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Integrated Redox Imbalance and Endothelial Injury in Acute Myocardial Infarction: Clinical Evidence from Sulfhydryl Depletion, Ceruloplasmin–Haptoglobin Suppression, ADMA Accumulation, and Nitric Oxide Loss

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Abstract

Background

Acute myocardial infarction (AMI) is increasingly recognized as a complex redox-mediated cardiovascular injury. Although oxidative stress and nitric oxide (NO) impairment are established contributors to myocardial damage, the integrated relationship between endogenous antioxidant defense and the asymmetric dimethylarginine (ADMA)–NO axis across STEMI and NSTEMI remains incompletely understood. This study aimed to characterize the combined redox–endothelial injury profile in acute coronary ischemia.

Methods

In this prospective case–control study, 93 participants were enrolled, including STEMI (n = 31), NSTEMI (n = 31), and healthy controls (n = 31). Serum total sulfhydryl status (TSH), ceruloplasmin, haptoglobin, ADMA, and NO concentrations were quantified. Intergroup differences were evaluated using Kruskal–Wallis H and Mann–Whitney U tests. Spearman correlation and multivariate ANOVA (MANOVA) adjusting for age, gender, hypertension, and diabetes mellitus were performed. ROC analysis evaluated discriminatory performance.

Results

All biomarkers differed significantly among groups ($p < 0.001$). AMI patients exhibited marked depletion of TSH, ceruloplasmin, haptoglobin, and NO, with the most pronounced impairment in STEMI patients (TSH: 12.32 ± 0.13 μmol/L; ceruloplasmin: 2.16 ± 0.04 mg/dL; haptoglobin: 5.18 ± 0.06 mg/dL; NO: 0.54 ± 0.03 μmol/L). ADMA was significantly elevated, reaching highest levels in NSTEMI (15.25 ± 0.17 μmol/L). Spearman correlation revealed strong positive inter-correlations among TSH, ceruloplasmin, haptoglobin, and NO ($r = 0.881–0.906$, $p < 0.001$), while ADMA was negatively correlated with all protective biomarkers ($r = -0.437$ to -0.448, $p < 0.001$). All biomarkers correlated significantly with Troponin I and LVEF. MANOVA confirmed group differences remained significant after covariate adjustment (Pillai's Trace = 1.978, $F = 1473.95$, $p < 0.001$), with no covariate having a significant effect ($p > 0.05$). ROC analysis demonstrated perfect discriminatory performance (AUC = 1.000).

Conclusions

AMI is associated with profound antioxidant depletion, ADMA accumulation, and NO loss. The coordinated biomarker alterations and their independence from traditional risk factors support an integrated redox–endothelial injury axis. Combined assessment of these biomarkers provides a mechanistically informative signature; however, perfect separation observed in ROC analysis limits immediate clinical diagnostic application, and validation in unselected populations is required.

INTRODUCTION

Acute myocardial infarction (AMI) remains a leading cause of mortality, heart failure, and long-term disability worldwide despite substantial advances in reperfusion strategies, antithrombotic therapy, and secondary prevention. Although coronary artery occlusion initiates myocardial ischemia, growing evidence indicates that the extent of myocardial injury is determined not only by ischemic burden but also by the magnitude of oxidative stress, endothelial injury, inflammatory activation, and impairment of endogenous cardioprotective pathways (1–3). Contemporary translational and clinical investigations have identified redox imbalance as a critical determinant of infarct expansion, adverse ventricular remodeling, and progression toward heart failure following acute coronary events (4, 5).

Oxidative stress develops when reactive oxygen species (ROS) generation exceeds the neutralizing capacity of endogenous antioxidant defense systems. During myocardial ischemia and reperfusion, excessive production of superoxide anions, hydrogen peroxide, hydroxyl radicals, and peroxynitrite promotes lipid peroxidation, protein oxidation, mitochondrial dysfunction, DNA damage, inflammatory activation, and cardiomyocyte death (6). Mitochondria, NADPH oxidases, xanthine oxidase, and uncoupled endothelial nitric oxide synthase (eNOS) represent major sources of ROS generation within ischemic myocardium (7, 8). Beyond their role as by-products of cellular injury, ROS actively regulate redox-sensitive signaling pathways involved in endothelial dysfunction, ferroptosis, vascular injury, and maladaptive ventricular remodeling, emphasizing their central role in AMI pathophysiology (9, 10).

Among endogenous antioxidant defense mechanisms, thiol-containing compounds constitute one of the most important extracellular redox buffering systems. Sulfhydryl (-SH) groups participate in scavenging reactive oxygen species, preserving protein structure, regulating enzymatic activity, and maintaining cellular redox homeostasis (11). Disturbances in thiol/disulfide balance have been associated with acute coronary syndromes, impaired coronary perfusion, increased infarct size, and unfavorable cardiovascular outcomes (12, 13). Consequently, total sulfhydryl status (TSH) has emerged as a promising indicator of systemic antioxidant reserve and oxidative stress burden during acute myocardial injury.

Ceruloplasmin and haptoglobin represent complementary components of endogenous antioxidant and iron-regulatory defense systems. Ceruloplasmin is a copper-dependent ferroxidase that limits iron-catalyzed oxidative reactions through oxidation of ferrous iron while contributing to vascular homeostasis and inflammatory regulation (14). Haptoglobin protects tissues against hemoglobin-mediated oxidative injury through sequestration and clearance of free hemoglobin, thereby reducing iron-dependent radical generation (15). Increasing evidence suggests that dysregulation of iron metabolism and ferroptosis contribute significantly to ischemia-reperfusion injury and adverse cardiac remodeling, highlighting ceruloplasmin and haptoglobin as potential biomarkers of redox-mediated cardiovascular injury (10, 16).

Endothelial dysfunction represents another fundamental component of acute coronary syndromes. Nitric oxide (NO), synthesized by endothelial nitric oxide synthase, exerts vasodilatory, anti-inflammatory, antithrombotic, and antiproliferative effects essential for vascular integrity and myocardial perfusion (17). Reduced NO bioavailability has been associated with impaired coronary microvascular function, platelet activation, vascular inflammation, endothelial injury, and adverse cardiovascular outcomes (18, 19). Consequently, disruption of endothelial NO signaling is increasingly recognized as a critical contributor to infarct progression and post-infarction remodeling.

A key regulator of endothelial NO production is asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase. Elevated ADMA concentrations impair endothelial NO synthesis, promote oxidative stress, accelerate atherosclerotic progression, and independently predict cardiovascular morbidity and mortality (20, 21). Importantly, oxidative stress and ADMA participate in a bidirectional pathogenic interaction. Reactive oxygen species increase ADMA accumulation through inhibition of dimethylarginine dimethylaminohydrolase activity, whereas elevated ADMA further suppresses NO bioavailability and exacerbates endothelial dysfunction (22). This self-amplifying cycle links redox imbalance, vascular injury, endothelial dysfunction, and myocardial damage.

ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) represent distinct clinical phenotypes of acute coronary syndromes characterized by differences in coronary occlusion, ischemic burden, inflammatory activation, and myocardial injury severity. However, the extent to which integrated redox imbalance and endothelial injury pathways differ between these entities remains incompletely understood. Identification of readily measurable biomarkers capable of simultaneously reflecting oxidative stress burden, antioxidant defense exhaustion, and endothelial dysfunction may facilitate biological phenotyping and improve mechanistic understanding of acute coronary ischemia.

Although oxidative stress and endothelial dysfunction have been extensively investigated in cardiovascular disease, most previous studies have examined individual biomarkers separately. Consequently, the integrated interaction between extracellular antioxidant defense systems, iron-dependent redox regulation, and nitric oxide signaling during acute myocardial infarction remains insufficiently characterized. To our knowledge, no previous clinical study has simultaneously evaluated total sulfhydryl status, ceruloplasmin, haptoglobin, asymmetric dimethylarginine, and nitric oxide within a unified redox–endothelial injury framework in both STEMI and NSTEMI patients. Such an integrated approach may provide novel insights into the biological interplay among oxidative injury, ferroptosis-associated pathways, endothelial dysfunction, and ischemic myocardial damage.

Therefore, the present study aimed to characterize the integrated redox–endothelial injury profile associated with acute myocardial infarction by simultaneously assessing serum total sulfhydryl status, ceruloplasmin, haptoglobin, asymmetric dimethylarginine, and nitric oxide concentrations in patients with STEMI and NSTEMI. Furthermore, we sought to determine whether coordinated alterations in these biomarkers reflect progressive impairment of antioxidant defense systems and endothelial homeostasis during acute coronary ischemia and whether this composite biochemical signature may contribute to future biomarker-guided risk stratification and mechanistically targeted cardiovascular therapies.

MATERIALS AND METHODS

Study Design and Population

This prospective case–control study was conducted to investigate the integrated relationship between oxidative stress, endogenous antioxidant defense systems, and endothelial injury in patients presenting with acute myocardial infarction (AMI).

Blood samples were collected from participants during routine clinical care and stored at –80°C for potential future research. The study hypothesis and the specific biomarker panel (total sulfhydryl status, ceruloplasmin, haptoglobin, asymmetric dimethylarginine, and nitric oxide) were developed after sample collection. Ethical approval was obtained prior to the initiation of any laboratory analyses.

Consecutive patients admitted to the Department of Cardiology between April 2025 and March 2026 were screened for eligibility. A total of 93 participants were enrolled and allocated into three groups: ST-segment elevation myocardial infarction (STEMI; n = 31), non-ST-segment elevation myocardial infarction (NSTEMI; n = 31), and age- and gender-matched healthy controls (n = 31). Baseline demographic and clinical characteristics are summarized in Table 1.

The diagnosis of STEMI and NSTEMI was established according to the 2023 European Society of Cardiology Guidelines for Acute Coronary Syndromes using clinical presentation, electrocardiographic findings, cardiac biomarker elevation, and coronary angiographic assessment. STEMI was defined as persistent ST-segment elevation accompanied by biochemical evidence of myocardial necrosis, whereas NSTEMI was defined as acute myocardial injury without persistent ST-segment elevation but with elevated cardiac troponin concentrations and compatible clinical findings.

Healthy control subjects were recruited from individuals undergoing routine cardiovascular evaluation. Control participants had no history of coronary artery disease, heart failure, inflammatory disorders, autoimmune disease, malignancy, chronic liver disease, chronic kidney disease, or antioxidant supplementation.

Exclusion criteria included previous myocardial infarction, prior coronary revascularization, chronic heart failure, severe renal impairment (estimated glomerular filtration rate <30 mL/min/1.73 m^2), chronic hepatic disease, active infection, autoimmune disease, malignancy, hematological disorders, and current antioxidant treatment.

Ethical Approval

The study protocol was approved by the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (Decision No. 251079; Approval Date: 07 April 2026). All procedures conformed to the ethical principles outlined in the Declaration of Helsinki.

Blood samples were collected as part of routine clinical care and stored at –80°C for potential future research. The study hypothesis and the specific biomarker panel were developed after sample collection. Ethical approval was obtained prior to the initiation of any laboratory measurements or data analyses. Written informed consent for the use of stored biological samples for future research was obtained from all participants at the time of enrollment, in compliance with institutional biobanking regulations.

This study was conducted as a non-interventional observational investigation; therefore, it was not registered in a clinical trial registry.

Clinical Assessment and Sample Collection

Demographic characteristics, cardiovascular risk factors, medication history, physical examination findings, and routine laboratory parameters were recorded at hospital admission.

Peripheral venous blood samples were obtained immediately after diagnosis and before coronary intervention, thrombolytic treatment, or reperfusion therapy. Blood samples were centrifuged at 3000 rpm for 10 min within 30 min of collection. Serum aliquots were separated and stored at −80°C until biochemical analysis.

To minimize analytical bias, laboratory personnel were blinded to clinical diagnosis and group allocation throughout the study.

Assessment of Oxidative Stress Biomarkers

Total Sulfhydryl Status

Serum total sulfhydryl status (TSH) was determined using the spectrophotometric method described by Ellman. The assay is based on the reaction between free sulfhydryl groups and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), generating a chromophore measured at 412 nm (Catalog Number: NBP3-25870). TSH was selected as an indicator of systemic antioxidant reserve and extracellular redox buffering capacity.

Ceruloplasmin

Serum ceruloplasmin concentrations were measured using an automated immunonephelometric assay according to the manufacturer's recommendations (Catalog Number: BIO4718). Ceruloplasmin was evaluated because of its ferroxidase activity and its role in limiting iron-catalyzed oxidative reactions.

Haptoglobin

Serum haptoglobin concentrations were quantified using an automated immunoturbidimetric assay (Catalog Number: H3536). Haptoglobin was included because of its protective role against hemoglobin-mediated oxidative injury and iron-dependent radical formation.

Assessment of Endothelial Injury Biomarkers

Asymmetric Dimethylarginine

Serum asymmetric dimethylarginine (ADMA) concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Catalog Number: MBS161697). ADMA is an endogenous nitric oxide synthase inhibitor and a well-established marker of endothelial dysfunction.

Nitric Oxide

Nitric oxide bioavailability was assessed indirectly through measurement of serum nitrate and nitrite metabolites using the Griess reaction method (Catalog Number: MAK454). Because of the short biological half-life of nitric oxide, determination of its stable metabolites is widely accepted as an indirect measure of endothelial nitric oxide production and vascular homeostasis.

Assay Performance Characteristics

All samples were measured in duplicate, and the mean values were used for statistical analysis. Assay-specific catalog numbers are provided in the respective method sections above.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Data distribution was assessed using the Shapiro–Wilk test together with visual examination of histograms and Q–Q plots. Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are presented as frequencies and percentages.

Since most biomarker variables did not follow a normal distribution, non-parametric tests were used for group comparisons. Comparisons among STEMI, NSTEMI, and control groups were performed using the Kruskal–Wallis H test, followed by Mann–Whitney U tests with Bonferroni correction for pairwise comparisons. Categorical variables were compared using the χ^2 test.

Effect sizes for ANOVA were quantified using eta-squared (η^2) statistics. According to conventional criteria, η^2 values greater than 0.14 were considered indicative of large effects.

Spearman's rank correlation coefficients were calculated to evaluate the relationships among biomarkers and with clinical severity markers (Troponin I, LVEF, CRP).

Multivariate analysis of variance (MANOVA) was performed to assess the effect of group allocation on biomarker levels after adjusting for potential confounders, including age, gender, hypertension, and diabetes mellitus. Partial eta-squared (η^2) values were reported as measures of effect size.

Receiver operating characteristic (ROC) curve analyses were performed to evaluate the discriminatory performance of oxidative stress and endothelial injury biomarkers for identifying acute myocardial infarction. Areas under the curve (AUCs), optimal cutoff values, sensitivities, specificities, and corresponding 95% confidence intervals were calculated.

All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

RESULTS

Baseline Clinical Characteristics

Baseline demographic and clinical characteristics of the study population are presented in Table 1. Patients with acute myocardial infarction exhibited a higher prevalence of conventional cardiovascular risk factors than healthy controls. Hypertension and diabetes mellitus were more frequent in both STEMI and NSTEMI groups, whereas HDL-C concentrations were significantly lower and LDL-C and C-reactive protein (CRP) levels were significantly higher compared with controls (all $p < 0.05$). Troponin I concentrations were markedly elevated in both myocardial infarction groups, confirming acute myocardial injury. In addition, left ventricular ejection fraction (LVEF) was significantly reduced in STEMI and NSTEMI patients compared with healthy controls ($p < 0.001$).

Oxidative Stress Biomarkers

Significant differences in oxidative stress biomarkers were observed among STEMI patients, NSTEMI patients, and healthy controls (Table 2, Figure 1). Total sulfhydryl status (TSH), ceruloplasmin, and haptoglobin concentrations were significantly lower in patients with acute myocardial infarction than in controls (all $p < 0.001$, Kruskal–Wallis H test).

TSH concentrations decreased from 27.87 ± 0.46 μmol/L in controls to 14.15 ± 0.07 μmol/L in NSTEMI patients and 12.32 ± 0.13 μmol/L in STEMI patients. Similarly, ceruloplasmin concentrations were lower in STEMI (2.16 ± 0.04 mg/dL) and NSTEMI (3.13 ± 0.05 mg/dL) patients than in controls (5.99 ± 0.09 mg/dL). Haptoglobin concentrations were also reduced, decreasing from 12.10 ± 0.04 mg/dL in controls to 6.06 ± 0.03 mg/dL in NSTEMI patients and 5.18 ± 0.06 mg/dL in STEMI patients.

Mann–Whitney U post-hoc analyses demonstrated significant differences among all pairwise group comparisons for TSH, ceruloplasmin, and haptoglobin (all $p < 0.001$; Table 3).

Endothelial Dysfunction Biomarkers

Significant alterations in endothelial dysfunction biomarkers were observed among study groups (Table 2, Figure 1). ADMA concentrations were significantly higher in patients with acute myocardial infarction than in healthy controls ($p < 0.001$, Kruskal–Wallis H test). The highest ADMA concentrations were observed in NSTEMI patients (15.25 ± 0.17 μmol/L), followed by STEMI patients (14.19 ± 0.05 μmol/L), whereas controls exhibited substantially lower values (7.27 ± 0.15 μmol/L).

Conversely, nitric oxide concentrations were significantly reduced in myocardial infarction patients. NO concentrations decreased from 1.25 ± 0.03 μmol/L in controls to 0.64 ± 0.02 μmol/L in NSTEMI patients and 0.54 ± 0.03 μmol/L in STEMI patients ($p < 0.001$).

Mann–Whitney U post-hoc analyses confirmed significant differences among all pairwise comparisons for both ADMA and NO (all $p < 0.001$; Table 3).

Integrated Oxidative Stress–Endothelial Dysfunction Profile

Integrated assessment of oxidative stress and endothelial dysfunction biomarkers demonstrated a coordinated pattern of antioxidant depletion and endothelial impairment in acute myocardial infarction (Figures 1 and 2). Compared with healthy controls, AMI patients exhibited lower concentrations of TSH, ceruloplasmin, haptoglobin, and NO together with higher ADMA concentrations.

Among patients with myocardial infarction, STEMI patients generally demonstrated greater reductions in antioxidant biomarkers and NO concentrations than NSTEMI patients. Figure 2 summarizes the proposed mechanistic relationship linking oxidative stress, antioxidant depletion, endothelial dysfunction, and myocardial injury.

Effect Size Analysis

Effect size analysis demonstrated strong associations between disease status and biomarker concentrations (Table 4). Eta-squared (η^2) values ranged from 0.992 to 1.000, indicating that a substantial proportion of biomarker variability was explained by study-group allocation.

The largest effect size was observed for haptoglobin ($\eta^2 = 1.000$), followed by ceruloplasmin and ADMA ($\eta^2 = 0.999$), TSH ($\eta^2 = 0.998$), and nitric oxide ($\eta^2 = 0.992$).

Correlation Analysis

Spearman's rank correlation analysis revealed significant inter-correlations among biomarkers and with clinical severity markers (Table 5). TSH, ceruloplasmin, haptoglobin, and nitric oxide demonstrated very strong positive correlations with each other, with correlation coefficients ranging from 0.881 to 0.906 (all $p < 0.001$), confirming that these biomarkers move in a coordinated manner as components of an integrated antioxidant–endothelial defense network. Conversely, ADMA was significantly negatively correlated with all protective biomarkers, with coefficients ranging from -0.437 to -0.448 (all $p < 0.001$).

All biomarkers were significantly correlated with Troponin I, which served as a marker of ischemic severity. TSH ($r = -0.824$, $p < 0.001$), ceruloplasmin ($r = -0.816$, $p < 0.001$), haptoglobin ($r = -0.819$, $p < 0.001$), and nitric oxide ($r = -0.847$, $p < 0.001$) all demonstrated strong negative correlations with Troponin I, whereas ADMA showed a moderate positive correlation ($r = 0.559$, $p < 0.001$). Similarly, all biomarkers were significantly associated with left ventricular ejection fraction (LVEF). TSH ($r = 0.444$, $p < 0.001$), ceruloplasmin ($r = 0.407$, $p < 0.001$), haptoglobin ($r = 0.417$, $p < 0.001$), and nitric oxide ($r = 0.424$, $p < 0.001$) demonstrated moderate positive correlations with LVEF, while ADMA showed a moderate negative correlation ($r = -0.333$, $p = 0.001$). C-reactive protein (CRP) showed a weak but significant negative correlation with LVEF ($r = -0.249$, $p = 0.016$) and a weak positive correlation with Troponin I ($r = 0.218$, $p = 0.036$).

Diagnostic Performance Analysis

Receiver operating characteristic (ROC) analyses demonstrated perfect discriminatory performance for all investigated biomarkers in differentiating patients with acute myocardial infarction from healthy controls (Table 7, Figure 3). All biomarkers achieved an area under the curve (AUC) of 1.000 (95% CI: 1.000–1.000), reflecting the exceptionally strong and consistent separation between patients and controls. The optimal cut-off value for total sulfhydryl status (TSH) was determined as ≤12.16 µmol/L, yielding a sensitivity of 96.8% and a specificity of 100%. For ceruloplasmin, the optimal cut-off was ≤2.10 mg/dL, with 96.8% sensitivity and 100% specificity. Haptoglobin demonstrated a cut-off of ≤5.12 mg/dL, providing 95.2% sensitivity and 100% specificity. For asymmetric dimethylarginine (ADMA), the optimal cut-off was ≥14.12 µmol/L, with 95.2% sensitivity and 100% specificity. Nitric oxide (NO) showed a cut-off of ≤0.50 µmol/L, yielding 93.5% sensitivity and 100% specificity. While these findings demonstrate the fundamental biological separation of the redox–endothelial injury axis, they should be interpreted with caution as they do not reflect real-world diagnostic performance in unselected patient populations presenting with suspected acute coronary syndrome.

DISCUSSION

The present study demonstrates that acute myocardial infarction is associated with a coordinated disruption of antioxidant defense systems and endothelial homeostasis. The principal findings were: (i) significant

reductions in total sulfhydryl status (TSH), ceruloplasmin, haptoglobin, and nitric oxide (NO) concentrations in both STEMI and NSTEMI patients; (ii) marked elevation of asymmetric dimethylarginine (ADMA); (iii) progressively greater biomarker abnormalities in patients with more severe ischemic injury; (iv) strong inter-correlations among biomarkers supporting an integrated redox–endothelial injury axis; and (v) perfect discriminatory performance of these biomarkers for distinguishing patients with acute myocardial infarction from healthy controls. Collectively, these observations support the existence of an integrated oxidative stress–endothelial dysfunction axis contributing to myocardial injury during acute coronary syndromes.

Oxidative stress has emerged as a central mechanism in the pathogenesis of myocardial ischemia and reperfusion injury. Beyond the primary ischemic insult caused by coronary artery occlusion, excessive production of reactive oxygen species (ROS) contributes to lipid peroxidation, protein oxidation, mitochondrial dysfunction, DNA damage, inflammatory activation, and cardiomyocyte death (4–9). Experimental studies have demonstrated that mitochondrial electron leakage, NADPH oxidase activation, xanthine oxidase activity, and uncoupled endothelial nitric oxide synthase (eNOS) collectively amplify oxidative burden during acute myocardial infarction (5–8). In turn, oxidative stress promotes infarct expansion, adverse ventricular remodeling, and progression toward heart failure (3–5). The marked biochemical disturbances observed in the present study are consistent with this established redox-mediated model of myocardial injury.

Importantly, these alterations may also be interpreted within the broader framework of redox-mediated cardiovascular toxicity. Excessive ROS generation not only reflects oxidative imbalance but also exerts direct toxic effects on vascular and myocardial tissues through disruption of cellular signaling pathways, mitochondrial bioenergetics, membrane integrity, and endothelial function. The observed biomarker profile may therefore represent a clinically relevant manifestation of cardiovascular toxicity driven by persistent redox dysregulation during acute myocardial ischemia. Such toxicity may contribute to both acute cardiomyocyte injury and subsequent adverse cardiac remodeling.

Among the investigated biomarkers, TSH demonstrated one of the most prominent alterations. Thiol-containing compounds represent a major extracellular antioxidant defense system responsible for maintaining redox balance through direct scavenging of ROS and regulation of redox-sensitive signaling pathways (11). Previous studies have shown that disturbances in thiol/disulfide homeostasis are associated with acute coronary syndromes and adverse cardiovascular outcomes (12,13). The substantial depletion of TSH observed in our cohort likely reflects accelerated oxidation and consumption of sulfhydryl-containing antioxidants in response to increased oxidative stress. The progressive decline from controls to NSTEMI and STEMI patients further suggests that thiol depletion may mirror increasing ischemic severity and oxidative burden.

A particularly important finding was the marked reduction in ceruloplasmin and haptoglobin concentrations. Ceruloplasmin functions as a copper-containing ferroxidase that limits iron-catalyzed free radical formation by converting ferrous iron (Fe^{2+}) into the less reactive ferric form (Fe^{3+}), whereas haptoglobin protects tissues from hemoglobin-mediated oxidative toxicity by binding free hemoglobin and facilitating its clearance (14,15). Both proteins play essential roles in maintaining iron homeostasis and preventing iron-dependent oxidative injury. Their depletion in the setting of acute myocardial infarction may therefore indicate exhaustion of endogenous iron-regulatory antioxidant defenses.

Recent advances in cardiovascular toxicology have highlighted ferroptosis as a critical mechanism of ischemic myocardial injury. Ferroptosis is a regulated form of cell death characterized by iron-dependent lipid peroxidation and accumulation of toxic lipid radicals (10,16). Experimental studies have demonstrated that ferroptotic signaling contributes to ischemia-reperfusion injury, cardiomyocyte death, ventricular remodeling, and heart failure progression (10,16). Because ceruloplasmin and haptoglobin are closely involved in iron sequestration and detoxification, their depletion may increase susceptibility to ferroptosis-mediated cellular damage. Loss of these protective mechanisms may facilitate the accumulation of redox-active iron, promote lipid peroxidation, and amplify myocardial toxicity. Accordingly, our findings support a potential mechanistic link between oxidative stress, impaired iron regulation, ferroptotic injury, and acute myocardial infarction.

Furthermore, the combined reduction of ceruloplasmin and haptoglobin may represent more than a simple consequence of oxidative stress. From a toxicological perspective, impairment of iron-buffering systems may create a permissive environment for iron-driven cardiovascular toxicity characterized by uncontrolled radical generation, membrane damage, mitochondrial dysfunction, and cardiomyocyte loss. This concept is supported by emerging evidence demonstrating that ferroptosis-associated pathways contribute substantially to myocardial injury and adverse post-infarction remodeling. Consequently, the observed biomarker alterations may reflect activation of a ferroptosis-related toxicological cascade operating during acute coronary ischemia.

Another major observation was the reciprocal alteration of ADMA and NO concentrations. Endothelial dysfunction represents a key pathological component of acute coronary syndromes and contributes to impaired coronary microvascular perfusion, inflammation, thrombosis, and myocardial injury (17–19). Nitric oxide is a critical mediator of vascular homeostasis and exerts vasodilatory, anti-inflammatory, antiplatelet, and cytoprotective effects (17,18). Reduced NO bioavailability therefore represents a hallmark of endothelial dysfunction and has been associated with adverse cardiovascular outcomes (18,19).

ADMA serves as an endogenous competitive inhibitor of nitric oxide synthase and provides an important mechanistic connection between oxidative stress and endothelial dysfunction (20–22). Elevated ADMA concentrations inhibit NO synthesis, promote eNOS uncoupling, and enhance ROS generation. Furthermore, oxidative stress suppresses dimethylarginine dimethylaminohydrolase (DDAH), the primary enzyme responsible for ADMA degradation, thereby promoting ADMA accumulation (19–22). This bidirectional interaction establishes a self-amplifying cycle in which oxidative stress increases ADMA, elevated ADMA further reduces NO production, and endothelial dysfunction subsequently aggravates ischemic injury. The simultaneous increase in ADMA and decrease in NO observed in our patients strongly supports the presence of this pathogenic pathway during acute myocardial infarction.

Integrated assessment of biomarkers provides formal statistical support for the proposed redox–endothelial injury axis. Spearman correlation analysis revealed very strong positive inter-correlations among TSH, ceruloplasmin, haptoglobin, and NO ($r = 0.881–0.906$, all $p < 0.001$), confirming that these biomarkers move in a coordinated manner as components of an integrated antioxidant–endothelial defense network. Conversely, ADMA was significantly negatively correlated with all protective biomarkers ($r = -0.437$ to -0.448, all $p < 0.001$), supporting its role as a reciprocal marker of endothelial inhibition. Importantly, all biomarkers demonstrated significant correlations with Troponin I and LVEF, indicating their association with ischemic severity. These findings support the concept that oxidative stress and endothelial dysfunction should not be viewed as isolated biological phenomena, but rather as components of a coordinated redox–vascular injury network.

Furthermore, multivariate analysis of covariance (MANOVA) confirmed that group differences remained highly significant after adjusting for age, gender, hypertension, and diabetes mellitus (Pillai's Trace = 1.978, $F = 1473.95$, $p < 0.001$, partial $\eta^2 = 0.989$). Notably, none of the covariates had a significant effect on biomarker levels (all $p > 0.05$), indicating that the observed differences are independent of traditional cardiovascular risk factors. This finding strengthens the biological specificity of the redox–endothelial injury axis and suggests that these biomarkers reflect pathophysiological processes beyond conventional risk factor burden.

Receiver operating characteristic analyses demonstrated perfect discriminatory performance for all investigated biomarkers, with AUC values of 1.000 for TSH, ceruloplasmin, haptoglobin, ADMA, and NO. While these findings indicate substantial biological separation between patients and controls, the perfect separation observed in the present study should be interpreted with caution. This degree of separation is exceptional in clinical biomarker research and likely reflects the profound biological differences in the redox–endothelial injury axis rather than real-world diagnostic utility. The diagnostic performance observed in the present study does not establish clinical diagnostic performance in patients presenting with suspected acute coronary syndrome. Validation in larger, multicenter cohorts with clinically relevant comparison groups—such as patients with chest pain or nonischemic myocardial injury—is necessary before clinical implementation can be considered. Accordingly, we have moderated our diagnostic claims and emphasize that these biomarkers are mechanistically informative rather than ready for clinical application.

Several limitations should be acknowledged. First, this was a single-center study with a relatively limited sample size. Second, biomarker measurements were obtained at a single time point and therefore do not reflect temporal changes during infarct evolution. Third, long-term clinical outcomes were not available, precluding assessment of prognostic significance. Fourth, the study did not include direct measurements of ROS generation, lipid peroxidation products, ferroptosis-related mediators, or endothelial function testing. Fifth, the perfect separation observed in ROC analysis, while supportive of the biological framework, limits the generalizability of these biomarkers as stand-alone diagnostic tests in unselected patient populations. Finally, the observational design does not permit causal inference regarding the relationships among oxidative stress, endothelial dysfunction, ferroptosis, and myocardial injury.

In conclusion, acute myocardial infarction is characterized by substantial depletion of antioxidant defense mechanisms, disruption of iron-regulatory protective pathways, accumulation of ADMA, and reduced nitric oxide bioavailability. These coordinated biochemical alterations support the existence of an integrated oxidative stress–endothelial dysfunction axis linking redox imbalance, ferroptosis-related mechanisms, cardiovascular toxicity, vascular injury, and myocardial damage. Simultaneous assessment of TSH, ceruloplasmin, haptoglobin, ADMA, and NO may provide a mechanistically informative biomarker profile in acute myocardial infarction and warrants further investigation in larger prospective studies.

CONCLUSIONS

This study demonstrates that acute myocardial infarction is associated with a profound and coordinated disruption of redox homeostasis, characterized by depletion of endogenous antioxidant defense mechanisms (TSH, ceruloplasmin, haptoglobin), accumulation of asymmetric dimethylarginine (ADMA), and reduced nitric oxide (NO) bioavailability. The strong inter-correlations among TSH, ceruloplasmin, haptoglobin, and NO, together with their reciprocal relationship with ADMA, provide formal statistical support for the existence of an

integrated oxidative stress–endothelial dysfunction axis linking redox imbalance, vascular injury, and myocardial damage during acute coronary ischemia.

Importantly, multivariate analysis confirmed that these biomarker alterations are independent of age, gender, hypertension, and diabetes mellitus, underscoring their biological specificity and suggesting that they reflect pathophysiological processes beyond conventional cardiovascular risk factors. The observed reductions in total sulfhydryl status, ceruloplasmin, and haptoglobin suggest exhaustion of endogenous antioxidant and iron-buffering systems, whereas elevated ADMA and decreased nitric oxide concentrations indicate substantial endothelial dysfunction. Together, these findings are consistent with emerging evidence implicating oxidative stress, impaired iron homeostasis, and ferroptosis-related mechanisms in the pathogenesis of ischemic myocardial injury.

Simultaneous assessment of TSH, ceruloplasmin, haptoglobin, ADMA, and nitric oxide provided a comprehensive biochemical profile that effectively distinguished patients with acute myocardial infarction from healthy controls. While the perfect separation observed in ROC analysis reflects the fundamental biological strength of this pathway, these biomarkers should be viewed as mechanistically informative rather than ready for clinical diagnostic application. Validation in larger, multicenter studies incorporating longitudinal follow-up and clinically relevant comparison groups—such as patients with non-ischemic chest pain—is necessary to clarify their clinical utility.

In conclusion, the coordinated biomarker alterations identified in this study support the existence of an integrated redox–endothelial injury axis in acute myocardial infarction. These findings may facilitate future biomarker-guided mechanistic research and the development of targeted redox-based therapeutic strategies for acute coronary syndromes.

Declarations

Funding

The authors received no specific funding for this study.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Approval

The study protocol was approved by the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee (Decision No. 251079; Approval Date: 07 April 2026). Written informed consent was obtained from all participants prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. Due to ethical restrictions and institutional data protection policies, the data are not

publicly available but can be accessed by qualified researchers who submit a reasonable request to the corresponding author.

Consent for publication: Not applicable, as this manuscript does not contain any individual person's data, images, or any other personal or clinical details that would compromise participant anonymity.

Author Contributions

M.A.I.: Conceptualization, methodology, patient recruitment, clinical assessment, data acquisition, interpretation of findings, supervision, manuscript review and editing.

H.D.: Biochemical analyses, laboratory investigations, formal analysis, statistical analysis, visualization, drafting of the manuscript, and critical revision.

All authors reviewed and approved the final version of the manuscript.

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Tables

Table 1. Baseline Clinical Characteristics of the Study Population

Variable	STEMI (n=31)	NSTEMI (n=31)	Control (n=31)	p value
Age (years)	60.9 ± 12.9	67.3 ± 11.9	62.5 ± 10.8	0.041
Male sex, n (%)	30 (96.8)	23 (74.2)	19 (61.3)	0.048
Hypertension, n (%)	19 (61.3)	22 (71.0)	8 (25.8)	0.021
Diabetes mellitus, n (%)	14 (45.2)	23 (74.2)	4 (12.9)	<0.001
Current smoking, n (%)	18 (58.1)	15 (48.4)	6 (19.4)	0.034
LDL-C (mg/dL)	111.3 ± 31.7	106.6 ± 29.8	92.4 ± 21.6	0.018
HDL-C (mg/dL)	34.8 ± 7.1	37.3 ± 8.4	49.6 ± 9.3	<0.001
CRP (mg/L)	23.2 ± 16.8	21.0 ± 14.5	2.8 ± 1.6	<0.001
Troponin I (ng/mL)	18,924 ± 9,875	3,352 ± 2,914	Not detectable	<0.001
LVEF (%)	54.3 ± 8.1	53.6 ± 7.9	62.8 ± 4.7	<0.001

Data are presented as mean ± SD or n (%). Categorical variables were compared using χ^2 test; continuous variables were compared using one-way ANOVA. **Abbreviations:** STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; LVEF, left ventricular ejection fraction.

Table 2. Oxidative Stress and Endothelial Dysfunction Biomarkers

Biomarker	Unit	STEMI (n=31)	NSTEMI (n=31)	Control (n=31)	F	p	η^2
TSH	µmol/L	12.32 ± 0.13	14.15 ± 0.07	27.87 ± 0.46	28833.84	<0.001	0.998
Ceruloplasmin	mg/dL	2.16 ± 0.04	3.13 ± 0.05	5.99 ± 0.09	30341.43	<0.001	0.999
Haptoglobin	mg/dL	5.18 ± 0.06	6.06 ± 0.03	12.10 ± 0.04	192617.34	<0.001	1.000
ADMA	µmol/L	14.19 ± 0.05	15.25 ± 0.17	7.27 ± 0.15	31552.73	<0.001	0.999
NO	µmol/L	0.54 ± 0.03	0.64 ± 0.02	1.25 ± 0.03	5871.81	<0.001	0.992

Values are mean ± SD. Intergroup differences were evaluated using one-way ANOVA; effect sizes were quantified using eta-squared (η^2). **Abbreviations:** TSH, total sulfhydryl status; ADMA, asymmetric dimethylarginine; NO, nitric oxide.

Table 3. Pairwise Comparisons of Biomarkers (Mann-Whitney U Test with Bonferroni Correction)

Biomarker	STEMI vs Control	NSTEMI vs Control	STEMI vs NSTEMI
TSH	<0.001	<0.001	<0.001
Ceruloplasmin	<0.001	<0.001	<0.001
Haptoglobin	<0.001	<0.001	<0.001
ADMA	<0.001	<0.001	<0.001
NO	<0.001	<0.001	<0.001

Data are presented as p values after Bonferroni correction. Pairwise comparisons were performed using Mann-Whitney U test. **Abbreviations:** TSH, total sulfhydryl status; ADMA, asymmetric dimethylarginine; NO, nitric oxide.

Table 4. Correlation Matrix of Biomarkers and Clinical Severity Markers (Spearman's Rho)

	TSH	Ceruloplasmin	Haptoglobin	ADMA	NO	EF	Troponin
TSH	1.000	0.894**	0.895**	-0.448**	0.906**	0.444**	-0.824**
Ceruloplasmin	0.894**	1.000	0.887**	-0.445**	0.881**	0.407**	-0.816**
Haptoglobin	0.895**	0.887**	1.000	-0.437**	0.905**	0.417**	-0.819**
ADMA	-0.448**	-0.445**	-0.437**	1.000	-0.443**	-0.333**	0.559**
NO	0.906**	0.881**	0.905**	-0.443**	1.000	0.424**	-0.847**
EF	0.444**	0.407**	0.417**	-0.333**	0.424**	1.000	-0.539**
Troponin	-0.824**	-0.816**	-0.819**	0.559**	-0.847**	-0.539**	1.000

p < 0.01. **Abbreviations: TSH, total sulfhydryl status; ADMA, asymmetric dimethylarginine; NO, nitric oxide; EF, ejection fraction.

Table 5. Diagnostic Performance of Biomarkers for Identifying Acute Myocardial Infarction

Biomarker	AUC (95% CI)	Optimal Cutoff	Sensitivity (%)	Specificity (%)	p
TSH (µmol/L)	1.000 (1.000–1.000)	≤12.16	96.8	100	<0.001
Ceruloplasmin (mg/dL)	1.000 (1.000–1.000)	≤2.10	96.8	100	<0.001
Haptoglobin (mg/dL)	1.000 (1.000–1.000)	≤5.12	95.2	100	<0.001
ADMA (µmol/L)	1.000 (1.000–1.000)	≥14.12	95.2	100	<0.001
NO (µmol/L)	1.000 (1.000–1.000)	≤0.50	93.5	100	<0.001

Abbreviations: AUC, area under the curve; CI, confidence interval; TSH, total sulfhydryl status; ADMA, asymmetric dimethylarginine; NO, nitric oxide.

Table 6. Multivariate Analysis of Variance (MANOVA) with Covariates

Multivariate Tests (Group Effect)					
Effect	**Pillai's Trace**	**F**	**df**	**p**	**Partial η²**
HastaGrubu	1.978	1473.95	(10, 166)	<0.001	0.989
Tests of Between Subjects Effects					
Biomarker	**F (HastaGrubu)**	**p**	**Partial η²**		
TSH	21958.74	<0.001	0.998		
Ceruloplasmin	25277.72	<0.001	0.998		
Haptoglobin	148667.59	<0.001	1.000		
ADMA	24313.49	<0.001	0.998		
NO	4549.58	<0.001	0.991		

Covariates (all p > 0.05): Age, Sex, Hypertension, Diabetes Mellitus. **Abbreviations:** TSH, total sulfhydryl status; ADMA, asymmetric dimethylarginine; NO, nitric oxide.

Table 7 is not available with this version.

Figures

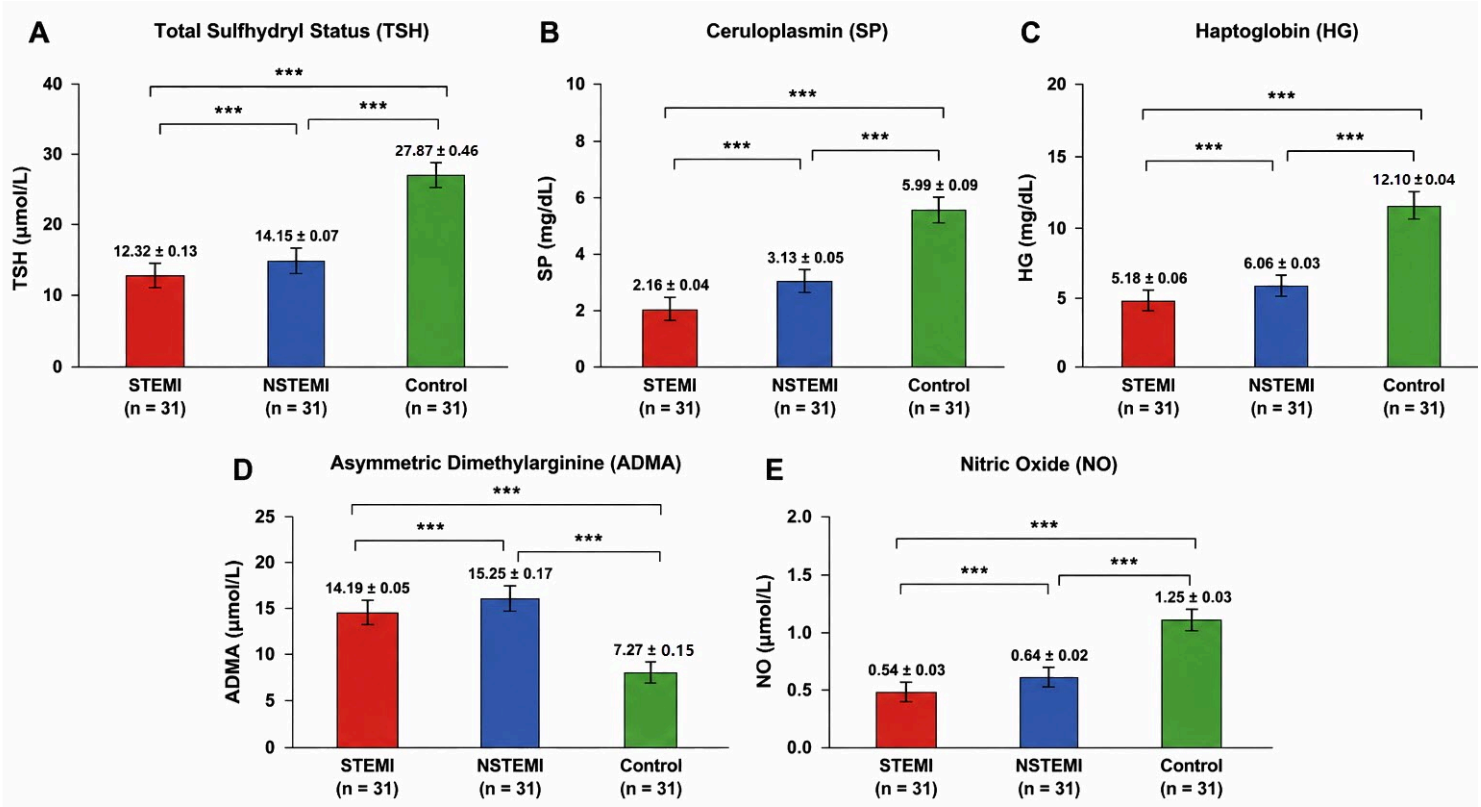


Figure 1. Oxidative Stress and Endothelial Dysfunction Biomarkers in STEMI, NSTEMI, and Healthy Controls

Mean ± SD concentrations of (A) total sulfhydryl status (TSH), (B) ceruloplasmin (SP), (C) haptoglobin (HG), (D) asymmetric dimethylarginine (ADMA), and (E) nitric oxide (NO) in patients with ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and healthy controls. Patients with acute myocardial infarction exhibited significantly lower TSH, ceruloplasmin, haptoglobin, and NO concentrations together with significantly higher ADMA levels compared with controls. ***p < 0.001 for all pairwise comparisons (Kruskal-Wallis H test with Mann-Whitney U post-hoc and Bonferroni correction).
Error bars indicate standard deviation.

Figure 1

See image above for figure legend

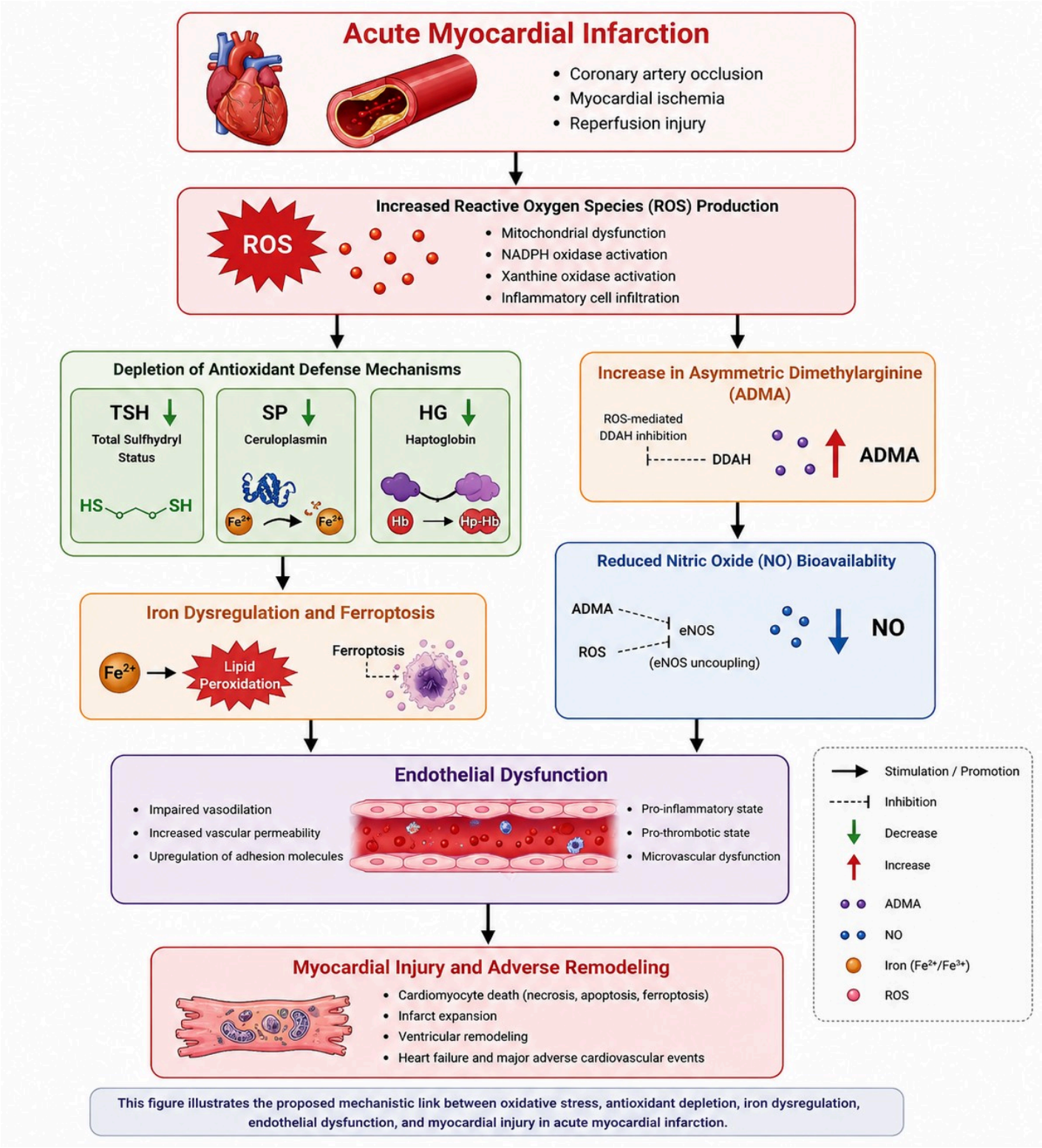


Figure 2

Proposed Mechanistic Relationship between Oxidative Stress, Antioxidant Depletion, Endothelial Dysfunction, and Myocardial Injury in Acute Myocardial Infarction

Legend

Schematic illustration of the proposed pathophysiological pathway linking oxidative stress and endothelial dysfunction in acute myocardial infarction. Increased reactive oxygen species (ROS) production is associated with depletion of endogenous antioxidant defense mechanisms, reflected by reduced total sulfhydryl status (TSH), ceruloplasmin (SP), and haptoglobin (HG) concentrations. Oxidative stress is accompanied by increased asymmetric dimethylarginine (ADMA) levels and reduced nitric oxide (NO) bioavailability, resulting in endothelial dysfunction and subsequent myocardial injury. The figure summarizes the biological framework underlying the observed biochemical alterations.

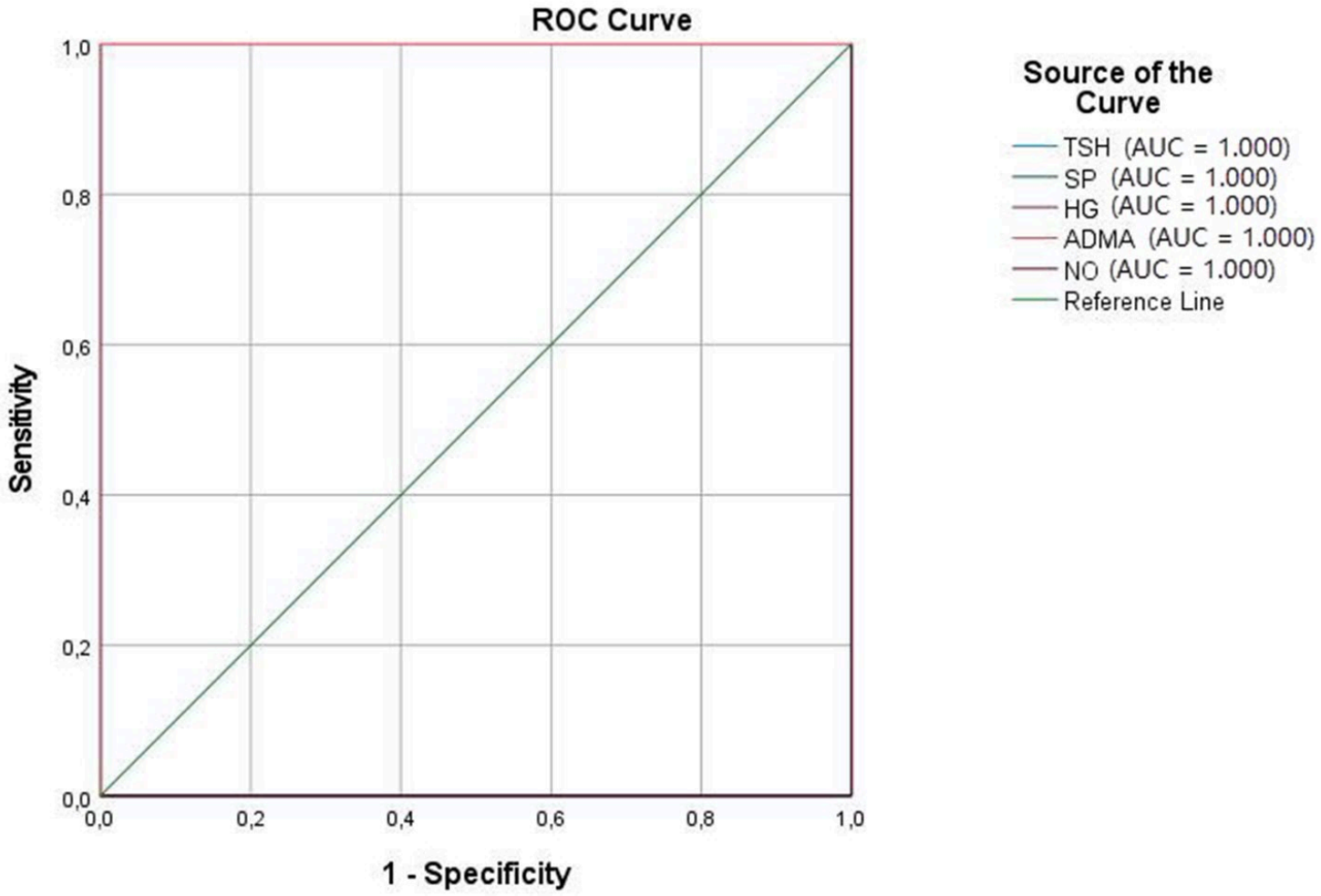


Figure 3

Receiver Operating Characteristic (ROC) Curve Analysis of Oxidative Stress and Endothelial Dysfunction Biomarkers for Identification of Acute Myocardial Infarction

Legend

Receiver operating characteristic (ROC) curves evaluating the discriminatory performance of total sulfhydryl status (TSH), ceruloplasmin (SP), haptoglobin (HG), asymmetric dimethylarginine (ADMA), and nitric oxide (NO) for distinguishing patients with acute myocardial infarction from healthy controls. All biomarkers demonstrated perfect discriminatory performance, with an area under the curve (AUC) of 1.000 (95% CI: 1.000–1.000) . The

diagonal dashed line represents the line of no discrimination (AUC = 0.50). Detailed AUC values, optimal cutoff points, sensitivities, and specificities are presented in Table 5.