C-Reactive Protein and N-Terminal Pro-brain Natriuretic Peptide Levels Correlate With Impaired Cardiorespiratory Fitness in Patients With Heart Failure Across a Wide Range of Ejection Fraction

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Background: Impaired cardiorespiratory fitness (CRF) is a hallmark of heart failure (HF). Serum levels of C-reactive protein (CRP), a systemic inflammatory marker, and of N-terminal pro-brain natriuretic peptide (NT-proBNP), a biomarker of myocardial strain, independently predict adverse outcomes in HF patients. Whether CRP and/or NT-proBNP also predict the degree of CRF impairment in HF patients across a wide range of ejection fraction is not yet established.

Methods: Using retrospective analysis, 200 patients with symptomatic HF who completed one or more treadmill cardiopulmonary exercise tests (CPX) using a symptom-limited ramp protocol and had paired measurements of serum high-sensitivity CRP and NT-proBNP on the same day were evaluated. Univariate and multivariate correlations were evaluated with linear regression after logarithmic transformation of CRP ($\log_{10}$) and NT-proBNP ($\log_N$).

Results: Mean age of patients was 57 ± 10 years and 55% were male. Median CRP levels were 3.7 [1.5–9.0] mg/L, and NT-proBNP levels were 377 [106–1,464] pg/ml, respectively. Mean peak oxygen consumption (peak VO_2) was 16 ± 4 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$. CRP levels significantly correlated with peakVO_2 in all patients ($R = -0.350$, $p < 0.001$) and also separately in the subgroup of patients with reduced left ventricular ejection fraction (LVEF) (HFrEF, $N = 109$) ($R = -0.282$, $p < 0.001$) and in those with preserved EF (HFpEF, $N = 57$) ($R = -0.459$, $p < 0.001$). NT-proBNP levels also significantly correlated with peak VO_2 in all patients ($R = -0.330$, $p < 0.001$) and separately in patients with HFrEF ($R = -0.342$, $p < 0.001$) and HFpEF ($R = -0.275$, $p = 0.032$). CRP and NT-proBNP did not correlate with each other ($R = 0.05$, $p = 0.426$), but independently predicted peak VO_2 ($R = 0.421$, $p < 0.001$ and $p < 0.001$, respectively).

Conclusions: Biomarkers of inflammation and myocardial strain independently predict peak VO$_2$ in HF patients. Anti-inflammatory therapies and therapies alleviating myocardial strain may independently improve CRF in HF patients across a large spectrum of LVEF.

Keywords: heart failure, biomarker, systemic inflammation, myocardial strain, cardiorespiratory fitness, cardiopulmonary exercise testing

INTRODUCTION

Heart failure (HF) is a syndrome that presents clinically with dyspnea, fatigue, and/or edema caused by structural or functional cardiac defects that lead to reduced cardiac output and/or increased cardiac pressure at rest or during stress. Impaired cardiorespiratory fitness (CRF) is a hallmark of heart failure (HF) (1). CRF is defined as the ability of the circulatory, respiratory, and muscular systems to supply oxygen during sustained physical activity (2). CRF is expressed in metabolic equivalents (METs) and measured by peak oxygen uptake (peak VO$_2$) using exercise tests (3). CRF is both an objective measure of habitual physical activity, as well as a prognostic indicator in HF.

C-reactive protein (CRP), a marker for systemic inflammation, is produced by the hepatocytes upon inflammation, infection, or tissue injury (4). Patients with HF show signs of chronic systemic inflammation, as shown by elevated serum levels of CRP (5). Increased levels of CRP are associated with an increased risk for CVD events and for mortality (6, 7). Higher CRP levels are also associated with worse cardiopulmonary exercise performance in patients with ischemic heart disease and systolic HF (8, 9).

Natriuretic peptides are peptide hormones that function as counter-regulatory mechanisms for the Renin-Angiotensin-Aldosterone-system (RAAS), and therefore cause a decrease in arterial pressure, central venous pressure, pulmonary capillary wedge pressure, cardiac output, and total blood volume through natriuresis and diuresis (10). Brain natriuretic peptide (BNP) is produced by the ventricles in response to an increase in myocardial stretch, damage, or ischemia (10). N-terminal pro-brain natriuretic peptide (NT-proBNP) is the biologically inactive peptide that is cleaved off the pro-hormone, proBNP (11). The diagnostic and prognostic power of both BNP and NT-proBNP

is similar, however, NT-proBNP is less sensitive to breakdown than BNP is, which results in a more accurate measurement and reproducibility (11). Plasma levels of NT-proBNP have shown to relate to a low peak oxygen uptake (peak VO$_2$) in HF patients (12).

Serum levels of CRP and NT-proBNP have shown to predict adverse outcomes in patients with HF, both in HF with reduced ejection fraction (HFrEF, LVEF <50%) and in HF with preserved ejection fraction (HFpEF, LVEF >50%). Although CRP and NT-proBNP provide independent and complementary insight into CRF, whether CRP and/or NT-proBNP also independently predict the degree of CRF impairment in HF patients is not yet established. We hypothesize that two biomarkers, CRP, and NT-proBNP, by acting as surrogates for different pathophysiologic mechanisms, inflammation and myocardial strain, respectively, will independently predict the degree of CRF impairment in patients with HF across the spectrum of LVEF including both HFrEF and HFpEF. The objective of this study was to investigate whether CRP and/or NT-proBNP can independently predict CRF impairment, defined as reduced peak VO$_2$ in patients with HF across a wide range of ejection fraction.

MATERIALS AND METHODS

Study Design

We retrospectively queried a database of de-identified data that was prospectively collected data from patients with symptomatic HF who completed one or more cardiopulmonary exercise tests (CPX) using a symptom-limited ramp protocol on a treadmill and had paired measurements of serum high-sensitivity CRP and NT-proBNP on the same day. All members of the research team have completed training on the ethical conduct of research on human subjects. The VCU Institutional Review Board approved of the study, which was conducted according to the International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki.

Cardiopulmonary Exercise Testing

All patients underwent maximal CPX with a certified exercise physiologist under the supervision of a physician with a metabolic cart connected to a treadmill (Vmax Encore, Viasys, Yora Linda, CA) using a ramp protocol, as described before (13). Oxygen and carbon dioxide sensors were calibrated before the test with known oxygen, nitrogen, and carbon dioxide concentrations and the flow sensor was calibrated using a 3-L syringe. Subjects were asked to exercise to maximal fatigue. Twelve-lead ECG measurements were done at baseline,

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Abbreviations: CRF, Cardiorespiratory Fitness; HF, Heart Failure; HFrEF, Heart Failure with Reduced Ejection Fraction; HFpEF, Heart Failure with Preserved Ejection Fraction; METs, Metabolic Equivalents; VO$_2$, Oxygen Consumption; CRP, C-Reactive Protein; NT-proBNP, N-Terminal Pro-Brain Natriuretic Peptide; WBC, White Blood Cell; NLR, Neutrophil to Leukocyte Ratio; ANP, Atrial Natriuretic Peptide; CVD, Cardiovascular Disease; CVP, Central Venous Pressure; RAAS, Renin Angiotensin Aldosterone System; LVEF, Left Ventricular Ejection Fraction; LVEDV, Left Ventricular End Diastolic Volume; LVESV, Left Ventricular End Systolic Volume; CPX, Cardiopulmonary Exercise Test; TET, Treadmill Exercise Time; Peak VO$_2$, Peak Oxygen Consumption; VE/VCO$_2$, Ventilatory Efficiency; ACS, Acute Coronary Syndrome; MI, Myocardial Infarction; ARNI, Angiotensin Receptor-Neprilysin Inhibitor; ACEI, Angiotensin-Converting-Enzyme Inhibitor; PARADIGM-HF, Prospective Comparison of ARNI with ACEI to Determine Impact on Global; Mortality and Morbidity in Heart Failure; CANTOS, Canakinumab Antiinflammatory Thrombosis Outcome Study; ROC, Receiver Operating Characteristic; AUC, Area Under the Curve.

throughout the test, and during recovery. Every 2 min blood pressure was measured using an automated exercise compatible device (Tango, SunTech Medical). Expired gases were sampled during exercise with a mouthpiece-mounted sensor and were analyzed continuously to measure oxygen consumption (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE). The peak VO_2 ($mlO_2 \bullet kg^{-1} \bullet min^{-1}$) during exercise was defined as the highest 10-s average value for VO_2 during the last 30 s of the exercise. Peak VO_2 measured during exercise is the most objective variable for assessment of functional capacity as is an important prognostic indicator (3). American Heart Association/American College of Cardiology guidelines for exercise testing contraindications and termination criteria were followed.

Doppler Echocardiography

Subjects underwent transthoracic Doppler echocardiogram. Echocardiography was performed according to the American Society of Echocardiography measurement guidelines and provides information on both cardiac dimensions and function (14). LV end-diastolic and end-systolic volumes (LVEDV, LVESV), EF, and early transmitral E wave velocities were obtained. Early mitral annulus (e′) velocities obtained by tissue Doppler were averaged between lateral and septal e′ and tricuspid annulus plane systolic excursion. The E/e′ ratio provides information on diastolic function and was calculated to estimate LV filling pressures (15, 16).

Biomarker Analysis

We analyzed differential comprehensive metabolic profile and plasma levels of biomarkers including high-sensitivity CRP and NT-proBNP. CRP, a marker for systemic inflammation, is increased in HF and has shown to relate to poor exercise performance (8, 9). CRP values <3.0 mg/L were considered within normal range. CRP values >3.0 mg/L have shown to be associated with an increased risk of cardiovascular disease (6). N-terminal pro-brain natriuretic peptide (NT-proBNP), a biomarker of myocardial strain, correlates with exercise capacity in HF (12). NT-proBNP values <300 ng/ml were considered within normal range (17). Both CRP and NT-proBNP independently predict adverse outcomes in patients with HF (7, 18, 19). Furthermore, we analyzed White Blood Cell (WBC) count, absolute neutrophils, and leukocyte count. Neutrophil to leukocyte ratio (NLR) is a measure for systemic inflammation (20). A NLR >4 has prognostic value in cardiovascular disease (21).

Data Analysis

For the data analysis, each of the tests were considered as a separate measurement for data analysis. We assessed the correlation between different clinical parameters and peak oxygen consumption (peak VO_2), as the preferred measures of CRF obtained during effort-limited maximal cardiopulmonary exercise testing (CPX). This was done for the entire HF group and then analyzed separately for 2 groups of patients stratified according to LVEF <50% (HFrEF) or ≥50% (HFpEF). According to the Fick principle, peak VO_2 is determined by stroke volume,

heart rate, and arterial-venous oxygen difference and therefore reflects both cardiac, vascular, and peripheral skeletal muscle components. The cause of exercise intolerance has been proposed to be different in HFrEF and HFpEF patients, therefore we stratified according to LVEF (22).

Statistical Analysis

Data was tested for deviation from Gaussian distribution using the Kolmogorov–Smirnov test, and due to a lack of deviation, is presented as mean and standard deviation. Data that deviated from Gaussian distribution is presented as median and interquartile range. For normally distributed continuous variables, differences between groups were evaluated using independent-samples t-test, and for not normally distributed variables with the Mann–Whitney U-test. Correlations between continuous variables were assessed using linear regression. Multivariate analysis using stepwise linear regression was used to assess predictors of CRF with available clinical parameters. A ROC curve analysis was performed to evaluate whether the biomarkers had discriminative value for reduced CRF defined as peak VO_2 <10 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ and peak VO_2 <14 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$. SPSS Statistics 24.0 (IBM, Armonk, NY) statistical software package was used for all analyses. No missing data imputation method used. A p-value < 0.05 was considered statistically significant.

TABLE 1 | Clinical characteristics in all patients.

Patient characteristics ($N = 200$)	
Age, y	57 (10)
Male sex, n (%)	110 (55%)
BMI, kg/m^2	35 (8)
BIOMARKERS	
CRP, mg/L	7.1 (8.9) 3.7 [1.5–9.0]
WBC, $\times 10^9$/L	7.2 (2.4)
Absolute Neutrophils, $\times 10^9$/L	4.0 [3.1–5.2]
Absolute Lymphocytes, $\times 10^9$/L	1.9 (0.6)
Neutrophil-to-Lymphocyte ratio	2.3 [1.6–3.1]
NT-proBNP, ng/ml	1306 (3092) 377 [106–1464]
DOPPLER ECHOCARDIOGRAPHY PARAMETERS	
LVEF, %	44 (14)
E/e'	15 (8)
CARDIOPULMONARY EXERCISE PARAMETERS	
Treadmill exercise time, min	8.7 (2.8)
Peak VO_2, $mlO_2 \bullet kg^{-1} \bullet min^{-1}$	16 (4)
Peak VO_2 % of predicted	54 (16)
VE/VCO$_2$ slope	33 (7)
DASI score	30 (16)
MLWHF score	46 (26)

BMI, Body Mass Index; WBC, White Blood Cell Count; NLR, Neutrophil to Leukocyte Ratio; DASI, Duke Activity Status Index; MLWHF, Minnesota Living With Heart Failure; LVEF, Left Ventricular Ejection Fraction; VO$_2$, Oxygen uptake; VE, Minute Ventilation; VCO$_2$, Carbon Dioxide output. Data shown as mean and (standard deviation) or median and [interquartile range].

RESULTS

We evaluated a total of 366 CPX from 200 different patients (2.0 ± 1.3 studies per patient) of which the clinical characteristics are shown in **Table 1** for all patients. Clinical characteristics are shown separately for patients with HF with reduced LVEF (LVEF <50%) and for patients with HF with preserved LVEF HFpEF (LVEF ≥50%) in **Table 2**. Mean age of patients was 57 ± 10 years and 110 (55%) were male. Mean LVEF was 44 ± 14% (HFrEF, $N = 109$ and HFpEF, $N = 57$) (**Figure 1**), with a mean LVEF of 36 ± 11% in HFrEF, and 58 ± 6% in HFpEF. Median high-sensitivity CRP levels were 3.7 [1.5–9.0] mg/L, respectively and median NT-proBNP levels were 377 [106–1,464] pg/ml, respectively. In HFrEF, median CRP levels were 4.2 [1.9–9.2] mg/L, and median NT-proBNP levels were 1,029 [280–2,263] pg/ml. In HFpEF, median CRP levels were 3.7 [0.6–10.8] mg/L and median NT-proBNP levels were 102 [46–183] pg/ml, respectively ($P < 0.001$ for NT-proBNP, $P = 0.03$ for CRP between HFpEF and HFrEF). The distribution of LVEF, CRP, and NT-proBNP is shown in **Figure 1**. Mean White Blood Cell (WBC) count and median Neutrophil to Leukocyte Ratio (NLR) was 7.2 (2.4) $\times 10^9$/L and 2.3 [1.6–3.1], with a mean WBC count and median NLR of 7.4 (2.6) $\times 10^9$/L and 2.0 [1.5–2.7] in HFpEF, and 6.9 (2.1) $\times 10^9$/L and 2.3 [1.6–3.2] in HFrEF, respectively, ($P = 0.198$ for WBC count, $P = 0.07$ for NLR). Mean peak oxygen consumption (peak VO$_2$) was 16 ± 4 mlO$_2 \bullet$ kg$^{-1} \bullet$ min^{-1} and mean treadmill exercise time (TET) was 8.7 ± 2.8 min.

CRP as a Predictor of CRF

CRP levels significantly and inversely correlated with peak VO$_2$ ($R = -0.350$, $p < 0.001$, $N = 316$), and with TET ($R = -0.342$, $p < 0.001$, $N = 314$) as shown in **Figure 2**. The association between CRP and peak VO$_2$ and between CRP and TET remained significant when the analysis was limited to HFrEF patients ($R = -0.282$, $p < 0.001$, $N = 192$ for peak VO$_2$ and $R = 0.336$, $p < 0.001$, $N = 190$ for TET) and to HFpEF patients ($R = -0.459$, $p < 0.001$, $N = 90$ for peak VO$_2$ and $R = -0.345$, $p < 0.01$, $N = 90$ for TET).

NT-proBNP as a Predictor of CRF

NT-proBNP levels significantly and inversely correlated with peak VO$_2$ ($R = -0.330$, $p < 0.001$, $N = 258$) and with TET ($R = -0.412$, $p < 0.001$, $N = 256$) as shown in **Figure 3**. The association between NT-proBNP and peak VO$_2$ and between NT-proBNP and TET remained significant when the analysis was limited to HFrEF patients ($R = -0.354$, $p < 0.001$, $N = 168$ for peak VO$_2$ and $R = -0.437$, $p < 0.001$, $N = 166$ for TET) as well as in HFpEF patients ($R = -0.275$, $p = 0.032$, $N = 61$ for peak VO$_2$ and $R = -0.459$, $p < 0.001$, $N = 61$ for TET).
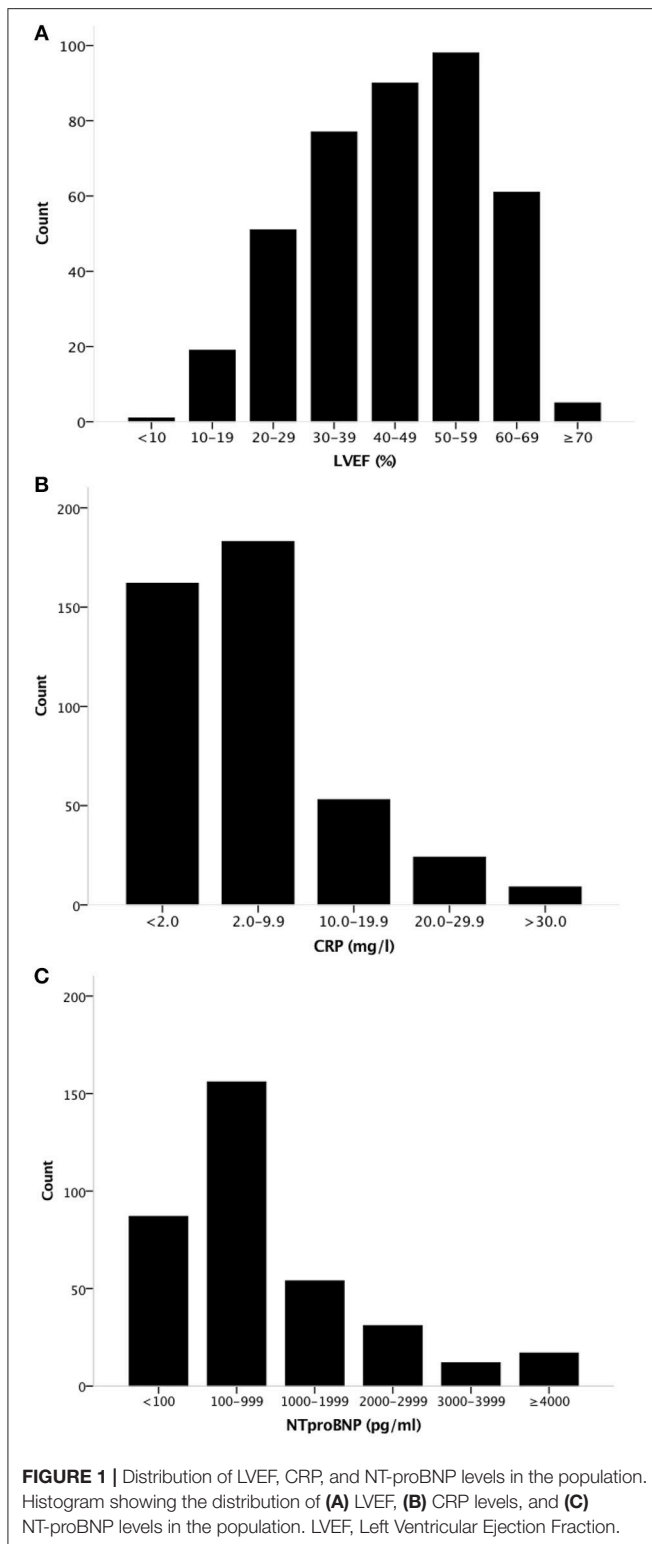
Multivariate Analysis

Multivariate analysis including CRP and NT-proBNP showed that both CRP and NT-proBNP independently predicted peak VO$_2$ ($R = 0.421$, $p < 0.001$ and $p < 0.001$, respectively), and TET ($R = 0.478$, $p < 0.001$, and $p < 0.001$, respectively). CRP and NT-proBNP did not exhibit collinearity ($R = +0.05$,

TABLE 2 | Clinical characteristics in HFpEF and HFrEF patients.

	HFpEF (N = 57)	HFrEF (N = 109)	
Age, y	53 (9)	57 (10)	$P = 0.03$
Male sex, n (%)	19 (33%)	78 (72%)	$P < 0.001$
BMI, kg/m^2	40 (8)	34 (8)	$P < 0.001$
BIOMARKERS			
CRP, mg/L	6.4 (7.1) 3.7 [0.6–10.8]	7.7 (9.7) 4.2 [1.9–9.2]	$P = 0.03$
NT-proBNP, ng/ml	172 (266) 102 [46–183]	1906 (3693) 1029 [280–2263]	$P < 0.001$
HgB, g/dl	13.1 (1.8)	13.2 (1.7)	$P = 0.75$
HBA1c, %	7.7 (1.9)	6.8 (1.4)	$P < 0.001$
WBC, $\times 10^9$/L	7.4 (2.6)	6.9 (2.1)	$P = 0.198$
Absolute Neutrophils, $\times 10^9$/L	4.1 [3.0–5.4]	4.0 [3.1–5.2]	$P = 0.71$
Absolute Lymphocytes, $\times 10^9$/L	2.1 (0.6)	1.8 (0.6)	$P = 0.002$
Neutrophil-to-Lymphocyte ratio	2.0 [1.5–2.7]	2.3 [1.6–3.2]	$P = 0.07$
DOPPLER ECHOCARDIOGRAPHY PARAMETERS			
LVEF, %	58 (6)	36 (11)	$P < 0.001$
LVEDV, ml	110 (32)	172 (62)	$P < 0.001$
LVESV, ml	47 (19)	115 (55)	$P < 0.001$
E'	8.1 (2.4)	6.5 (3.6)	$P = 0.01$
E/e'	11.3 (4.7)	17.2 (8.3)	$P < 0.001$
CARDIOPULMONARY EXERCISE PARAMETERS			
Treadmill exercise time, min	9.4 (2.6)	8.4 (2.8)	$P = 0.005$
Peak VO$_2$, mlO$_2 \bullet$ kg$^{-1} \bullet$ min^{-1}	16.5 (4.6)	15.4 (4.3)	$P = 0.04$
Peak VO$_2$ % of predicted	55 (17)	52 (14)	$P = 0.356$
VE/VCO$_2$ slope	30 (5)	33 (7)	$P < 0.001$

BMI, Body Mass Index; HgB, Hemoglobin; HBA1c, Hemoglobin A1c; WBC, White Blood Cell Count; NLR, Neutrophil to Leukocyte Ratio; LVEF, Left Ventricular Ejection Fraction; LVEDV, Left Ventricular End Diastolic Volume; LVESV, Left Ventricular End Systolic Volume; VO$_2$, Oxygen uptake; VE, Minute Ventilation; VCO$_2$, Carbon Dioxide output. Data shown as mean and (standard deviation) or median and [interquartile range].

FIGURE 1 | Distribution of LVEF, CRP, and NT-proBNP levels in the population. Histogram showing the distribution of **(A)** LVEF, **(B)** CRP levels, and **(C)** NT-proBNP levels in the population. LVEF, Left Ventricular Ejection Fraction.

patients, NT-proBNP predicted peak VO_2 independent from CRP ($R = 0.287$, $p = 0.5$ for CRP, $p = 0.04$ for NT-proBNP) and TET ($R = 0.459$, $p = 0.988$ for CRP, $p < 0.001$ for NT-proBNP). Furthermore, multivariate analysis including NT-proBNP, BMI, age, and sex significantly predicted peak VO_2 ($R = 0.631$, $p < 0.001$, $p < 0.001$, $p = 0.04$, respectively, with a trend for sex ($p = 0.08$). NT-proBNP, CRP, BMI, age, and sex significantly predicted TET ($R = 0.651$, $p < 0.001$, $p = 0.006$, $p < 0.001$, $p < 0.001$, $p = 0.029$, respectively). When the analysis was limited to HFrEF patients, BMI, age, sex, NT-proBNP, and CRP predicted Peak VO_2 ($R = 0.669$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.03$, respectively) and TET ($R = 0.716$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.002$, respectively). When the analysis was limited to HFpEF patients, BMI, and age significantly predicted peak VO_2 ($R = 0.669$, $p < 0.001$, and $p < 0.001$, respectively) and BMI, age, and NT-proBNP predicted TET ($R = 0.680$ $p < 0.001$, $p = 0.017$, $p = 0.03$, respectively).

ROC Curve Analysis

A ROC curve analysis was performed to evaluate whether CRP and NT-proBNP have discriminative value for reduced CRF, defined as peak VO_2 <10 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ and peak VO_2 <14 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ (**Figure 4**). For peak VO_2 < 10 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$, the area under the curve (AUC) $= 0.660$ 95% CI [0.544–0.776], $P = 0.014$ for CRP and AUC $= 0.749$ 95% CI [0.669–0.829], $P < 0.001$ for NT-proBNP. For peak VO_2 <14 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$, AUC $= 0.658$ 95% CI [0.597–0.718], $P < 0.001$ for CRP and AUC $= 0.608$ 95% CI [0.537–0.678] $P = 0.003$ for NT-proBNP.

DISCUSSION

CRF is an important determinant of quality of life and prognostic indicator in patients with HF (23, 24). In the current study, we show that systemic inflammation, as measured by elevated CRP levels, and myocardial strain, as indicated by elevated NT-proBNP levels, independently predict impaired CRF in patients with HF, reflected in reduced peak VO_2. Both CRP and NT-proBNP show a high discriminative value for reduced CRF as defined by peak VO_2 <10 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ and peak VO_2 <14 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$.

Systemic Inflammation in Heart Failure

HF is characterized by systemic inflammation, as shown by elevated circulating levels of inflammatory biomarkers in patients that increase with progression of the disease (25). For one, systemic inflammation may be the result of HF by means of tissue hypoperfusion and neurohormonal activation (26), or inflammation may play a pathophysiologic role in HF (27, 28). It is suggested that the pro-inflammatory state contributes to the development and progression of HF, not only by impairing myocardial function but also by affecting other organs and tissues and thereby adding to other aspects of the HF syndrome including cachexia and anemia (25).

The preferred inflammatory biomarker in cardiovascular disease is CRP (29, 30). HF patients have elevated CRP levels and such levels tend to increase with clinical decompensation

$p = 0.426$, $N = 255$). When the analysis was limited to HFrEF patients, CRP and NT-proBNP predicted peak VO_2 ($R = 0.432$, $p < 0.001$, $p < 0.001$, respectively) and TET ($R = 0.496$, $p < 0.001$, $p < 0.001$, respectively). When the analysis was limited to HFpEF

and predict worse outcomes (5, 31, 32). Elevated CRP levels reflect inflammatory and immune deregulation in HF (31, 33). Elevated CRP levels also correlate with worse cardiac function (34, 35), and worse functional capacity in patients with ischemic heart disease and systolic HF (8, 9). The association between elevated CRP levels and HF is however complex and likely only incompletely understood. CRP is produced in the liver in response to cytokines such as Interleukin-6 (IL-6) (36). IL-6 is considered a secondary cytokine produced by myeloid cells in response to Interleukin-1 (IL-1) and Tumor Necrosis Factor-α (TNF-α) (36). Elevated IL-1 and TNF-α levels have been reported in patients with HF (33, 37). Moreover, IL-1 and TNF-α are also known as soluble cardiodepressant factors in patients with sepsis (38). More recently enhanced IL-1 activity has been described in patients with acute decompensated HF (31). IL-1 induces a reversible left ventricular systolic dysfunction in the mouse that is characterized by β-adrenergic receptor desensitization and impaired contractile reserve (39). This data points to an active role of IL-1 in the pathophysiology of HF.

Myocardial Strain

Elevated NT-proBNP levels reflect myocardial strain due to increased pressure, however, levels may also increase in response to other insults such as ischemia or inflammatory cytokines. NT-proBNP has shown to predict adverse outcomes in HF patients (10). Although BNP exerts protective effects on the heart during HF, the circulating levels of BNP or NT-proBNP reflect worse hemodynamics (elevated filling pressures) and neurohormonal activation (40). A lack of endogenous BNP response facilitates the onset of HF in animal experimental models (41), whereas potentiation of the BNP effects using recombinant BNP or neprylisin inhibitors help prevent HF (42).

Limitations

The retrospective nature of the study limited the power of our analyses, specifically in the HFpEF cohort for which the sample size was smaller than for HFrEF patients. Another limitation of our study is that every visit in the data analysis was used as separate data point, which results in some patients being represented more than once in the database. Having patients represented more than once can alter the representation of the patient population, as a single patient could have provided more than one data entry leading to an over-representation of their specific clinical characteristics. Lastly, we were not able to address the role of other biomarkers for inflammation in HF in our study.

Potential Implications for Diagnosis, Risk Stratification, and Treatment

According to our findings, both elevated CRP and NT-proBNP levels are independently predictive of impaired CRF in HF and predictive of moderately or severe reduction in peak VO_2. A scoring system that would include both biomarkers is therefore likely to yield a better discrimination than only one of the markers. In non-ST elevation ACS, CRP, and BNP in combination with troponin I were predictive of mortality, MI

and CHF. Further, a combination of the biomarkers provided additional prognostic value (43). These observations were validated in a cohort of 1,635 patients in the TACTICS-TIMI 18 study, and after adjustment for known clinical predictors, the number of elevated biomarkers remained predictive of the composite end point. Specifically, patients with one, two, and three elevated biomarkers had a 2.1, 3.1, and 3.7-fold increase in the risk of death, MI, or development of CHF after 6 months (43). A scoring system that could utilize the prognostic power of both CRP and NT-proBNP would allow for risk stratification beyond that solely provided by each of the markers used separately to predict CRF in patients with HF across a wide range of LVEF.

Inhibiting systemic inflammation with anti-inflammatory therapies and alleviating myocardial strain may represent two independent therapeutic strategies to improve CRF in patients with HF. Phase II studies have started exploring the effects of Interleukin-1 blockers in HF. A pilot feasibility study in 7 patients was conducted to test the efficacy of Anakinra on CP exercise performance in patients with HF and evidence of CRP. CRP levels were greatly reduced and peak VO_2 significantly improved (31). In the REDHART sub study, 60 patients with HFrEF and elevated CRP were randomly assigned to daily subcutaneous injections of Anakinra 100 mg for weeks, 12 weeks, or placebo (44). Treatment with Anakinra did not affect peak VO_2 or VE/VCO$_2$ slope at 2 weeks; however, patients showed improvement in peak VO_2 when assigned to the 12-week group. Further, the incidence of death or rehospitalization for HF at 24 weeks was 6, 31, and 30% for the Anakinra 12-week, Anakinra 2-week, and placebo groups, respectively. In the D-HART pilot study, the effects of IL-1 blockade with Anakinra on aerobic exercise capacity and CRP in patients with HFpEF were examined (45). Anakinra led to a statistically significant improvement in peak VO_2 consumption and a significant reduction in plasma CRP levels.

In a follow up study, the Diastolic Heart Failure Anakinra Response Trial-2 (DHART-2), patients with stable symptomatic HFpEF were treated with Anakinra to confirm the effects on peak VO_2 and CRP and observe its effects on serum NT-proBNP (46). Twenty-eight patients completed two visits or more and Anakinra was found to significantly reduce CRP as well as NT-proBNP levels. After 12 weeks of IL-1 blockade with Anakinra, NT-proBNP was reduced at a magnitude that correlated with CRP reduction. Anakinra however failed to increase peak VO_2 in the DHART2 study. The potential benefits of IL-1 blockade in patients with heart disease is further supported by the results of the phase III Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) (47), in which patients with prior acute myocardial infarction were randomized to canakinumab, IL-1β blocker, or placebo, and showed a significant reduction in major adverse cardiac events. A small single-center sub-study of the CANTOS trial showed a significant improvement in peak VO_2 in canakinumab-treated patients at 3 and 12 months (46).

Neprilysin inhibitors have also provided a novel therapeutic strategy to combat HF symptoms and promote CRF. Another
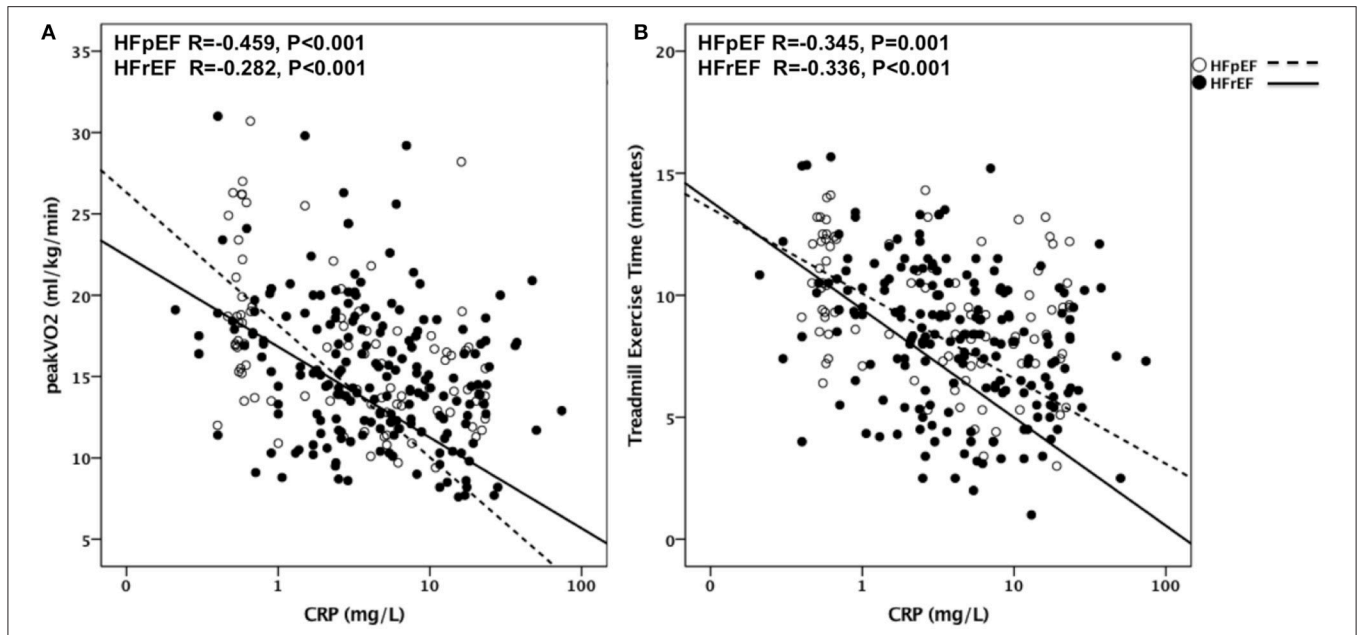
FIGURE 2 | CRP as predictor of CRF in HFrEF and HFpEF. Correlations are shown in **(A)** between CRP and Peak VO$_2$ in patients with HFpEF ($R = -0.459$, $P < 0.001$) and with HFrEF ($R = -0.282$, $P < 0.001$) and in **(B)** between CRP and TET in patients with HFpEF ($R = -0.345$, $P < 0.001$) and with HFrEF ($R = -0.336$, $P < 0.001$). VO$_2$, Oxygen uptake; HFpEF, Heart Failure with preserved Ejection Fraction; HFrEF, Heart Failure with reduced Ejection Fraction; TET, Treadmill Exercise Time.
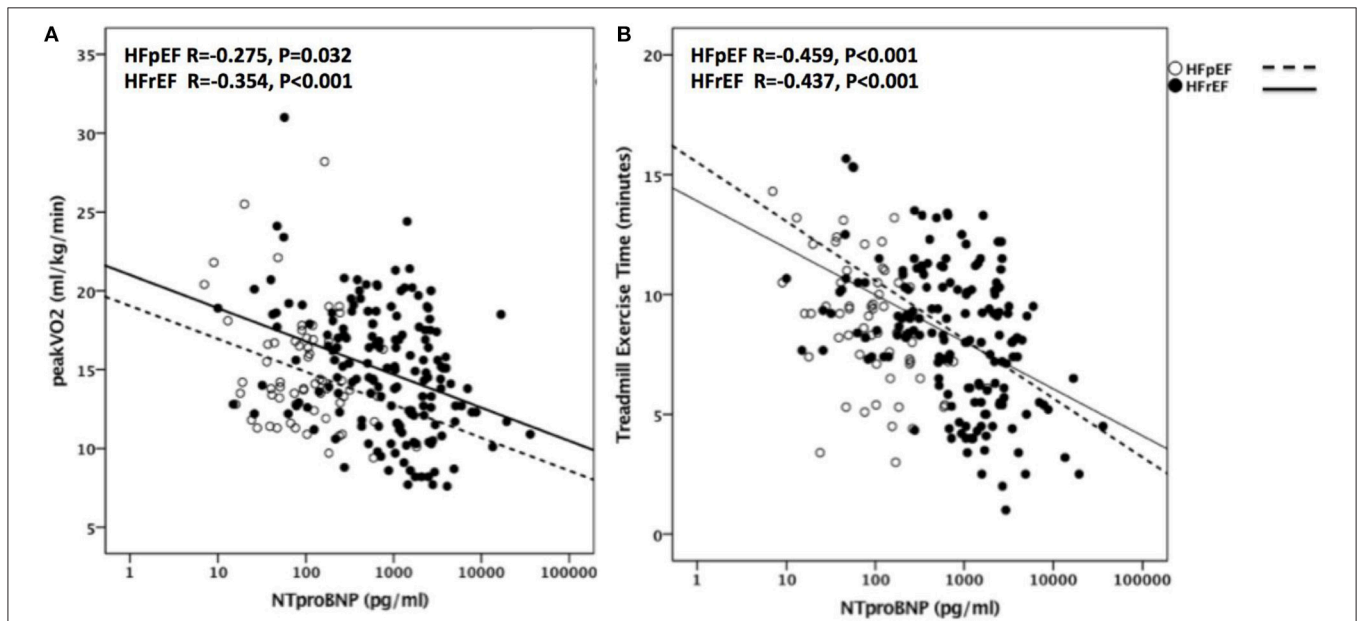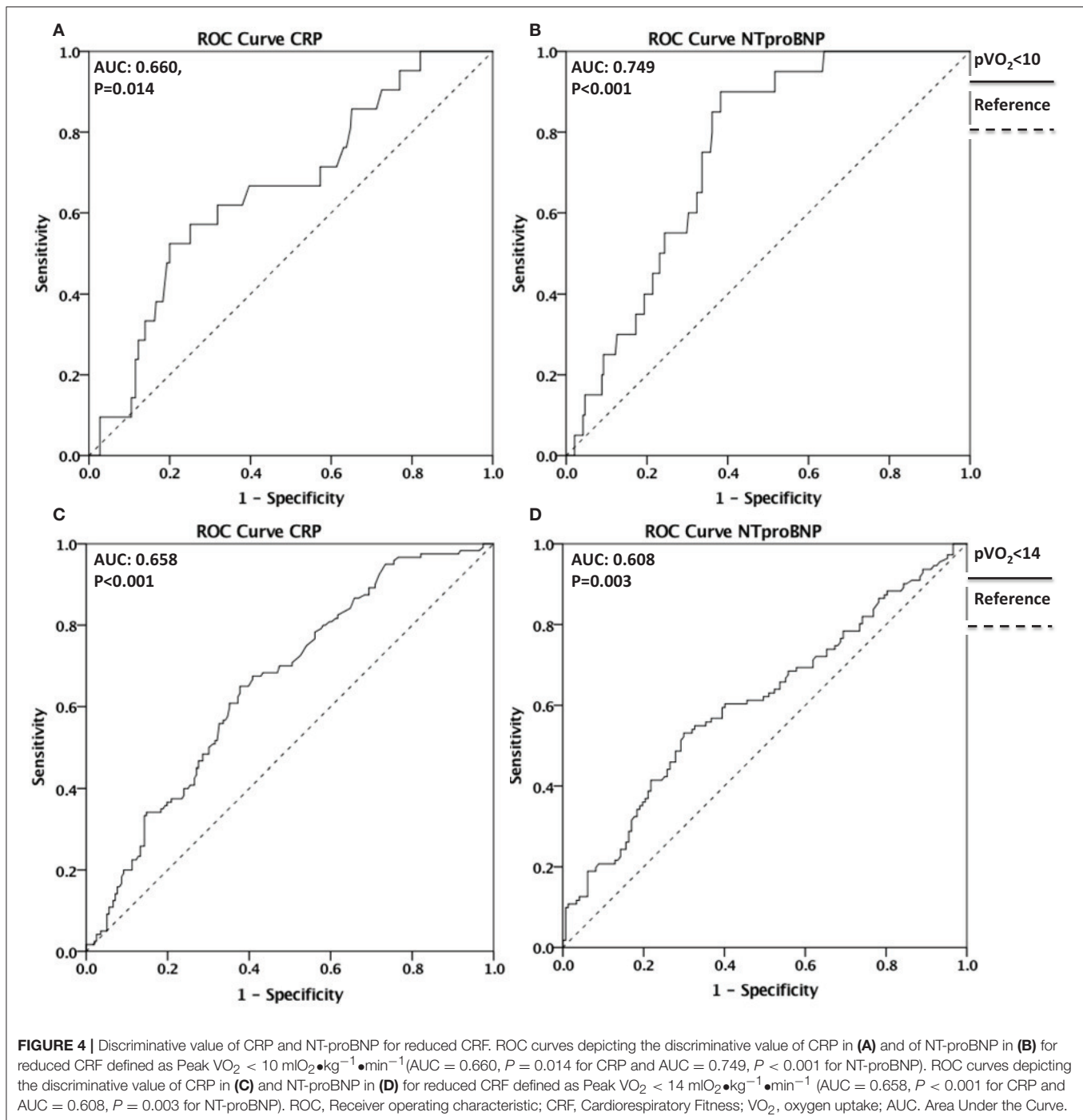

FIGURE 3 | NT-proBNP as predictor of CRF in HFrEF and HFpEF. Correlations are shown in **(A)** between NT-proBNP and Peak VO$_2$ in patients with HFpEF ($R = -0.275$, $P = 0.032$) and with HFrEF ($R = -0.354$, $P < 0.001$) and in **(B)** between NT-proBNP and TET in patients with HFpEF ($R = -0.459$, $P < 0.001$) and with HFrEF ($R = -0.437$, $P < 0.001$). VO$_2$, oxygen uptake; HFpEF, Heart Failure with preserved Ejection Fraction; HFrEF, Heart Failure with reduced Ejection Fraction; TET, Treadmill Exercise Time.

study was completed to compare the effects of Candoxatril (novel neutral endopeptidase inhibitor) with those of Furosemide in the treatment of patients with mild HF (37). Male patients with mild HF were randomly assigned to treatment with 20 mg of Furosemide twice a day, 200 mg of Candoxatril twice a day, or 400 mg of Candoxatril twice a day, for 9 days. For patients

FIGURE 4 | Discriminative value of CRP and NT-proBNP for reduced CRF. ROC curves depicting the discriminative value of CRP in **(A)** and of NT-proBNP in **(B)** for reduced CRF defined as Peak $VO_2 < 10$ $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ (AUC = 0.660, $P = 0.014$ for CRP and AUC = 0.749, $P < 0.001$ for NT-proBNP). ROC curves depicting the discriminative value of CRP in **(C)** and NT-proBNP in **(D)** for reduced CRF defined as Peak $VO_2 < 14$ $mlO_2 \bullet kg^{-1} \bullet min^{-1}$ (AUC = 0.658, $P < 0.001$ for CRP and AUC = 0.608, $P = 0.003$ for NT-proBNP). ROC, Receiver operating characteristic; CRF, Cardiorespiratory Fitness; VO_2, oxygen uptake; AUC. Area Under the Curve.

assigned to Furosemide, treadmill exercise capacity decreased by 30 ± 26 s compared to an increase of 12 ± 35 and of 35 ± 31 s for 200 mg of Candoxatril twice a day and 400 mg of Candoxatril twice a day, respectively (37). A pilot study was completed to evaluate the short-term effects of sacubitril/valsartan on maximal exercise capacity evaluated by peak VO_2 in stable patients with symptomatic HFrEF, with a secondary end point looking at changes in the VE/VCO_2 slope. When compared with baseline peak VO_2, patients experienced a significant increase in peak VO_2 at 30 days ($+0.92$ $mlO_2 \bullet kg^{-1} \bullet min^{-1}$), which corresponded to a 7.9% increase (38). These beneficial effects on CRF, fit well with the overall favorable effects of sacubitril/valsartan on major adverse cardiovascular events and cardiac death in the Prospective Comparison of ARNI (Angiotensin Receptor-Neprilysin Inhibitor) with ACEI (Angiotensin-Converting-Enzyme Inhibitor) to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial (48).

CONCLUSION

Biomarkers of inflammation and myocardial strain independently predict reduced peak VO_2 in HF patients. Anti-inflammatory therapies and therapies alleviating myocardial strain may independently improve CRF in HF patients across a large spectrum of LVEF.

ETHICS STATEMENT

The study was completed with a de-identified database available at VCU. The research was considered exempt from Institutional Review Board review as per VCU IRB guidelines.

AUTHOR CONTRIBUTIONS

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