and sTNF-R1 concentrations at baseline. The level of significance was set at $\alpha = 0.05$ for all tests. Heart failure participants without EF data were excluded from the subtype analyses.

After creating unadjusted and age-adjusted models, three additional models were made. Model 1 adjusted for demographics and behavioral covariates including age, gender,

race/ethnicity, smoking, and PA. Model 2 adjusted for the variables in Model 1 plus WC, BP, HDL, LDL, triglycerides, and self-reported family history of MI. Model 3, the fully adjusted model, included Model 2 covariates plus anti-inflammatory medication use including statins, oral steroids, aspirin, and NSAIDS (excluding aspirin).

In a separate analysis using proportional hazards regression, covariates included in the fully adjusted model were chosen using a stepwise backward elimination process. For each model that was tested, potential covariates that did not contribute significantly based on $p = 0.05$ were removed and excluded from subsequent analyses. A final parsimonious model was included to help elucidate the relationship between the independent and dependent variables.

RESULTS

During ~11.2 years of follow-up, 178 adults developed HF. Of these participants, 158 had the appropriate EF data for the subtype analysis, resulting in 87 categorized as HFrEF (55.1%) and 71 categorized as HFpEF (44.9%). Table 1 illustrates sample characteristics according to HF status and subtype.

Table 1. Baseline Characteristics of U.S. Adults by HF Status

Total (N=6,814)	Total HF N (%)	HFrEF N (%)	HFpEF N (%)	No HF N (%)
Total N	178 (2.61)	87 (1.28)	71 (1.05)	6,636 (97.39)
Age, Mean (SD)	68.9 (8.72)	67.7 (8.99)	70.1 (8.31)	61.9 (10.20)
Age Categories				
45-55	16 (8.99)	9 (10.34)	6 (8.45)	2130 (32.10)
56-65	43 (24.16)	24 (27.59)	14 (19.72)	1895 (28.56)
66-75	71 (39.89)	32 (36.78)	30 (42.25)	1853 (27.92)
> 75	48 (26.97)	22 (25.29)	21 (29.58)	758 (11.42)
Gender				
Male	106 (59.55)	61 (70.11)	36 (50.70)	3107 (46.82)
Female	72 (40.45)	26 (29.89)	35 (49.30)	3529 (53.18)
Race/Ethnicity				
Caucasian	69 (38.76)	33 (37.93)	29 (40.85)	2554 (38.49)
African American	61 (34.27)	35 (40.23)	20 (28.17)	1830 (27.58)
Hispanic	38 (21.35)	17 (19.54)	15 (21.13)	1458 (21.97)
Chinese	10 (5.62)	2 (2.30)	7 (9.86)	794 (11.97)
Smoking Status				
Never	72 (40.68)	35 (40.70)	28 (40.00)	3346 (50.58)
Former	77 (43.50)	33 (38.37)	37 (52.11)	2410 (36.43)
Current	28 (15.82)	18 (20.93)	8 (11.27)	859 (12.99)
Physical Activity Level				
≥ 500 MET-min/wk	93 (52.25)	45 (51.72)	39 (54.93)	4079 (61.47)
< 500 MET-min/wk	85 (47.75)	42 (48.28)	32 (45.07)	2557 (38.53)
Waist Circumference (cm)				
< 102, M; < 88 W	59 (33.15)	33 (37.93)	20 (28.17)	3032(45.69)
≥ 102, M; ≥ 88 W	119 (66.85)	54 (62.07)	51 (71.83)	3604 (54.31)
Blood Pressure				
Normal	16 (8.99)	9 (10.34)	5 (7.04)	2191 (33.03)
Elevated	10 (5.62)	3 (3.45)	6 (8.45)	532 (8.02)
Hypertensive	152 (85.39)	75 (86.21)	60 (84.51)	3911 (58.95)
Family history of MI				
No	84 (52.83)	34 (43.59)	41 (65.08)	3577 (57.36)
Yes	75 (47.17)	44 (56.41)	22 (34.92)	2659 (42.64)
Total Cholesterol (mg/dL)				
LDL-C≥ 130	75 (43.10)	37 (43.53)	28 (40.00)	3009 (45.92)
TG ≥ 150	87 (48.88)	41 (47.13)	35 (49.30)	2642 (39.95)
HDL-C < 40, M; < 50 W	96 (53.93)	52 (59.77)	34 (47.89)	3082 (46.46)
hs-CRP				
Normal	86 (56.58)	42 (53.85)	35 (59.32)	4235 (70.12)
Elevated	66 (43.42)	36 (46.15)	24 (40.68)	1805 (29.88)
IL-6				
Normal	87 (63.04)	47 (64.38)	31 (60.78)	4328 (75.31)
Elevated	51 (36.96)	26 (35.62)	20 (39.22)	1419 (24.69)
sTNF-R1				
Normal	35 (59.32)	15 (60.00)	15 (60.00)	1900 (75.31)
Elevated	24 (40.68)	10 (40.00)	10 (40.00)	623 (24.69)
Medications				
Statins	32 (17.98)	17 (19.54)	11 (15.49)	977 (14.73)

Oral Steroids	7 (3.93)	5 (5.75)	2 (2.82)	98 (1.48)
Aspirin	63 (35.39)	31 (35.63)	24 (33.80)	1639 (24.71)
NSAIDs (excluding aspirin)	31 (17.42)	21 (24.14)	9 (12.68)	1158 (17.46)

HF, heart failure; HFrEF, heart failure reduced ejection fraction (EF ≤ 50 or "Low"); HFpEF, heart failure preserved ejection fraction (EF > 50 or "Normal"); MI, myocardial infarction; hs-CRP, high sensitivity C-reactive protein (> 3–10 mg/L); IL-6, interleukin-6 (75_{th} percentile); sTNF-R1, tumor necrosis factor-α receptor-1 (75_{th} percentile); NSAID, non-steroidal anti-inflammatory drug; M; men, W; women. Blood pressure (mmHg): Normal; SBP < 120 and DBP < 80, Elevated; SBP 120-129 and DBP < 80, Hypertensive; SBP ≥ 130 or DBP ≥ 80; LDL-C, low-density lipoprotein; HDL-C high-density lipoprotein; TG, triglyceride.

The prevalence of HF overall in the sample was 2.6% (1.5% of men and 1.0% of women, $p = 0.01$). On average, those with HF were significantly older than those without HF, regardless of subtype. There was a significantly greater proportion of males than females in HF overall ($p = 0.01$) and in HFrEF ($p < 0.001$), however, the same was not true for those with HFpEF ($p = 0.90$). There was a greater proportion of African Americans with HFrEF and a greater proportion of Caucasians in HFpEF.

Table 2. HRs for Incident HF, HFrEF, and HFpEF in U.S. Adults with Elevated Inflammatory Biomarkers

	HF Overall					
	hs-CRP		IL-6		sTNF-R1	
	HR	95% CI	HR	95% CI	HR	95% CI
Unadjusted	1.80***	1.31-2.49	1.83***	1.29-2.58	2.12**	1.26-3.57
Age adjusted	1.86***	1.35-2.57	1.81***	1.28-2.56	2.08**	1.24-3.50
Model 1	1.85***	1.32-2.58	1.66**	1.16-2.36	1.88*	1.04-3.20
Model 2	1.77**	1.23-2.54	1.56*	1.06-2.29	1.72	0.95-3.12
Model 3	1.73**	1.20-2.50	1.50*	1.02-2.22	1.64	0.93-3.06
	HFrEF					
	hs-CRP		IL-6		sTNF-R1	
	HR	95% CI	HR	95% CI	HR	95% CI
Unadjusted	2.02***	1.29-3.16	1.73*	1.07-2.80	2.07	0.93-4.61
Age adjusted	2.06***	1.32-3.22	1.72*	1.07-2.78	2.04	0.92-4.56
Model 1	2.05**	1.29-3.26	1.49	0.91-2.44	1.68	0.73-3.88
Model 2	1.94**	1.17-3.22	1.47	0.86-2.51	1.77	0.70-4.44
Model 3	1.90**	1.14-3.15	1.39	0.81-2.38	1.69	0.67-4.24
	HFpEF					
	hs-CRP		IL-6		sTNF-R1	
	HR	95% CI	HR	95% CI	HR	95% CI
Unadjusted	1.62	0.96-2.73	2.02**	1.15-3.55	2.08	0.93-4.64
Age adjusted	1.72*	1.02-2.90	2.01**	1.14-3.53	2.07	0.93-4.61
Model 1	1.79*	1.04-3.09	2.02**	1.14-3.59	2.02	0.90-4.55
Model 2	1.77	0.98-3.18	1.70	0.91-3.17	1.89	0.78-4.59
Model 3	1.76	0.97-3.18	1.69	0.90-3.16	1.96	0.81-4.75

HF, heart failure; HFrEF, heart failure with reduced ejection fraction (EF < 50 or "Low"); HFpEF, heart failure with preserved ejection fraction (EF ≥ 50 or "Normal"); hs-CRP, high-sensitivity C-reactive protein (> 3-10 mg/L); IL-6, interleukin-6 (75th percentile); sTNF-R1, soluble tumor necrosis factor-α receptor-1 (75th percentile); HR, hazard ratio; CI, confidence interval.
*$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$
Model 1: Adjusted for age, gender, race/ethnicity, smoking, physical activity; Model 2: Model 1 + waist circumference, blood pressure, HDL-C, LDL-C, triglycerides, family history of myocardial infarction; Model 3: Model 2 + statins, oral steroids, aspirin, NSAIDs (excluding aspirin)

Risk of HF

Table 2 displays the multivariable adjusted HR obtained using the proportional hazards regression procedure for HF overall, HFrEF, and HFpEF. In the fully adjusted model, participants with elevated hs-CRP had a significantly greater risk of HF overall (HR 1.73; 95% Confidence Interval [CI] 1.20-2.50; $p \leq 0.01$). In those with elevated IL-6, the risk of HF overall

was attenuated after controlling for covariates but remained statistically significant in the in the fully adjusted model (HR 1.50; 95% CI 1.02-2.22; $p < 0.05$). In the age-adjusted model, adults with elevated sTNF-R1 concentrations had a significantly greater risk of HF overall however, after adjusting for anti-inflammatory medications, this relationship was borderline significant ($p = 0.06$).

Table 3 includes the HRs of HF overall in the parsimonious models. In this analysis, elevated hs-CRP, IL-6 and sTNF-R1 were all significantly associated with an increased risk of HF overall with the greatest risk seen in elevated sTNF-R1 (HR 1.91; 95% CI 1.08-3.38; $p < 0.05$). Females had a significantly lower risk of HF compared to males with the lowest risk seen in the hs-CRP model (HR 0.43; 95% CI 0.29-0.64; $p \leq 0.001$). Self-reported smokers had a two-time greater risk of HF whereas those who were hypertensive at baseline showed a three to four-time greater risk of HF in all three models. Overall, the significant contributors seen across all three models were the same apart from PA in IL-6, and WC and oral steroids in sTNF-R1, which did not significantly contribute to either model.

Table 3. HRs for Incident HF in U.S. Adults with Elevated Inflammatory Markers

	hs-CRP		IL-6		sTNF-R1	
	HR	95% CI	HR	95% CI	HR	95% CI
Biomarker						
Elevated	1.76	1.22-2.52**	1.57	1.07-2.30*	1.91	1.08-3.38*
Age	1.07	1.04-1.09***	1.07	1.04-1.09***	1.09	1.06-1.13***
Gender						
Male						
Female	0.43	0.29-0.64***	0.47	0.31-0.70***	0.54	0.30-0.99*
Smoking Status						
Never						
Former	1.07	0.72-1.58	1.21	0.81-1.82	0.99	0.52-1.91
Current	2.24	1.35-3.70**	2.50	1.45-4.33***	2.92	1.30-6.57**
Blood Pressure						
Normal						
Elevated	1.50	0.56-4.01	2.22	0.76-6.42	2.90	0.71-11.77
Hypertensive	3.16	1.71-5.83**	4.35	2.08-9.09***	4.03	1.41-11.51**
Physical Activity Level						
≥ 500 MET-min/wk						
< 500 MET-min/wk	1.44	1.01-2.04*			1.96	1.19-3.45**
Waist Circumference (cm)						
< 102, M; < 88 W						
≥ 102, M; ≥ 88 W	1.54	1.05-2.28*	1.62	1.08-2.43**		
Medications						
Oral Steroids	2.50	1.09-5.73*	3.33	1.45-7.63**		

HF, heart failure; hs-CRP, high sensitivity C-reactive protein (> 3-10 mg/L); IL-6, interleukin-6 (75^{th} percentile); sTNF-R1, soluble tumor necrosis factor-α receptor-1 (75^{th} percentile); HR, hazard ratio; CI, confidence interval; M; men, W; women. Blood pressure (mmHg): Normal; SBP < 120 and DBP < 80, Elevated; SBP 120-129 and DBP < 80, Hypertensive; SBP ≥ 130 or DBP ≥ 80. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

Risk of HFrEF

In the fully adjusted model, participants with elevated hs-CRP had a significant increased risk of HFrEF (HR 1.90; 95% CI 1.14-3.15; $p \leq 0.01$) (Table 2). For those with elevated baseline IL-6 concentrations, the age-adjusted model revealed a significant 72% increased risk of HFrEF (HR 1.72; 95% CI 1.07-2.78; $p < 0.05$), however, this relationship did not remain statistically significant in the fully adjusted model. Elevated sTNF-R1 was not significantly associated with risk of HFrEF in any model.

Table 4 includes the HRs of HFrEF in the parsimonious models. Elevated hs-CRP was associated with a significant two-time greater risk of HFrEF (HR 2.05; 95% CI 1.26-3.35; $p \leq 0.01$). For IL-6, when the smoking status variable was added to the model the relationship was attenuated and longer statistically significant, so the model was stratified by never smokers and current or former smokers. This revealed that for smokers or former smokers, IL-6 did not significantly contribute to the model, however, for never smokers it did (HR 1.71; 95% CI 0.88-3.32; $p > 0.05$, and HR 2.33; 95% CI 1.04-5.23; $p < 0.05$, respectively). Elevated sTNF-R1 was not significantly associated with risk of HFrEF in any model (data not shown).

Table 4. HRs for Incident HFrEF in U.S. Adults with Elevated Inflammatory Markers

	hs-CRP		IL-6			
			Never Smokers		Ever Smokers	
	HR	95% CI	HR	95% CI	HR	95% CI
Biomarker						
Elevated	2.05	1.26-3.35**	2.33	1. 04-5.23*	1.71	0.88-3.32
Age	1.04	1.02-1.07***	1.06	1.02-1.10**	1.05	1.01-1.09**
Gender						
Male						
Female	0.28	0.16-0.49***	0.29	0.12-0.67**	0.25	0.10-0.61**
Medications						
Oral Steroids	4.80	1.91-12.06***	8.81	1.99-38.98**		
Smoking Status						
Never						
Former	0.91	0.52-1.60				
Current	2.71	1.43-5.13**				
Family hx						
No						
Yes	1.89	1.16-3.07**				
Blood Pressure						
Normal						
Elevated	0.88	0.18-4.28				
Hypertensive	3.38	1.51-7.55**				

HFrEF, heart failure with reduced ejection fraction (EF < 50 or "Low"); hs-CRP, high-sensitivity C-reactive protein (> 3-10 mg/L); IL-6, interleukin-6 (75th percentile); HR, hazard ratio; CI, confidence interval; M; men, W; women. Blood pressure (mmHg): Normal; SBP < 120 and DBP < 80, Elevated; SBP 120-129 and DBP < 80, Hypertensive; SBP ≥ 130 or DBP ≥ 80. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

Risk of HFpEF

Those with elevated hs-CRP had a borderline 76% increased risk of HFpEF in the fully adjusted model (HR 1.76; 95% CI 0.97-3.18; $p = 0.06$). In IL-6 the age-adjusted model revealed a significant increased risk of HFpEF (HR 2.05; 95% CI 1.17-3.60; $p = 0.01$), however, after adjusting for WC this relationship did not remain statistically significant. Elevated sTNF-R1 was not significantly associated with risk of HFpEF in any model.

Table 5 includes the HRs of HFpEF in the parsimonious models. Elevated hs-CRP was associated with a significant 89% increased risk of HFpEF and nearly a five-times greater risk in hypertensive individuals compared to their normotensive counterparts. For IL-6, when the race/ethnicity variable was added to the model, the relationship was no longer significant, so the model was stratified by race/ethnicity. This revealed that African Americans with elevated IL-6 had almost a six-time significantly greater risk of HFpEF (HR 5.89; 95% CI 1.52-22.80; $p \leq 0.01$). There was not a significant relationship seen with elevated IL-6 in Caucasians, Hispanics, or Chinese Americans (data not shown). Elevated sTNF-R1 was not significantly associated with risk of HFrEF in any model (data not shown).

Table 5. HRs for Incident HFpEF in U.S. Adults with Elevated Inflammatory Markers

	hs-CRP		IL-6 African Americans	
	HR	95% CI	HR	95% CI
Inflammatory Biomarker				
Elevated	1.89	1.09-3.28*	5.89	1.52-22.80**
Age	1.09	1.05-1.13***	1.09	1.02-1.17**
Blood Pressure				
Normal				
Elevated	2.83	0.56-14.13		
Hypertensive	4.73	1.44-15.51**		

HFpEF, heart failure with preserved ejection fraction (EF ≥ 50 or "Normal"); hs-CRP, high-sensitivity C-reactive protein (> 3-10 mg/L); IL-6, interleukin-6 (75th percentile); HR, hazard ratio; CI, confidence interval. Normal; SBP < 120 and DBP < 80, Elevated; SBP 120-129 and DBP < 80, Hypertensive; SBP ≥ 130 or DBP ≥ 80 *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

DISCUSSION

Recent reports (11, 26, 50) have addressed the need for investigating the novel use of biomarkers in the evaluation and management of HF patients. In this multi-ethnic, population-based sample, elevated hs-CRP (> 3-10 mg/L), IL-6 (> 75th percentile) and sTNF-R1 (> 75th percentile) were associated with a significant increase in risk of HF. When examining risk by HF subtype, hs-CRP was a significant predictor in both HFrEF and HFpEF. Interestingly, baseline IL-6 concentrations were a significant predictor of HFrEF in nonsmokers only and of HFpEF in African Americans only. These findings add to the evidence demonstrating a positive relationship between biomarkers of inflammation in HF (32, 35, 51) and to the risk differences seen in HFrEF and HFpEF (17).

Many studies that have examined the associations between biomarkers of inflammation and HF have either included only those with HFrEF or did not stratify by HF subtype at all (28, 32, 35, 36). In a 2018 study (52), DuBrock et al. investigated the relationship between CRP and HFpEF (≥ 50%) in 216 outpatients with objective evidence of HF. They reported that CRP was

elevated in about 60% of patients in this sample, which is much higher than in our study (40%). This is likely because our sample was free from clinical cardiovascular disease at baseline when CRP values were measured whereas DuBrock et al.'s analysis measured CRP values in HF patients, who are already more likely to have elevated CRP (51).

Our findings also add to the research examining the race/ethnicities differences in HF and the HF subtypes, the latter being much less established. In a 2005 community cohort study (6), Galasko et al. examined ethnic differences in the prevalence and etiology of LV systolic dysfunction in 734 patients ≥ 45 years of age. The investigators found a similar overall prevalence of LV systolic dysfunction among white and non-white participants. However, though the aim was to examine ethnic differences, the sample was primarily white (71%) and the majority of the non-white patients were South Asian, which are more likely to have LV diastolic dysfunction (53).

In a 2008 study (32), Bahrami et al. examined race/ethnicity differences in 79 incident CHF patients after a median 4.0 years of follow-up in the MESA. African Americans were found to have the highest incidence rates of CHF and the greatest risk of developing CHF, however, this relationship was no longer significant after adjusting for hypertension and/or diabetes. Interestingly, African Americans had the highest proportion of CHF that was not preceded by clinical MI ($p = 0.06$). This is interesting because African Americans have the greatest risk of MI (54) and of the two HF subtypes, previous MI is a significantly stronger risk factor for HFrEF than HFpEF (17). The present study revealed a significant increased risk of HFpEF in African Americans with elevated IL-6 but not any other race/ethnicity. Studies that have examined IL-6 across difference race/ethnicities have been contradicting (55, 56) and this relationship warrants further investigation.

The predictive value of IL-6 in HFrEF was dependent on smoking status in this analysis. This is interesting since both IL-6 and smoking are associated with inflammation in HF patients (57, 58). Cigarette use is a well-known, strong risk factor in HFrEF, but in nonsmokers, there may be indication for IL-6 to take its place in predicting risk according to our analysis. The Health, Aging, and Body Composition Study (59), examined the predictive value of CRP, TNF-α, and IL-6 in incident HF. The investigators reported that adding IL-6 to their risk prediction model improved the performance of the model.

Due to the sequential discovery of HFrEF and HFpEF, many early clinical trials only included those with HFrEF and the life-prolonging therapies that have been successfully implemented over the past 30 years in this subtype have not been efficacious for those with HFpEF (13). In the 2016 guidelines for the diagnosis and treatment of acute and chronic HF (13), the European Society of Cardiology stated that no treatment to date has convincingly been shown to reduce morbidity or mortality in HFpEF patients. Current treatment recommendations for HFpEF focus on managing comorbidities and alleviating symptoms, however, there is emerging evidence that inflammation might be the target for prevention intervention (14, 59). Cytokines themselves may be a potential therapeutic target in some patients (60), however, in the prevention of HF, the role of lifestyle behaviors should also be considered. Greater achievement of the AHA's Life's Simple 7 guidelines (61) on smoking, body mass, PA, diet, cholesterol, BP, and glucose has been associated with a lower lifetime risk of HF as well as preservation of cardiac structure and function in old age. In the present study, PA levels significantly added the hs-CRP and sTNF-R1 models. Additionally, insufficient PA (< 500 MET·min/wk) in accordance to the 2018 DHHS PA guidelines (47) was also associated with an increased risk of HF overall. Chronic exercise training has been shown to have anti-

inflammatory effects, independent of body fat loss, in adults of any age and even with chronic conditions including HF and type 2 diabetes (62). Even acute 20-min bouts of exercise have been shown to decrease inflammatory responses via down regulation of TNF-α (63).

In a 2019 study, Upadhya et al. (14) reviewed several non-pharmacological interventions that have been done in HFpEF including exercise interventions. Despite exercise intolerance being a cardinal sign in HF, the exercise interventions revealed several positive outcomes including increased exercise capacity, peak oxygen consumption, quality of life and even diastolic dysfunction. Additionally, this review provides an exercise prescription for those with stable HFpEF, which could prove useful for clinicians working with HFpEF patients. Considering the lack of effective therapies in HFpEF, this evidence underscores the importance of PA in reducing inflammation and in the prevention and treatment of HF.

There are a few limitations to this study. Inherent to the design of the MESA to create an ethnically diverse sample, the sample is not representative of US adults, thus affecting the external validity of the results. Additionally, biomarkers of inflammation were measured at a single time point, therefore, it is unclear if elevated concentrations were indicative of acute or chronic inflammation. However, all outliers were excluded, and serial measurements of these biomarkers have been shown to add little information (64). Another limitation of this study is the small sample size among the HF samples. Additionally, only a subset of the population had measured sTNF-R1 values, which contributed to the lack of significant findings seen in this biomarker when stratified by HF subtype. Incident HF was defined as time to first HF event, therefore subsequent HF events and potential changes in EF over time were not included in the analysis.

In summary, the results of this study suggested that elevated hs-CRP, IL-6 and sTNF-R1 are positively associated with HF risk. Elevated hs-CRP was also positively associated with risk of HFrEF and HFpEF. Elevated IL-6 predicted HFrEF among nonsmokers only and HFpEF in African Americans only. Further research is necessary to determine the pathophysiological mechanisms behind these findings and to further elucidate the associations between HFrEF and HFpEF. The combined use of various biomarkers in determining the prognosis and diagnosis of HF should also be examined.

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Appendices

Appendix A

UNF
UNIVERSITY of NORTH FLORIDA.

Office of Research and Sponsored Programs
1 UNF Drive
Jacksonville, FL 32224-2665
904-620-2455 FAX 904-620-2457
Equal Opportunity/Equal Access/Affirmative Action Institution

MEMORANDUM

DATE: March 30, 2020

TO: Ms. Michelle Stone

VIA: Dr. James Churilla

DEPT: Clinical & Applied Movement Sciences

FROM: Dr. Jennifer Wesely, Chairperson
UNF Institutional Review Board

RE: Review conducted on behalf of the UNF Institutional Review Board
"Biomarkers of Inflammation in Heart Failure Patients with Reduced and Preserved Ejection Fractions: Multi-Ethnic Study of Atherosclerosis"

This is to advise you that your project, "Biomarkers of Inflammation in Heart Failure Patients with Reduced and Preserved Ejection Fractions: Multi-Ethnic Study of Atherosclerosis," was reviewed on behalf of the UNF Institutional Review Board and was declared "not research involving human subjects" based on the definitions provided in the U.S. Department of Health and Human Services Code of Federal Regulations found at 45 CFR 46.102. As such, this project qualifies for a Waiver of IRB Review.

Please note, this waiver does not absolve the Principal Investigator from complying with other federal, state, or local laws or institutional policies and procedures that may be applicable in the conduct of this project.

This waiver applies to your project in the form and content as submitted to the IRB for review. Any variations or modifications to this project involving the participation of human subjects must be approved by the IRB prior to implementing such changes. Please maintain a copy of this waiver for your records.

Thank you for submitting your project to the IRB for consideration. Should you have any questions or if we can be of further assistance, please contact the Research Integrity office at 904-620-2455, or IRB@unf.edu.

IRB Form Version 01.03.2019

Vita

Michelle L. Stone is a graduate teaching and research assistant in the Clinical and Applied Movement Sciences department at the University of North Florida (UNF) in Jacksonville, FL. There, she recently completed her master's degree in Exercise Science and Chronic Disease. Upon earning her Bachelor of Science in Health degree from UNF in 2014, where she majored in Exercise Science and graduated with Summa Cum Laude honors, she completed an internship in Durham, North Carolina at the Duke Molecular Physiology Institute and successfully obtained certification as an Exercise Physiologist from the American College of Sport's Medicine.

Originally from West Sacramento, California, Michelle currently lives in Jacksonville, FL with her partner, Travis, her cat, Duchess, and their dog, Nala. Her research interests consist of cardiovascular health, heart failure, physical activity, and translational research. Her future ambitions include attending medical school to help spread the knowledge of exercise science related research and implement this literature into her own practice and research endeavors.