

Biochemical Association of Lipid Peroxidation and Antioxidant Defense with Heart Failure Severity in Patients with Reduced Ejection Fraction

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ABSTRACT

Background: Redox imbalance is implicated in myocardial injury and in the clinical progression of heart failure. This study examined whether indices of lipid peroxidation and antioxidant capacity vary with disease severity in patients with heart failure with reduced ejection fraction (HFrEF).

Methods: In this prospective analytical study, 120 adults with HFrEF were enrolled from January 2025 through January 2026. Disease severity was characterized by New York Heart Association (NYHA) functional class, left ventricular ejection fraction (LVEF), and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Biochemical profiling included serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and total antioxidant capacity (TAC), measured with standardized laboratory procedures.

Results: Among the 120 participants, 68 were male (56.7%) and 52 were female (43.3%). Mean MDA rose from $2.84 \pm 0.72 \mu\text{mol/L}$ in NYHA II to $5.22 \pm 1.01 \mu\text{mol/L}$ in NYHA IV ($p < 0.001$), whereas SOD, GPx, catalase, and TAC decreased as functional class worsened. MDA was directly related to NYHA class ($p = 0.64$) and NT-proBNP ($p = 0.60$) and inversely related to LVEF ($r = -0.56$; all $p < 0.001$). Of the redox indices, the MDA/TAC ratio had the closest relationship with NYHA severity ($p = 0.69$, $p < 0.001$) and retained an independent association in the adjusted model (aOR 1.41; 95% CI 1.21–1.65).

Conclusion: More severe HFrEF was accompanied by greater lipid peroxidation and weaker antioxidant defenses. The MDA/TAC ratio may add biochemical information about systemic redox imbalance across the spectrum of HFrEF severity.

Keywords: antioxidant defense; heart failure; HFrEF; lipid peroxidation; malondialdehyde; oxidative stress; total antioxidant capacity

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INTRODUCTION

Heart failure (HF) is a clinical syndrome in which structural or functional cardiac disease compromises ventricular filling, ejection, or both, leading to a substantial burden of illness, repeated hospitalization, and death. Heart failure with reduced ejection fraction (HFrEF), generally defined by a left ventricular ejection fraction (LVEF) of 40% or less, is characterized by impaired contractile performance together with ventricular remodeling, neurohormonal activation, inflammation, and metabolic disturbance [1,2]. Although guideline-directed pharmacotherapy has improved outcomes, patients continue to experience appreciable residual risk; consequently, biological pathways and biomarkers that track continuing myocardial injury and clinical severity remain important areas of investigation [3].

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the ability of the antioxidant defense system, is a central biological process in HF that occurs when ROS production exceeds the antioxidant defense. In the failing heart ROS can be generated from mitochondrial dysfunction, nicotinamide adenine dinucleotide phosphate oxidases, xanthine oxidase, uncoupled nitric oxide synthase, and inflammation-related pathways [4]. Prolonged oxidant excess leads to modification of mitochondrial energy production, calcium homeostasis, endothelial signaling pathways, and redox-regulated pathways, and promotes cardiomyocyte damage, programmed cell death, extracellular-matrix modification, and fibrosis. These effects, when combined, can contribute to a worsening

of impaired contractility and adverse ventricular remodeling in HFrEF [5,6].

Lipid oxidation in the membranes is one of the most important downstream effects of excessive ROS. The polyunsaturated fatty acids of cellular and mitochondrial membranes are particularly vulnerable, and yield reactive aldehyde products such as malondialdehyde (MDA) and 4-hydroxynonenal [7]. MDA is frequently employed as a circulating marker for the oxidative damage to lipids as it is measurable in biological samples and it is a product of lipid degradation. These lipid-derived products can, in turn, exacerbate mitochondrial damage, protein modification, abnormal cell signaling, inflammation, and fibrosis. Current mechanistic work also links lipid peroxidation with mitochondrial failure and regulated oxidative cell-death pathways in cardiovascular disease [8,9].

The extent of lipid oxidation could be greater as HF progresses in humans. Previous research has indicated higher circulating MDA levels and lipid peroxide levels in HF and a progressive increase with increasing functional classes [10]. More recent investigations have again highlighted MDA, showing relationships between higher serum concentrations and adverse events or mortality even after adjustment for conventional clinical risk markers [7]. Thus, circulating MDA may reflect both oxidative injury and the overall biological burden of the HF syndrome [11]. Endogenous antioxidant systems oppose this oxidant burden. Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide, while catalase and glutathione peroxidase (GPx) participate in the subsequent removal of hydrogen peroxide and lipid hydroperoxides [12]. Non-enzymatic antioxidants in the circulation additionally contribute to total antioxidant capacity (TAC). Depletion or reduced activity of these defenses can leave the myocardium more vulnerable to oxidative injury. Alterations in SOD, catalase, GPx, glutathione, and related redox pathways have been described in HF, supporting simultaneous assessment of oxidant injury and antioxidant reserve rather than reliance on a single marker [3,8].

The link between oxidative biology and the clinical gradient of HFrEF, however, is not fully defined. Prior investigations have often combined different HF phenotypes, measured one redox biomarker at a time, or emphasized acute HF and mortality instead of biochemical variation across chronic HFrEF severity [13,14]. Evaluating lipid peroxidation together with several antioxidant systems against functional class, LVEF, and a validated marker of cardiac wall stress may therefore clarify how redox balance changes as disease advances. The reported independent association of circulating MDA with adverse HF

outcomes provides additional rationale for studying these markers in a clearly specified clinical phenotype [15].

Accordingly, this study evaluated the relationship between HFrEF severity and a panel comprising MDA, SOD, GPx, catalase, and TAC. Associations were examined with NYHA functional class, LVEF, and N-terminal pro-B-type natriuretic peptide. We hypothesized that progressively severe HFrEF would be accompanied by greater lipid oxidative damage and a parallel reduction in antioxidant capacity, indicating a shift toward systemic oxidant predominance [16].

MATERIALS AND METHODS

Study Design and Setting:

A prospective analytical observational design was used from January 2025 to January 2026 through collaboration between the Department of Biochemistry, Fatima Jinnah Medical University (FJMU), Lahore, Pakistan, and Khatam-un-Nabiyeen Trust (Regd.) Hospital, Sargodha, Punjab, Pakistan. Participant enrollment, cardiovascular evaluation, and echocardiography took place at the hospital, whereas assays of oxidative injury and antioxidant status were performed in the Department of Biochemistry at FJMU.

Study Population and Sample Size:

The study enrolled 120 consecutive patients meeting criteria for HFrEF by non-probability sampling. Individuals attending the cardiology outpatient service or undergoing hospital evaluation for chronic HF during the recruitment period were assessed for eligibility. The target sample was chosen to provide 80% statistical power at a 95% confidence level for clinically meaningful associations between redox biomarkers and measures of HF severity.

Eligibility Criteria:

Patients eligible for the trial were adults ages 18-80 years with a history of chronic HF for at least three months and an LVEF of $\leq 40\%$. Exclusion criteria included myocardial infarction or acute coronary syndrome (ACS) in the last three months, decompensated HF with need for intravenous inotropic support, advanced liver disease, active cancer, hematologic disease, or major surgery in the last three months. Individuals taking high-dose antioxidant preparations, including pharmacological vitamin C or E, were also excluded to limit treatment-related distortion of redox measurements.

Clinical Assessment:

Age, sex, body mass index, smoking, duration of HF, cause of HF, hypertension, diabetes mellitus, ischemic heart disease, prior myocardial infarction, and current cardiovascular treatment were recorded on a proforma. Standardized clinical methods were used to collect resting blood pressure, pulse rate, oxygen saturation, weight and height.

New York Heart Association (NYHA) functional classification was used for the symptomatic severity grading. Participants were categorized into classes II, III, and IV based on activity limitation and the presence of dyspnea or fatigue. The NYHA class was assigned by the treating cardiologist prior to the results of the redox laboratory tests.

Echocardiographic Assessment:

A trained cardiac sonographer used a standard cardiac ultrasound machine to take a transthoracic echocardiogram. LV ejection fraction (LVEF) was calculated from the apical four and two chamber views by the modified biplane Simpson method if the quality of the image was good enough. HFrEF was considered to be LVEF $\leq 40\%$. Left ventricular end-diastolic and end-systolic dimensions were measured when available and severe systolic dysfunction was prespecified as LVEF $\leq 30\%$ for secondary analyses.

Blood Collection and Sample Processing:

Each participant provided approximately 8–10 mL of venous blood under aseptic technique, preferably following an 8–12-hour overnight fast. Specimens were put into plain, EDTA, or tubes specific to the type of assay to be performed. Samples were centrifuged within 1 hour at approximately 3,000 rpm for 10–15 minutes and the serum and plasma were placed in the labeled tubes and were frozen at -80°C until the time of analysis, thereby minimizing degradation and multiple freeze-thaws.

Routine Laboratory Investigations:

Routine testing comprised complete blood count, fasting glucose, creatinine, estimated glomerular filtration rate, lipid profile, sodium, potassium, and albumin. NT-proBNP was quantified on an automated immunoassay platform in accordance with the assay manufacturer's instructions and served as the established biochemical indicator of HF severity.

Assessment of Lipid Peroxidation:

Lipid peroxidation was represented by serum malondialdehyde (MDA), quantified with a thiobarbituric acid-reactive substances spectrophotometric assay. In an acidic, heated reaction, MDA forms a chromogenic product with thiobarbituric acid that was measured at the assay-specified wavelength. Concentrations were reported in $\mu\text{mol/L}$. When feasible, duplicate measurements were obtained and their average was entered into the analysis.

Assessment of Antioxidant Defense:

Antioxidant status was characterized by superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and total antioxidant capacity (TAC). Erythrocyte SOD was quantified by a spectrophotometric inhibition method and reported as U/g hemoglobin. GPx was measured through a coupled enzymatic assay of glutathione-dependent

peroxide reduction and expressed as U/g hemoglobin. Catalase was determined from hydrogen peroxide decomposition kinetics and expressed as kU/g hemoglobin. Serum TAC was assessed with a validated colorimetric method and reported as mmol Trolox equivalents/L, or in the corresponding units specified by the assay.

Oxidative Balance Index:

An exploratory redox balance index was generated as the MDA/TAC ratio for each participant. Serum MDA was divided by TAC so that larger ratios denoted greater lipid oxidative burden in relation to systemic antioxidant capacity.

Quality Control:

Laboratory procedures were carried out in accordance with standard procedures. The persons who performed the assays had no knowledge of the NYHA class and echocardiographic results. Instruments were calibrated following laboratory procedures, and when applicable, internal control materials were included, and specimens from different severity groups were alternated within analytical batches to reduce systematic bias.

Study Outcomes:

The main analysis accounted for the differences in MDA and antioxidant markers according to the severity of HF (NYHA class). Secondary analyses assessed associations of MDA, SOD, GPx, catalase, TAC, and the MDA/TAC ratio with LVEF, NT-proBNP, and severe systolic dysfunction (LVEF $\leq 30\%$). Additionally, we investigated if there were any selected redox markers that could differentiate patients in the advanced symptomatic groups (NYHA III–IV).

Statistical Analysis:

Statistical analyses were performed in IBM SPSS Statistics 27.0. Distributional assumptions for continuous variables were evaluated with the Shapiro-Wilk test and inspection of distribution plots. Data with approximately normal distributions are presented as mean $\pm$ standard deviation; skewed data are summarized as median with interquartile range. Categorical variables are reported as counts and percentages.

Differences among NYHA classes were tested with one-way analysis of variance followed by a suitable post-hoc comparison for normally distributed measures, whereas the Kruskal-Wallis test was used for non-normal continuous variables. Categorical comparisons used the chi-square test or Fisher's exact test as appropriate.

Pearson or Spearman coefficients, selected according to distribution and scale, were used to quantify associations among redox biomarkers, LVEF, NYHA class, and NT-proBNP. Multivariable regression was then used to test whether MDA and antioxidant indices remained associated with greater HF severity after

accounting for age, sex, body mass index, smoking, hypertension, diabetes, ischemic heart disease, renal function, hemoglobin, and HF treatment. A further model added NT-proBNP to determine whether the redox measures retained information beyond this established cardiac biomarker.

Receiver operating characteristic analysis assessed the ability of MDA, TAC, and the MDA/TAC ratio to identify advanced symptomatic HF. Discrimination was summarized by the area under the curve and its 95% confidence interval. Statistical testing was two-sided, and $p < 0.05$ defined significance.

Ethical Considerations:

The investigation followed the Declaration of Helsinki. Approval was obtained from the applicable Institutional Review Board/Ethics Committee, and every participant provided written informed consent before study procedures and phlebotomy. Identifiers were removed from study data, which were maintained under confidential handling throughout the project.

RESULTS

Baseline Characteristics:

The final analysis included 120 participants with HFrEF: 68 males (56.7%) and 52 females (43.3%), with a mean age of 59.8 ± 10.9 years. NYHA class II accounted for 44 patients (36.7%), class III for 49 (40.8%), and class IV for 27 (22.5%). Sex distribution did not differ significantly across functional classes ($p = 0.91$).

A clear clinical gradient was evident across NYHA groups. Mean LVEF fell from $35.1 \pm 3.6\%$ in class II to $24.9 \pm 4.3\%$ in class IV ($p < 0.001$), while median NT-proBNP rose from 1,080 to 4,860 pg/mL ($p < 0.001$). Hemoglobin and estimated glomerular filtration rate were also lower in NYHA IV than in the less symptomatic groups (Table 1).

Table 1. Clinical profile across NYHA functional classes

Variable	NYHA II (n=44)	NYHA III (n=49)	NYHA IV (n=27)	p value
Age, years	56.9 ± 10.2	60.1 ± 10.5	64.0 ± 11.1	0.018
Male, n (%)	24 (54.5)	28 (57.1)	16 (59.3)	0.91
Female, n (%)	20 (45.5)	21 (42.9)	11 (40.7)	0.91
BMI, kg/m ²	27.2 ± 4.1	26.5 ± 4.0	25.9 ± 4.4	0.39

Hypertension, n (%)	24 (54.5)	29 (59.2)	15 (55.6)	0.89
Diabetes mellitus, n (%)	13 (29.5)	19 (38.8)	12 (44.4)	0.42
Ischemic etiology, n (%)	23 (52.3)	30 (61.2)	17 (63.0)	0.54
Heart rate, beats/min	78.6 ± 9.8	84.2 ± 11.1	89.8 ± 12.4	<0.001
LVEF, %	35.1 ± 3.6	30.2 ± 4.2	24.9 ± 4.3	<0.001
Hemoglobin, g/dL	13.5 ± 1.5	12.8 ± 1.6	12.1 ± 1.7	0.002
eGFR, mL/min/1.73 m ²	76.2 ± 17.4	68.8 ± 18.1	61.9 ± 17.7	0.004
NT-proBNP, pg/mL, median (IQR)	1,080 (710–1,670)	2,420 (1,690–3,520)	4,860 (3,410–6,520)	<0.001

Values are reported as mean $\pm$ SD, median (IQR), or n (%). BMI: body mass index; LVEF: left ventricular ejection fraction; eGFR: estimated glomerular filtration rate.

Lipid Peroxidation and Antioxidant Defense:

Lipid oxidative burden increased across worsening functional classes. Mean serum MDA was 2.84 ± 0.72 μ mol/L in NYHA II, 4.01 ± 0.88 μ mol/L in NYHA III, and 5.22 ± 1.01 μ mol/L in NYHA IV ($p < 0.001$).

Antioxidant measures changed in the opposite direction. SOD, GPx, catalase, and TAC were each lower with more advanced NYHA class, with TAC decreasing from 1.25 ± 0.19 mmol/L in NYHA II to 0.84 ± 0.17 mmol/L in NYHA IV ($p < 0.001$). Across the same groups, the MDA/TAC ratio rose to nearly three times its class II value, consistent with a progressively less favorable balance between lipid oxidation and antioxidant reserve (Table 2).

Table 2. Redox biomarkers across NYHA functional classes

Biomarker	NYHA II	NYHA III	NYHA IV	p value
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MDA, $\mu\text{mol/L}$	2.84 $\pm$ 0.72	4.01 $\pm$ 0.88	5.22 $\pm$ 1.01	<0.001
SOD, U/g Hb	1,190 $\pm$ 205	981 $\pm$ 194	790 $\pm$ 181	<0.001
GPx, U/g Hb	50.4 $\pm$ 9.8	41.2 $\pm$ 9.1	33.0 $\pm$ 8.3	<0.001
Catalase, kU/g Hb	49.0 $\pm$ 9.1	40.6 $\pm$ 8.3	33.5 $\pm$ 7.8	<0.001
TAC, mmol/L	1.25 $\pm$ 0.19	1.04 $\pm$ 0.18	0.84 $\pm$ 0.17	<0.001
MDA/TAC ratio	2.34 $\pm$ 0.71	3.98 $\pm$ 1.08	6.40 $\pm$ 1.61	<0.001

MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase; TAC: total antioxidant capacity; Hb: hemoglobin.

Sex-Based Comparison:

Redox profiles did not materially differ by sex. Mean MDA was $3.91 \pm 1.31 \mu\text{mol/L}$ in males and $3.86 \pm 1.28 \mu\text{mol/L}$ in females ($p=0.83$), and no significant sex differences were detected for SOD, GPx, catalase, TAC, the MDA/TAC ratio, LVEF, or NT-proBNP (Table 3). Thus, the severity-related redox pattern was evident in both sexes.

Table 3. Clinical and biochemical measures stratified by sex

Variable	Male (n=68)	Female (n=52)	p value
Age, years	59.2 $\pm$ 10.8	60.6 $\pm$ 11.1	0.49
LVEF, %	30.6 $\pm$ 6.1	31.2 $\pm$ 6.4	0.60
MDA, $\mu\text{mol/L}$	3.91 $\pm$ 1.31	3.86 $\pm$ 1.28	0.83
SOD, U/g Hb	1,006 $\pm$ 245	1,021 $\pm$ 238	0.74
GPx, U/g Hb	42.0 $\pm$ 10.7	42.8 $\pm$ 10.5	0.68
Catalase, kU/g Hb	41.2 $\pm$ 9.8	41.8 $\pm$ 9.4	0.74
TAC, mmol/L	1.06 $\pm$ 0.24	1.08 $\pm$ 0.23	0.64

MDA/TAC ratio	3.96 $\pm$ 1.72	3.85 $\pm$ 1.65	0.72
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Correlation With Heart Failure Severity:

MDA increased in parallel with NYHA class ($\rho=0.64$, $p<0.001$) and NT-proBNP ($\rho=0.60$, $p<0.001$), but decreased as LVEF increased ($r=-0.56$, $p<0.001$). Antioxidant indices displayed inverse directional patterns.

TAC was lower at higher NYHA class ($\rho=-0.57$, $p<0.001$) and higher NT-proBNP ($\rho=-0.51$, $p<0.001$), while it rose with LVEF ($r=0.48$, $p<0.001$). The MDA/TAC ratio showed the largest correlation coefficients overall: $\rho=0.69$ with NYHA class, $\rho=0.64$ with NT-proBNP, and $r=-0.60$ with LVEF (Table 4).

Table 4. Correlations between redox biomarkers and indices of HF severity

Biomarker	NYHA class	LVEF	NT-proBNP
MDA	0.64	-0.56	0.60
SOD	-0.53	0.43	-0.45
GPx	-0.49	0.40	-0.42
Catalase	-0.46	0.37	-0.39
TAC	-0.57	0.48	-0.51
MDA/TAC ratio	0.69	-0.60	0.64

Every correlation shown was significant at $p<0.001$.

Association With Severe Systolic Dysfunction:

Thirty-eight participants (31.7%) met the prespecified definition of severe systolic dysfunction (LVEF $\leq 30\%$). Compared with those whose LVEF was $>30\%$, this subgroup had higher MDA (4.89 ± 1.09 vs $3.47 \pm 1.06 \mu\text{mol/L}$; $p<0.001$), lower TAC (0.91 ± 0.19 vs $1.15 \pm 0.22 \text{ mmol/L}$; $p<0.001$), and a higher MDA/TAC ratio (5.55 ± 1.75 vs 3.18 ± 1.24 ; $p<0.001$).

Multivariable Analysis:

In the model adjusted for age, sex, BMI, smoking, hypertension, diabetes, ischemic etiology, renal function, hemoglobin, and HF therapy, each increase in MDA remained independently related to higher NYHA severity (aOR 1.92; 95% CI 1.45–2.55; $p<0.001$). The association remained significant when NT-proBNP was added to the model (aOR 1.58; 95% CI 1.18–2.12; $p=0.002$).

Greater TAC was independently linked to lower severity after NT-proBNP adjustment (aOR 0.82 per 0.1 mmol/L increase; 95% CI 0.72–0.94; $p=0.004$). The MDA/TAC ratio retained the largest independent

association with an increase in NYHA class (aOR 1.41 per unit; 95% CI 1.21–1.65; $p < 0.001$) (Table 5).

Table 5. Adjusted associations between redox biomarkers and increasing NYHA severity

Biomarker	Adjusted OR (95% CI)*	p value	NT-proBNP-adjusted OR (95% CI)†	p value
MDA, per 1 $\mu\text{mol/L}$	1.92 (1.45–2.55)	<0.001	1.58 (1.18–2.12)	0.002
SOD, per 100 U/g Hb	0.83 (0.74–0.93)	0.001	0.88 (0.78–0.99)	0.035
GPx, per 10 U/g Hb	0.74 (0.58–0.94)	0.014	0.81 (0.63–1.04)	0.096
Catalase, per 10 kU/g Hb	0.77 (0.60–0.98)	0.034	0.84 (0.65–1.09)	0.18
TAC, per 0.1 mmol/L	0.77 (0.68–0.87)	<0.001	0.82 (0.72–0.94)	0.004
MDA/TAC ratio, per unit	1.56 (1.33–1.82)	<0.001	1.41 (1.21–1.65)	<0.001

*Model adjusted for age, sex, BMI, smoking, hypertension, diabetes, ischemic etiology, eGFR, hemoglobin, and HF therapy. †The second model additionally included NT-proBNP.

ROC Analysis:

For identifying NYHA III–IV status, MDA yielded an AUC of 0.82 (95% CI 0.75–0.89) and TAC an AUC of 0.80 (95% CI 0.72–0.87). The MDA/TAC ratio performed best among the oxidative indices, with an AUC of 0.87 (95% CI 0.81–0.93); a cutoff >3.25 provided 82.9% sensitivity and 79.5% specificity. NT-proBNP produced an AUC of 0.89 (Table 6).

Table 6. ROC performance for identifying NYHA class III–IV

Marker	AUC	95% CI	Cutoff	Sensitivity (%)	Specificity (%)
MDA	0.82	0.75–0.89	$>3.65 \mu\text{mol/L}$	78.9	75.0
TAC	0.80	0.72–0.87	$<1.08 \text{ mmol/L}$	76.3	72.7
MDA/TAC ratio	0.87	0.81–0.93	>3.25	82.9	79.5
NT-proBNP	0.89	0.83–0.95	$>1,850 \text{ pg/mL}$	85.5	81.8

Taken together, increasing HFrEF severity coincided with a higher lipid oxidative burden and diminishing antioxidant capacity in both sexes. MDA and the MDA/TAC ratio rose with worse NYHA class and higher NT-proBNP and were inversely related to LVEF, whereas SOD, GPx, catalase, and TAC showed the reverse pattern. Among the redox measures, the MDA/TAC ratio had the strongest overall association with clinical and echocardiographic severity and remained significant after multivariable adjustment.

DISCUSSION

This study identified a graded shift toward oxidative imbalance as HFrEF became clinically more severe [1]. Advancing NYHA class was accompanied by rising serum MDA and falling SOD, GPx, catalase, and TAC. The same pattern was reflected in objective disease markers: MDA was higher at lower LVEF and higher NT-proBNP, whereas antioxidant measures changed in the opposite direction. Similar associations were seen in males and females and remained evident after adjustment for major clinical covariates [2,3]. The stepwise rise in MDA from NYHA II through IV is consistent with increasing membrane lipid oxidation during progression of HF [4,5]. MDA is a product of the oxidative degradation of polyunsaturated lipids and thus is a measure of the total amount of lipid damage. There is evidence from a systematic review that MDA is elevated in HF as compared to non-HF controls, supporting a role for oxidative stress in the syndrome. The present findings are an extension of

that observation in that they show a gradient of severity in a cohort of patients with HFrEF, with the highest level of lipid-peroxidation burden occurring in those with the most symptomatic and most systolic impairment [6].

There is also a possible mechanistic explanation for the negative relationship between MDA and LVEF. Overproduction of ROS leads to dysfunction of mitochondria energetic, calcium handling, endothelial and intracellular signaling, and to apoptosis, inflammation, fibrosis, and adverse remodeling. Recent evaluation of redox biomarkers in the various HF phenotypes has also found that elevated MDA correlates with worse systolic function, and that it is a circulating marker of myocardial oxidative injury rather than a laboratory marker of less specificity [7,8]. The results were also consistent with the observation of high levels of circulating MDA in chronic HFrEF, which have been associated to poor outcomes. These findings suggest that MDA may be a marker of the severity of the disease process, as well as the presence of oxidative stress. Nevertheless, a larger population that is followed prospectively is needed to confirm that MDA provides prognostic information that is clinically useful in everyday practice [9,10].

The simultaneous decrease in SOD, GPx, catalase and TAC gives the complementary side of this redox profile. SOD is used to reduce the amount of superoxide and produces hydrogen peroxide which is partially eliminated by catalase and GPx. A decrease in the function of these enzymes may extend the duration of ROS exposure and consequently the amount of tissue damage resulting from oxidation. The decrease in TAC is especially revealing as it indicates that the whole circulating antioxidant pool is depleted, and not only one enzymatic pathway [11,12].

Close correlation between redox markers in the circulation and ventricular performance has not been consistently reported in the literature. The discrepancy in observations may be due to differences in the HF phenotype, sample size, clinical stage, assay method, the presence of covariates, or concomitant therapy. By restricting enrollment to reduced-ejection-fraction HF and assessing several oxidant and antioxidant measures across predefined NYHA categories, the present study was positioned to detect a graded biochemical pattern that can be obscured in more heterogeneous cohorts [13].

The MDA/TAC ratio emerged as the redox variable most closely related to NYHA class, LVEF, and NT-proBNP. Its associations were stronger than those of MDA or TAC considered separately. This finding is biologically coherent because net oxidative injury depends on the relation between oxidant burden and protective capacity. A ratio incorporating both processes may therefore summarize systemic redox

status more effectively, although the threshold observed here is exploratory and requires validation in external populations [14,15].

As expected, NT-proBNP remained a strong indicator of HF severity and showed slightly greater discrimination than the individual oxidative markers. The redox measures should therefore be viewed as complementary rather than as substitutes for established HF biomarkers. They may capture an additional pathophysiological axis involving membrane oxidative injury and depletion of antioxidant defenses. Persistence of the MDA and TAC associations after inclusion of NT-proBNP in the regression model supports this interpretation [14,16]. Male and female participants had comparable concentrations and activities of the measured redox markers, and adjustment for sex did not materially change the severity associations. Within this cohort, the biochemical gradient therefore appeared to track HFrEF severity more closely than sex. Dedicated studies with larger sex-specific samples are still needed to determine whether smaller biological differences exist [17].

Several design features strengthen the analysis: lipid peroxidation and multiple antioxidant components were measured together; enrollment was limited to HFrEF; both sexes were represented; biochemical and echocardiographic indices of severity were examined concurrently; and the adjusted models incorporated relevant clinical covariates [18]. The findings should nevertheless be interpreted in light of important limitations. An observational design cannot establish causality, and blood redox markers may not reproduce the oxidative environment within myocardial tissue. MDA estimates can also depend on assay methodology, while diet and other unmeasured metabolic influences may affect antioxidant status. In addition, the sample size was modest and recruitment was limited to the participating centers, reducing external generalizability. Multicenter longitudinal studies using standardized assays are needed to define the prognostic value, reproducibility, and potential therapeutic relevance of these markers [19,20].

CONCLUSION

More advanced HFrEF was accompanied by a progressive shift toward lipid oxidative injury and reduced antioxidant capacity. MDA and the MDA/TAC ratio increased with worse NYHA class, lower LVEF, and higher NT-proBNP, whereas SOD, GPx, catalase, and TAC displayed the opposite relationships. These patterns were similar in males and females and remained significant after adjustment for relevant clinical characteristics. The MDA/TAC ratio had the closest overall association with HF severity, indicating that combined consideration of oxidant burden and antioxidant reserve may add information

about systemic redox status in HFrEF. Prospective validation is necessary before these measures are incorporated into routine risk stratification.

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Competing Interests: The authors declare no competing interests.

Data Availability: Data are available from the corresponding author upon reasonable request.

Author Contributions: MZ conceptualized and supervised the study. IA and RS contributed to data collection and biochemical analysis. ME and HA contributed to methodology and data interpretation. WA contributed to cardiological assessment and clinical interpretation. MZ drafted the manuscript. All authors reviewed and approved the final manuscript.

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