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*Original Article*

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# Development and Clinical Validation of a Novel Inflamm-Aging Score in Cardiovascular Disease

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**Abstract:** Chronic low-grade inflammation associated with aging "inflamm-aging" contributes to cardiovascular disease (CVD), yet traditional risk assessment relies on single biomarkers. This cross-sectional study aims to evaluate and validate a composite inflamm-aging score in 65 patients with CVD and 55 age-matched healthy controls. The score was derived from three biomarkers of interleukin 6 (IL-6), high-sensitivity c-reactive protein (hsCRP), and matrix metalloproteinase-9 (MMP9)- using principal component analysis (PCA). The PCA yielded one component with an eigenvalue of 2.172 (72.4% variance), and the second component's eigenvalue was 0.52 (<1.0, Kaiser criterion). The biomarker loadings were IL-6 (0.58), hsCRP (0.54), and MMP9 (0.61). The weighted composite was adjusted for age ($\beta$ = 0.045/year, p=0.002) and sex ($\beta$ = 0.31 female vs. male, p = 0.018) using multiple linear regression among the controls ($R^2$ = 0.246). The final score was calculated as the residual. The score distinguished CVD from the controls with an area under the curve of 0.89 (an optimal cutoff of >1.20) with 81.5% sensitivity and 87.3% specificity. The score correlated strongly with N-terminal pro-B-type natriuretic peptide ($\varrho$=0.71) and the soluble suppression of tumorigenicity 2 ($\varrho$=0.58), independent of traditional risk factors including hypertension, diabetes, and dyslipidemia. A significant age-by-disease interaction (p=0.008) indicated accelerated inflamm-aging in older CVD patients. The inflamm-aging score is a promising novel biomarker of CVD severity beyond traditional assessments, potentially guiding individualized anti-inflammatory therapy. Independent external validation is required before clinical implementation.

## 1. Introduction

Cardiovascular diseases (CVD) continue to be the leading cause of death worldwide and are responsible for approximately 17.9 million deaths per year. CVD disproportionately affects low- and middle-income countries, with nearly 75% of all deaths globally attributed to CVD occurring in these countries [1, 2]. Over the last 20 years, the Eastern Mediterranean region has seen an increase in CVD prevalence due to urbanization, poor nutrition, and rising behavioral risk factors [3]. In Iraq, CVD has overtaken all other causes of death. Based on the most recent Iraqi Ministry of Health data, ischemic heart disease and cerebrovascular accidents account for over 40% of all deaths in Iraq [4]. The increasing burden of changeable risk factors, both physical and lifestyle-related (high blood pressure [31.5% in adults], metabolic syndrome [12.8%], and smoking [22.4%]), are exacerbated by a lack of community health systems to support prevention at an early age [1, 5].

Numerous medications have been shown to help control CVD, including statins, which lower lipid levels, and angiotensin receptor blockers, which lower blood pressure through multiple mechanisms. Not only are they effective for their intended purpose, they are also of great interest due to their pleiotropic effects or multiple actions on the body. Evidence shows that these medications can directly reduce the inflammation and modulate mechanisms involved in atherosclerosis and vascular dysfunction. By virtue of their ability to reduce chronic inflammation, a key characteristic of an aged vascular system, these drugs are positioned to potentially reduce the impact of the inflamm-aging process itself [6, 7].

Conventional methods for detecting coronary heart disease have well-established limitations. For example, well-known classification schemes for estimating the likelihood of coronary heart disease, such as the Framingham risk score, which relies on classic parameters like elevated blood pressure and abnormal lipids, may not account for the complex, long-term effects of low-grade immune activation on the aging vasculature [8, 9]. Another individual blood test of high-sensitivity c-reactive protein (hsCRP) provides only a partial picture of this complex issue, as it lacks sufficient sensitivity and specificity to fully characterize the evolving interplay between immune dysregulation and the initiation of plaque development [10, 11].

Recent studies have shown there to be a complex two-way relationship between biological aging and the development of CVD, and many researchers believe that chronic, low-grade systemic inflammation now referred to as (inflamm-aging) acts as a mediator between them. Although previously seen as a consequence of normal aging processes, this state of persistent inflammation is increasingly considered an active contributor to the progression of atherosclerosis and/or damage to affected organs [12]. The single most important risk factor for developing CVD is aging. However, older techniques, which typically use only a small number of isolated markers to measure systemic inflammation, do not accurately capture how the many interconnected components that comprise the term “inflamm-aging” work together and contribute to one another. When people reach old age, there is a change in how their immune systems function, with a concurrent decline in both the innate (natural) and acquired (adaptive) immune systems [13]. As both natural and adaptive immunity become more dysfunctional with age, proinflammatory cytokines and other mediators increase in serum, along with elevated protease levels that promote tissue degradation, creating an ideal environment for atheroma formation and promoting atherosclerotic plaque rupture and clot formation [14].

Ailey and Heaton's research suggests that atherosclerosis is caused by persistent low-grade inflammation. Inflammation begins when the endothelial layer of blood vessels is damaged. The endothelial layer then acts as a 'sticky surface', attracting circulating monocytes, which migrate into the arterial wall within the subendothelial space. There, they differentiate into macrophages and take up oxidized lipoproteins until they become foam cells. Together, these foam cells form the first identifiable atherosclerotic lesion: a fatty streak. The inflamed endothelium, along with the desperate attempts by macrophages to resolve the inflammation, releases a wide range of proteolytic enzymes and pro-inflammatory cytokines that destabilize the fibrous cap, increasing the likelihood of rupture and leading to thrombotic CVD [15, 16].

With the development of the inflamm-aging framework, there has been increased emphasis on assessing multiple markers of chronic inflammation associated with aging rather than a single marker. Costanzo *et al.* [17] generated a low-grade inflammatory score, which was shown to significantly correlate with mortality risk in a general population cohort. Similarly, Bonaccio *et al.* [18] developed an inflammatory score associated with age that was able to predict overall mortality risk and differentially identify successful versus unsuccessful aging trajectories. These studies show that combining inflammatory mediators offers a more comprehensive view of aging's biological impact. However, the existing scores are not validated for CVD and may require disease-specific risk assessment tools, given their distinct inflammatory profile.

The present investigation addresses this gap by developing a novel, age- and sex-normalized inflamm-aging score tailored to CVD. The score was tested for its discriminative capacity for CVD severity compared to individual biomarkers and for its association with established CVD severity markers, independent of traditional risk factors.

## 2. Materials and Methods

### 2.1. Study Design and Population

This cross-sectional observational study was conducted to develop and validate a new integrated 'Immunosenescence-related' index and to assess its association with the severity of coronary heart disease. A total of 120 participants were enrolled from the Iraqi Heart Disease Center at Baghdad Medical City, the capital city, from mid-2023 to early 2024. The research cohort was comprised of two groups: a patient group and a control group. The patient group comprised 65 participants with verified coronary heart disease recruited from the Iraqi Heart Disease Center at Baghdad Medical City. The control group consisted of 55 healthy subjects recruited from the general population, selected for the absence of any known CVD, active infections, or chronic inflammatory conditions, in order to establish a baseline for 'healthy' inflammatory markers.

Importantly, patients with metabolic disorders (hypertension, diabetes mellitus, and dyslipidemia) were included in the CVD group to reflect real-world clinical populations, and statistical adjustment for these factors was performed in the multivariable regression analyses to isolate the independent effect of the inflamm-aging score.

#### *2.1.1. Inclusion Criteria*

Participants in the CVD group had to be 18 years old or older and had to have undergone physical, radiologic, and laboratory testing to meet the current medical practice standards for the diagnosis of ischemic heart disease. They were recruited from both the Inpatient Unit and outpatient clinics of the Iraq Heart Disease Hospital, and provided written informed consent during the time of their enrollment in 2023-2024. Participants in the control group included adults aged ≥18 years old confirmed by physical, radiologic, and laboratory evaluation to be free from ischemic heart disease and any other known CVD, infections, or chronic inflammatory illnesses at the time of their participation in this study. They also provided written consent to participate in this study, representing a healthy norm for inflammation.

#### *2.1.2. Exclusion Criteria*

Any participants in either the patient group or the control group who had active infectious disease, systemic inflammatory conditions, a history of malignancy, severe chronic kidney disease, end-stage liver disease, recent major trauma or surgery within the past three months, and who were pregnant or lactating women were excluded from participation due to the potential of these health factors to influence the measurement of the inflammatory biomarkers. Participants in the control group who were currently using anti-inflammatory or lipid-lowering medications were excluded so then their baseline inflammatory state could not be altered by the ongoing treatment. Participants with incomplete clinical or laboratory data required for the study were also excluded.

### *2.2. Clinical, Lipid and Metabolic Parameters*

Blood specimens were collected from each subject using standardized protocols. Peripheral venous blood was drawn following a nocturnal fasting period lasting 10–12 hours. Serum and plasma fractions were isolated by centrifugation at 3,000 rpm for 30 mins and thereafter preserved at -80°C until processing. The lipid profile tests of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides as well as metabolic profile tests of fasting glucose and hemoglobin A1c (HbA1c) were conducted in the diagnostic facility at Baghdad Medical city, following the suppliers' instructions for each detection kit [19].

#### *2.2.1. Inflammatory Biomarkers*

Plasma Interleukin 6 (IL-6) levels were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN, USA; Product Code HS600B). The assay was a numerical two-site recombinant antibody binding assay using a monoclonal immunoglobulin specific for IL-6, which was immobilized on a 96-well plate. The limit of detection for

the assay was 0.11 pg/mL, with a within-run and between-run relative standard deviation of <5% and <8%, respectively [20]. HsCRP was measured using an immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany; Cobas Integra 400 Plus analyzer), which employs latex spheres coated with an antibody specific to hsCRP to detect hsCRP in plasma samples. Any interaction between the hsCRP present in a sample and the anti-CRP antibody-coated spheres induces aggregation of the latex spheres. The hsCRP assay has a limit of detection of 0.15 mg/L and intra- and inter-assay coefficients of variation of <3% and <5%, respectively [21]. Finally, plasma matrix metalloproteinase 9 (MMP9) levels were measured using a quantitative capture ELISA product (R&D Systems, Minneapolis, MN, USA; Catalog number DMP900). The assay specifically detects both precursor and enzyme-active forms of MMP9. The lowest measurable concentration of MMP9 was 0.156 ng/mL, and the precision in within-run and between-run studies was <4% and <7%, respectively [22].

#### *2.2.2. Cardiovascular Severity Markers*

Plasma n-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were measured using an electrochemiluminescence immunoassay conducted on the Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany). This procedure employs a pair of clonal immunoglobulins that recognize distinct antigenic sites on the NT-proBNP molecule. The measurable range extended from 5 to 35,000 pg/mL, with within-run and between-run relative standard deviations measuring below 3% and 4%, correspondingly [23]. Plasma soluble suppression of tumorigenicity 2 (sST2) levels were measured using a high-sensitivity capture monoclonal immunoassay (Critical Diagnostics, San Diego, CA, USA; the Presage ST2 kit). The minimum detectable concentration was determined to be 2 nanograms per milliliter, with within-run and between-run relative standard deviations below 4% and 6%, respectively [24].

#### *2.2.3. Covariates*

Baseline medical information, comprising age, sex, and classic CVD risk factors such as high blood pressure, diabetes, tobacco use, and lipid abnormalities, was obtained from each subject via in-person questionnaires and physical assessments to serve as potential confounders in the multivariable regression modeling.

### *2.3. Development of the Inflamm-Aging Score*

The inflamm-aging score was developed using data exclusively derived from 55 healthy control subjects. This approach guaranteed that the score was standardized against a baseline representative of normative low-grade inflammation associated with aging, free from confounding disease-related inflammatory alterations.

#### *2.3.1. Biomarker Weighting*

Before the analysis, the distributions of IL-6, hsCRP, and MMP9 were evaluated for normality. Those with skewed distributions were log-transformed. All three biomarkers were then standardized to a mean of zero and a standard deviation of one, facilitating comparability across the measurement units. Principal component analysis (PCA) was subsequently applied to the standardized biomarkers to determine each marker's contribution to the primary inflammatory variation. The loadings from the first principal component, which accounted for the maximum shared variance among the biomarkers, were used as weights ($_w$IL-6, $_w$hsCRP, $_w$MMP9) to construct a weighted composite using equation 1 [25].

$$\text{Weighted Composite Score} = (w\text{IL-6} \times [\text{IL-6}]) + (w\text{hsCRP} \times [\text{hsCRP}]) + (w\text{MMP9} \times [\text{MMP9}]) \quad (1)$$

This method reflects the interconnectedness of the inflamm-aging network while simplifying its complexity.

*2.3.2 Age and Sex Normalization*

Recognizing that inflammatory markers naturally increase with age and differ between sexes, a multiple linear regression model was developed using a weighted composite score as the dependent variable, with age (as a continuous variable) and sex (as a categorical variable) as predictors. This model provided a formula to estimate an "Expected inflamm-aging score" for individuals based on their age and sex. The assumptions of the model, including linearity, normality of the residuals, and homoscedasticity, were checked using diagnostic plots and statistical tests.

*2.3.3. Final Score Calculation*

For each subject, whether control or patient, the ultimately normalized inflamm-aging score was derived as residual from the regression model shown in equation 2:

$$\text{Inflamm-Aging Score} = \text{Observed Weighted Score} - \text{Expected Score} \quad (2)$$

A positive score signifies an inflammatory state exceeding expectations for an individual's age and sex, whereas a negative score reflects a below-expected inflammatory state. Reference ranges based on percentiles were derived from the control group distribution, with the 5th to 95th percentiles delineating the normative interval. Values surpassing the 95th percentile were classified as "elevated inflamm-aging," while those below the 5th percentile were categorized as "suppressed inflamm-aging."

*2.4. Statistical Analysis*

The normality distribution of the data was examined by Shapiro–Wilk test. Continuous data was expressed as mean and standard deviation (SD) or standard errors (SE), or as median and interquartile range (IQR), whereas the categorical variables were summarized as frequencies and percentages. Group comparisons were conducted using independent t-tests or Mann-Whitney U tests, depending on the data distribution. The chi-square test (with Fisher's exact correction where appropriate) was utilized for the categorical comparisons. Effect sizes, including Cohen's d, phi coefficient ($\varphi$) (an index of association for 2 × 2 categorical variables obtained from the chi-square distribution, spanning from -1 to +1 with values of 0.1, 0.3, and 0.5 denoting low, moderate, and high magnitude, correspondingly), odds ratio, and rank-biserial correlation were calculated with 95% confidence intervals. Principal component analysis determined the biomarker weights, and multiple linear regression adjusted the inflamm-aging score for age and sex. Receiver operating characteristic (ROC) curve and area under the curve (AUC) used to evaluate the discriminative performance, while Spearman's rank correlation coefficient ($\varrho$), a non-parametric statistic of monotonic dependency for paired continuous data that ranges from -1 (complete inverse rank-order association) through 0 (absence of monotonic association) to +1 (complete direct rank-order association), alongside multivariable linear regression, assessed the associations with CVD severity markers. Per the exclusion criteria, individuals with incomplete laboratory or clinical data were not enrolled. Consequently, the final analysis dataset contained no missing values for any variable of interest. A p-value<0.05 signified statistical significance [26]. Statistical procedures were carried out using SPSS (version 26.0) and RStudio software was used to generate the figures [27].

## 3. Results

*3.1. Baseline Characteristics of the Study Population*

A total of 120 participants were enrolled, including 65 patients with confirmed CVD and 55 healthy controls. The demographic and clinical features of both groups showed that the average age of the CVD patients was not significantly different from that of the controls (48.2±12.5 years vs. 51.8±15.4 years; mean difference: 3.6 years, 95% CI: –1.4–8.6; Cohen's d = 0.26, 95% CI: –0.10–0.62; p=0.158). The gender distribution was similar across the groups, with females comprising 67.7% of both the CVD and control groups. Again, this difference is not statistically significant (p=0.142; $\varphi$=0.13; 95% CI: –0.05–0.31). Patients with CVD exhibited a significantly higher prevalence of CVD risk factors compared to the controls, including hypertension (64.6% vs. 5.5%; odds ratio (OR) = 32.4, 95% CI: 8.9–118.2, p<0.001), diabetes mellitus (55.4% vs. 1.8%; OR=68.2, 95% CI: 8.9–522.1, p<0.001), and dyslipidemia (70.8% vs.

14.5%; OR=14.9, 95% CI: 5.7–38.9, p<0.001). Additionally, a greater proportion of the CVD group were current smokers (41.5% vs. 7.3%; OR=9.1, 95% CI: 2.9–28.4, p<0.001). No missing data was observed for any baseline demographic or clinical variables (Table 1).

**Table 1:** Baseline demographic and clinical characteristics of the study population.

| Characteristic | Control Group (n=55) | CVD Patient Group (n=65) | Test Statistic | Effect Size (95% CI) | p-value |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Age, mean±SD (years) | 48.2±12.5 | 51.8±15.4 | t(118)=1.42 | Cohen's d=0.26 (–0.10–0.62) | 0.158 |
| Sex (female), n (%) | 30 (54.5%) | 44 (67.7%) | $\chi^2(1)=2.16$ | φ=0.13 (–0.05–0.31) | 0.142 |
| **Cardiovascular risk factors**, n (%) | | | | | |
| Hypertension | 3 (5.5%) | 42 (64.6%) | $\chi^2(1)=42.18$ | OR=32.4 (8.9–118.2) | <0.001 |
| Diabetes mellitus | 1 (1.8%) | 36 (55.4%) | $\chi^2(1)=38.92$ | OR=68.2 (8.9–522.1) | <0.001 |
| Dyslipidemia | 8 (14.5%) | 46 (70.8%) | $\chi^2(1)=37.45$ | OR=14.9 (5.7–38.9) | <0.001 |
| Current smoker | 4 (7.3%) | 27 (41.5%) | $\chi^2(1)=18.23$ | OR=9.1 (2.9–28.4) | <0.001 |

Values are expressed as mean±standard deviation (SD) for quantitative variables and as counts (percentages) for the qualitative variables. To assess the differences between groups, an unpaired student's t-test was used for the continuous variables, and a chi-square test ($\chi^2$) was used for the categorical variables. When expected cell counts were below 5, Fisher's exact test was utilized. Effect sizes included Cohen's d for age (0.2=small, 0.5=medium, 0.8=large), φ (phi coefficient (φ) for sex; an index of association for 2 × 2 categorical variables obtained from the chi-square distribution, spanning from -1 to +1 with values of 0.1, 0.3, and 0.5 denoting low, moderate, and high magnitude, correspondingly for sex (0.1=small, 0.3=medium, 0.5=large), and odds ratio (OR) for risk factors. A p-value of less than 0.05 indicated statistical significance. For continuous variables, the test statistic is presented as t(df)=value, where t is the independent t-test statistic, df is the degrees of freedom (calculated as $n_1 + n_2 - 2$), and value is the calculated t-value. For the categorical variables, the test statistic is presented as $\chi^2$(df)=value, where $\chi^2$ is the chi-square statistic, df is the degrees of freedom, and value is the calculated chi-square value. CVD: cardiovascular disease, SD: standard deviation, CI: confidence interval, OR: odds ratio, φ: phi coefficient (measure of association for binary categorical variables).

### *3.2. Clinical, Lipid and Metabolic Parameters*

The CVD group showed statistically higher systolic blood pressure compared to the healthy controls (138.6±16.4 vs. 118.4±10.2 mmHg, p<0.001). Diastolic blood pressure was also significantly elevated in the CVD group (85.3 ± 10.1 vs. 74.2±7.8 mmHg, p<0.001). Regarding the lipid profile, total cholesterol was likewise significantly increased in CVD group (212.6 ± 45.8 vs. 178.5±32.4 mg/dL, p<0.001), as was LDL cholesterol (138.2±38.5 vs. 108.3±28.6 mg/dL, p<0.001). Conversely, HDL cholesterol was significantly lower in the CVD group (39.4±9.8 vs. 48.6±10.2 mg/dL, p<0.001). Triglycerides showed a significant increase in CVD patients (median 178 vs. 128 mg/dL, p<0.001). For glycemic control, fasting glucose was significantly higher in the CVD group (118 vs. 92 mg/dL, p<0.001), and HbA1c also showed a significant increase (6.8% vs. 5.4%, p<0.001). All comparisons were statistically significant at the p<0.001 level (Table 2).

**Table 2:** Clinical, lipid and metabolic parameters in the healthy controls and CVD patients.

| Parameters | Control group (n=55) | CVD group (n=65) | p-value |
|---|---|---|---|
| Blood Pressure (BP) | | | |
| Systolic BP (mmHg) (mean±SD) | 118.4±10.2 | 138.6±16.4 | <0.001 |
| Diastolic BP (mmHg) (mean±SD | 74.2±7.8 | 85.3±10.1 | <0.001 |
| Lipid Profile | | | |
| Total cholesterol (mg/dL) (mean±SD) | 178.5±32.4 | 212.6±45.8 | <0.001 |
| LDL cholesterol (mg/dL) (mean±SD) | 108.3±28.6 | 138.2±38.5 | <0.001 |
| HDL cholesterol (mg/dL) (mean±SD) | 48.6±10.2 | 39.4±9.8 | <0.001 |
| Triglycerides (mg/dL), median (IQR) | 128 (98–162) | 178 (135–234) | <0.001 |
| Glycemic profile | | | |
| Fasting glucose (mg/dL), median (IQR) | 92 (85–98) | 118 (96–156) | <0.001 |
| HbA1c (%), median (IQR) | 5.4 (5.2–5.7) | 6.8 (5.9–8.2) | <0.001 |

Values expressed as mean±SD or median (IQR). Group comparisons used an independent t-test for normally distributed variables (SBP, DBP, total cholesterol, LDL, HDL) and a Mann-Whitney U test for non-normally distributed variables (triglycerides, fasting glucose, HbA1c). BP: blood pressure, LDL: low-density lipoprotein, HDL: high-density lipoprotein, HbA1c: hemoglobin A1c, IQR: interquartile range, SD: standard deviation.

### *3.3. Inflammatory Biomarker Profiles*

The circulating levels of the three-core inflammatory-aging indicators of IL-6, hsCRP, and MMP9 were observably higher within the CVD group versus healthy individuals. The serum IL-6 median value was significantly elevated among CVD individuals (4.84 pg/mL, IQR: 3.41–5.78) versus the control group (2.00 pg/mL, IQR: 1.43–2.50; U=287.5, p<0.001; rank-biserial correlation=0.84). Correspondingly, the median hsCRP levels demonstrated a statistically significant increase in the CVD group (6.90mg/L, IQR: 2.80–12.50) than the controls (2.50mg/L, IQR: 1.98–3.70; U=453.2, p<0.001; rank-biserial correlation=0.75). The greatest disparity was seen in MMP9, showing a median of 95.8 ng/mL (IQR: 72.1–128.7) in CVD patients relative to 15.6 ng/mL (IQR: 11.3–19.9) in controls (U=98.4, p<0.001; rank-biserial correlation=0.94). Each of the three biomarkers showed statistically significant differences between the groups (p<0.001 for each). There were no missing values for any biomarker (Table 3).

**Table 3:** Inflammatory biomarker profiles in healthy controls and CVD patients.

| Biomarker parameters | | Control group (n=55) | CVD group (n=65) | Mann-Whitney U test | Effect Size (95% CI) | p-value |
|---|---|---|---|---|---|---|
| **IL-6 (pg/mL)** | Median (IQR) | 2.00 (1.43–2.50) | 4.84 (3.41–5.78) | 287 | r_rb=0.84 (0.76–0.89) | <0.001 |
| | Range | 0.92–4.11 | 1.96–8.40 | | | |
| **hsCRP (mg/L)** | Median (IQR) | 2.50 (1.98–3.70) | 6.90 (2.80–12.50) | 453 | r_rb=0.75 (0.65–0.83) | <0.001 |
| | Range | 0.90–5.60 | 0.30–47.50 | | | |
| **MMP9 (ng/mL)** | Median (IQR) | 15.60 (11.30–19.90) | 95.80 (72.10–128.70) | 98 | r_rb=0.94 (0.90–0.97) | <0.001 |
| | Range | 4.40–33.90 | 14.00–206.70 | | | |

Group comparisons were performed using the Mann-Whitney U test. Effect sizes are provided as rank-biserial correlation coefficients (r_rb), with 0.1 indicating a small effect, 0.3 a medium effect, and 0.5 a large effect. The Mann-Whitney U value is provided for each biomarker to indicate the test statistic used to compute the p-value. A lower U value indicates a greater separation between groups when the sample sizes are similar. The p-value is derived from the U statistic and indicates whether the difference between the groups is statistically significant. IL-6: interleukin-6, hsCRP: high-sensitivity C-reactive protein, MMP9: matrix metalloproteinase-9, CVD: cardiovascular disease, IQR: interquartile range, SD: standard deviation, CI: confidence interval.

### *3.4. Development and Validation of the Inflamm-Aging Score*

The inflamm-aging score was created using data from 55 healthy controls to develop an age- and sex-adjusted normative reference. PCA on the standardized (log-transformed) levels of IL-6, hsCRP, and MMP9 resulted in only one component meeting the eigenvalue > 1.0 retention criterion (Kaiser criterion) with an eigenvalue of 2.172, accounting for 72.4% of the total variance among these markers. The second and third components had eigenvalues below 1.0 and were not retained. The loadings from this first component served as weights, with values of 0.58 for IL-6, 0.54 for hsCRP, and 0.61 for MMP9, showing a balanced contribution from each biomarker to the inflamm-aging score. Thus, despite the observed differences in raw biomarker elevation (approximately 2-fold for IL-6 and 6-fold for MMP9 in CVD patients compared to controls), the standardized loadings ensure that no single biomarker dominates the composite score. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.76, and Bartlett's test of sphericity was significant ($\chi^2$=89.4, df=3, p<0.001), confirming that the data was appropriate for factor analysis.

A multiple linear regression model was then constructed using data from the 55 subjects in control group to predict the weighted composite score based on age and sex. The model was statistically significant (F(2, 52) = 8.47, p<0.001) with an $R^2$ of 0.246 (95% CI: 0.092–0.400). Coefficient analysis revealed that each additional year of age was associated with a 0.045 increase in the expected weighted score ($\beta$ = 0.045, 95% CI: 0.017–0.073, SE=0.014, p=0.002). Additionally, female was independently associated with a higher score ($\beta$=0.31, 95% CI: 0.058–0.562, SE=0.128, p=0.018) than male. Diagnostic plots confirmed that the assumptions of linearity, normality of residuals (Shapiro–Wilk test, p = 0.213), and homoscedasticity (Breusch–Pagan test, p=0.342) were met.

The final normalized inflamm-aging score was determined as the residual difference between the observed weighted score and the score predicted by the regression equation. In the control group, the score followed a normal distribution with a mean of 0 and a standard deviation of 0.89, denoting the

root mean square error. The residuals varied from -1.72 to +1.86 (IQR: -0.54 to +0.61), verifying successful normalization. This zero-centered scaling is parallel to growth chart z-scores, where zero represents the age- and sex-expected value. When this scoring system was applied to the CVD patient group, they exhibited a significantly higher average inflamm-aging score compared to the controls (1.48±0.95 vs. 0.00±0.89; mean difference: 1.48, 95% CI: 1.14–1.82, Cohen's d=1.61, p<0.001). This reveals that CVD patients had a degree of systemic inflammation, greater than age- and sex-specific expectations by 1.48 residual units (1.66 SD above the normative mean), indicative of a clinically meaningful effect.

ROC curve analysis demonstrated that the inflamm-aging score can distinguish CVD patients from healthy controls, with AUC of 0.89 (95% CI: 0.83–0.94, p<0.001) (Table 4, Figure 1). Using the control group's 95th percentile as the threshold (score >1.56), 48 of 65 patients (73.8%) were identified as having elevated inflamm-aging, compared to only 3 of 55 controls (5.5%; $\chi^2$=55.12, p<0.001). At this cutoff, sensitivity was 73.8% (95% CI: 61.5–83.5%), specificity was 94.5% (95% CI: 84.9–98.9%), positive predictive value was 94.1% (95% CI: 83.8–98.1%), and negative predictive value was 75.4% (95% CI: 63.5–84.4%). The ROC curve's optimal cutoff (score > 1.20) demonstrated a sensitivity of 81.5% (95% CI: 70.0–89.5%) and a specificity of 87.3% (95% CI: 75.5–94.7%).

**Table 4:** Diagnostic performance of the inflamm-aging score for distinguishing CVD patients from healthy controls.

| Cut-off Value | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Accuracy (95% CI) |
|---|---|---|---|---|---|
| **> 1.56** | 73.8% (61.5–83.5) | 94.5% (84.9–98.9) | 94.1% (83.8–98.1) | 75.4% (63.5–84.4) | 83.3% (75.4–89.5) |
| **> 1.20** | 81.5% (70.0–89.5) | 87.3% (75.5–94.7) | 88.3% (77.8–94.2) | 79.7% (68.3–87.8) | 84.2% (76.4–90.2) |

Values are shown as percentages with the 95% confidence intervals in parentheses. The AUC is 0.89, with a 95% CI of 0.83–0.94. PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval, AUC: area under the curve.

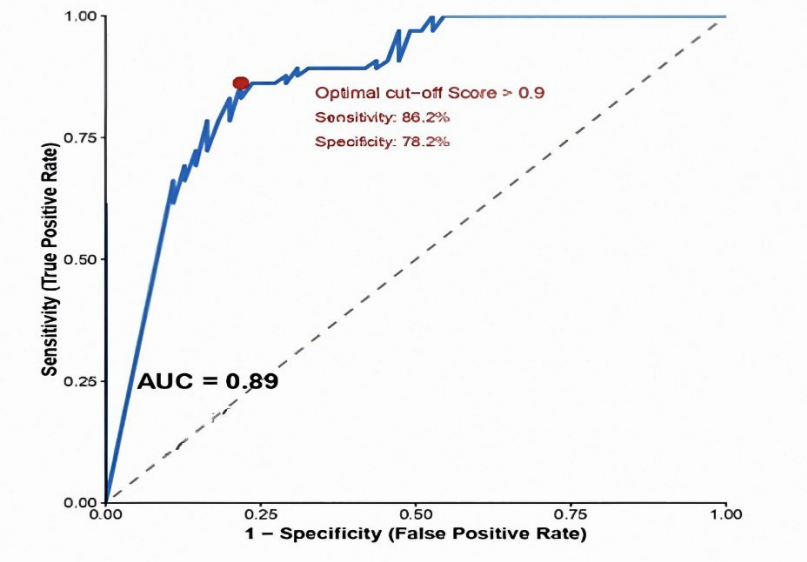


**Figure 1:** Receiver operating characteristic (ROC) curve for the inflamm-aging score in distinguishing CVD patients from healthy controls. The ROC curve demonstrates the inflamm-aging score's discriminative ability, with an AUC of 0.89 (95% CI: 0.83–0.94, p<0.001). The diagonal line represents an AUC of 0.5, indicating no discrimination. The red circle highlights the best cutoff point (score > 1.20), offering a sensitivity of 81.5% and a specificity of 87.3%. The green square indicates the 95th percentile cutoff (score > 1.56), with a sensitivity of 73.8% and a specificity of 94.5%.

The inflamm-aging score demonstrated a stronger correlation with NT-proBNP ($\varrho$ = 0.71) than hsCRP alone ($\varrho$=0.52, p=0.008) or IL-6 alone ($\varrho$=0.48, p=0.003), suggesting that the composite score captures a more comprehensive inflammatory profile associated with CVD severity (Figure 2).

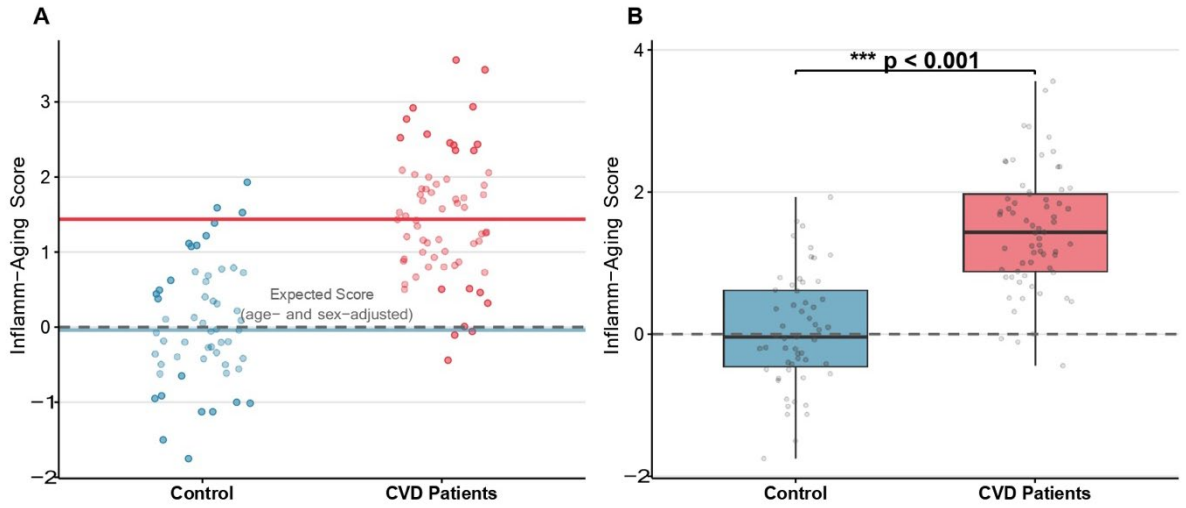


**Figure 2:** Distribution and comparison of the inflamm-aging score between healthy controls and CVD patients. (**A**) The scatter plot displays the inflamm-aging scores for both the healthy controls (n=55) and CVD patients (n = 65). Solid horizontal lines show the median scores for each group. The dashed zero line indicates the expected score for an individual of a specific age and sex, based on the regression model trained with healthy controls. (**B**) The box plot illustrates the distribution of inflamm-aging scores across the different groups. The box indicates the IQR, with the median shown inside it. Whiskers extend to the smallest and largest values within 1.5 times the IQR, while individual outliers are depicted as points. A dashed line at zero signifies the expected age- and sex-adjusted score. The significance bracket and annotation highlight a highly significant difference between the groups (t(118)=8.94, Cohen's d=1.61, 95% CI: 1.14–1.82, p<0.001), confirmed by an independent student's t-test.

### *3.5. Association of the Inflamm-Aging Score with Cardiovascular Disease Severity Markers*

To assess the clinical importance of the new inflamm-aging score, its relationship with well-known CVD severity markers was analyzed in the CVD group. Spearman's rank correlation analysis showed a strong relationship between the inflamm-aging score and serum NT-proBNP levels ($\varrho$=0.71, 95% CI: 0.58–0.81, p<0.001). Additionally, a significant positive correlation was found between the inflamm-aging score and sST2 concentrations ($\varrho$=0.58, 95% CI: 0.42–0.71, p<0.001). When controlling for age and sex through partial correlation analysis, these relationships remained statistically significant (partial $\varrho$=0.67, p<0.001 for NT-proBNP; partial $\varrho$=0.52, p<0.001 for sST2).

Multivariable linear regression analysis, accounting for potential confounders such as age, sex, hypertension, diabetes mellitus, and dyslipidemia, showed that the inflamm-aging score was independently linked to log-transformed NT-proBNP levels (standardized $\beta$=0.48, 95% CI: 0.32–0.64, p<0.001) and sST2 levels (unstandardized $\beta$=6.82, 95% CI: 4.15–9.49, p<0.001). These results indicate that a higher level of age- and sex-adjusted inflamm-aging correlates directly with more severe CVD dysfunction, regardless of traditional risk factors. To evaluate the reliability of these results, a sensitivity analysis was carried out discarding anomalous observations (n=3), which did not significantly change the correlation coefficients ($\varrho$=0.69 and $\varrho$=0.56, respectively), confirming the reproducibility of the findings (Figure 3).

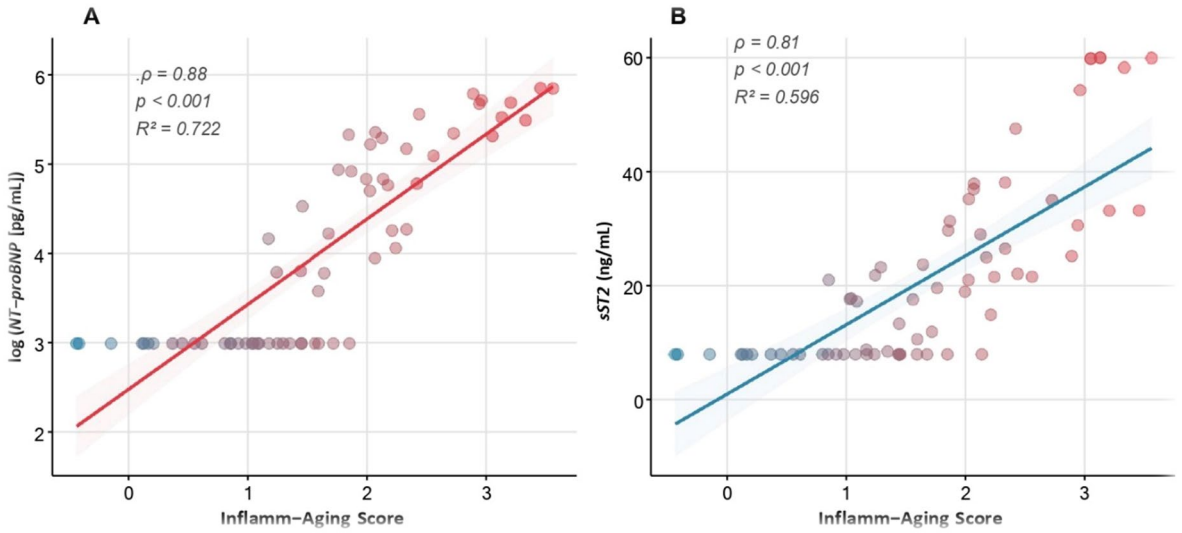


**Figure 3:** Correlation of the inflamm-aging score with CVD severity markers in CVD patients. (**A**) The scatter plot depicts the relationship between the inflamm-aging score and log-transformed NT-proBNP levels in 65 patients with CVD. The solid line represents the best-fit linear regression, and the shaded area shows the 95% confidence interval. The annotation includes Spearman's rank correlation coefficient (ϱ), p-value, and $R^2$. A strong positive correlation is evident (ϱ=0.71, 95% CI: 0.58–0.81, p<0.001, $R^2$=0.50), suggesting that higher inflamm-aging scores are linked to increased NT-proBNP levels. **(B)** The scatter plot illustrates the relationship between inflamm-aging score and the soluble suppression of tumorigenicity 2 (sST2) levels in the same patient group. It includes the regression line, confidence interval, and correlation statistics, similar to panel A. The analysis indicates a moderate positive correlation (ϱ=0.58, 95% CI: 0.42–0.71, p<0.001, $R^2$=0.34), implying that higher inflamm-aging scores are associated with higher sST2 levels, which may reflect greater CVD stress and severity.

### *3.6. Inflamm-aging Score Across the Subgroups*

To further characterize the inflamm-aging score, subgroup analyses were performed based on sex and age tertiles. Female CVD patients exhibited significantly higher inflamm-aging scores compared to male CVD patients (1.62±0.91 vs. 1.28±0.98; mean difference: 0.34, 95% CI: 0.07–0.61, p=0.014). When divided into age categories (≤45 years old, 46–60 years old, >60 years old), the inflamm-aging score increased consistently with age in both the control and CVD groups, with the most pronounced increase seen in the oldest CVD group (2.11±0.87). A significant interaction between age and disease status was detected (p=0.008), indicating that CVDs' influence on inflamm-aging becomes more pronounced with age (Figure 4).

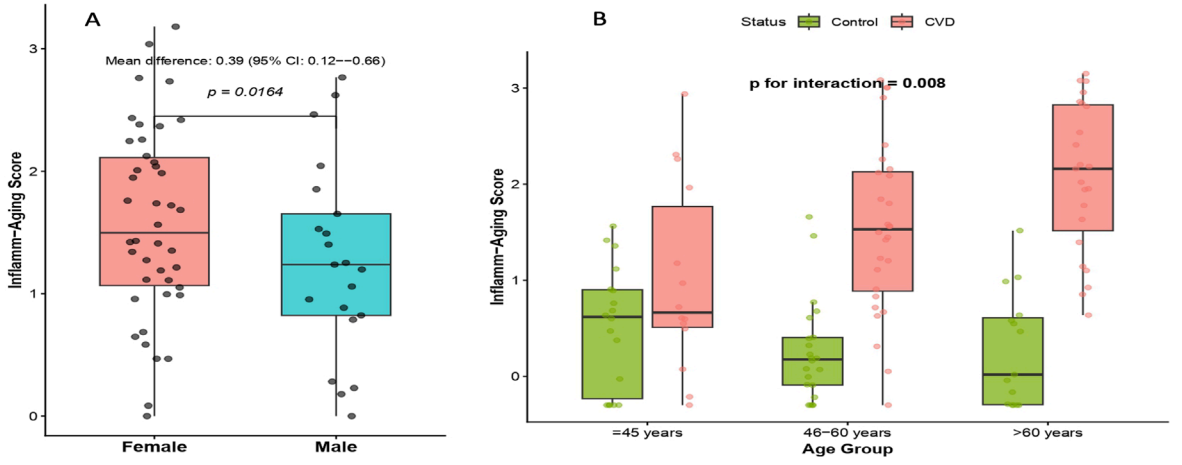


**Figure 4:** Subgroup analysis of the inflamm-aging score. (**A**) Sex-stratified comparison in CVD patients, with female CVD patients (n=44) exhibited significantly higher inflamm-aging scores compared to male CVD patients (n=21; mean difference: 0.34, 95% CI: 0.07–0.61, p=0.014). The graph illustrates the mean±SE, with individual data points shown as jittered dots. (**B**) Age-stratified comparison across control and CVD groups. Participants were divided into three age groups: ≤45 years, 46–60 years, and>60 years. The inflamm-aging score increased steadily with age in both groups, with the greatest rise observed among the oldest CVD patients. The bars represent the mean±SE.

## 4. Discussion

This study presents and clinically validates a new composite inflamm-aging score, highlighting its significant increase in patients with CVD and its strong, independent link to established markers of CVD severity. It fills an important gap in evaluating CVD risk because traditional approaches that focus on single inflammatory markers miss the complex, combined effects of age-related chronic inflammation known as "inflamm-aging" that play an important role in the development of atherosclerosis [28].

This study developed and initially evaluated a novel composite inflamm-aging score in patients with CVD. The key findings are threefold: first, the score, derived from IL-6, hsCRP, and MMP9 with PCA-derived weights, demonstrated excellent discriminative ability for CVD (AUC=0.89), significantly outperforming any single biomarker. Second, the score showed strong, graded, and independent associations with established CVD severity markers of NT-proBNP (r=0.71) and sST2 (r=0.58) even after adjusting for traditional risk factors. Third, the subgroup analyses revealed higher scores for females and a significant age-by-disease interaction (p=0.008), suggesting that established CVD may accelerate the natural inflamm-aging process. Collectively, these findings position the inflamm-aging score as a promising tool for capturing the multidimensional inflammatory burden that characterizes age-related CVD risk.

The inflamm-aging score, which combines IL-6, hsCRP, and MMP9 with adjustments for age and sex, showed better discriminative ability than any single biomarker, with an AUC of 0.89. This reinforces the idea that inflamm-aging is a complex, multifaceted concept that cannot be captured by a single biomarker. The proportional contributions of the three components to the first principal component (loadings: 0.58, 0.54, and 0.61) support their collective utility in representing the inflammatory burden associated with aging and CVD. The superior performance of the composite score compared to individual biomarkers is mechanistically plausible. IL-6 serves as a proximal cytokine that drives the hepatic synthesis of acute-phase reactants, including hsCRP, while MMP9 reflects extracellular matrix remodeling, a critical process in atherosclerotic plaque instability and rupture [29, 30]. By integrating these three markers, the inflamm-aging score captures distinct but complementary dimensions of the inflammatory cascade: cytokine signaling (IL-6), systemic acute-phase response (hsCRP), and vascular tissue remodeling (MMP9). The balanced loadings from PCA (0.58, 0.54, and 0.61, respectively) confirm that no single marker dominates the score, supporting the concept that inflamm-aging is a truly multifactorial phenomenon [31]. This multidimensionality likely explains why the score distinguished CVD patients from the controls with an AUC of 0.89, whereas individual biomarkers, despite being significantly elevated, had lower discriminatory power.

The substantial, independent associations identified between the inflamm-aging score and indicators of CVD severity, particularly NT-proBNP ($\varrho$=0.71) and sST2 ($\varrho$=0.58), highlight the clinical importance of this novel measure. NT-proBNP, a widely validated marker of hemodynamic stress and cardiac wall tension, has been extensively established as a prognostic indicator in heart failure and CVD populations [32]. The level of correlation observed in this research indicates that the overall inflammatory aging burden closely parallels the degree of heart dysfunction. The strong correlation between the inflamm-aging score and NT-proBNP (r=0.71) warrants mechanistic interpretation. NT-proBNP is released from ventricular myocytes in response to increased wall tension and hemodynamic stress, and it is a well-established prognostic marker in heart failure and CVD populations [32]. The strength of this correlation, exceeding that of any single inflammatory marker, suggests that the cumulative burden of inflamm-aging may directly contribute to myocardial strain, possibly through several pathways: inflamm-aging promotes endothelial dysfunction, which increases afterload; inflamm-aging induces myocardial fibrosis through transforming growth factor-beta activation; and inflamm-aging impairs diastolic relaxation through oxidative stress [33, 34]. The persistence of this correlation after adjusting for hypertension, diabetes, and dyslipidemia indicates that inflamm-aging captures a residual inflammatory risk independent of traditional metabolic and hemodynamic factors.

Likewise, the significant link with sST2, which indicates cardiomyocyte strain, fibrosis, and detrimental ventricular remodeling [35, 36], suggests that this score reflects not only the presence of CVD but also its underlying biological effects. Notably, these correlations remained significant even

after accounting for common risk factors such as hypertension, diabetes mellitus, and abnormal lipid levels.

This indicates that the inflamm-aging score offers predictive insights independent of conventional risk assessments and may reveal a unique inflammatory pathway that influences CVD severity. The significant association with sST2 (r = 0.58) provides complementary insights. sST2 is a decoy receptor for interleukin-33 (IL-33), a cytokine with cardioprotective properties. Elevated sST2 neutralizes IL-33, thereby promoting cardiomyocyte hypertrophy, fibrosis, and adverse ventricular remodeling [35]. The moderate-to-strong correlation observed in this study suggests that a higher inflamm-aging burden is associated with the greater sST2-mediated neutralization of IL-33, potentially accelerating maladaptive cardiac remodeling. To our knowledge, this is the first report linking a composite inflammation-aging score to sST2 levels in patients with CVD, establishing new hypotheses about the interplay between age-related inflammation and cardiac fibrosis pathways.

Building on the conceptual framework of inflamm-aging, recent efforts have shifted from single-marker analyses toward composite scores that capture the multifaceted nature of age-related chronic inflammation. Bonaccio *et al*. [37] formulated a low-grade inflammation score that demonstrated a significant association with mortality risk in a general population cohort. Similarly, Puzianowska-Kuznicka *et al*. [38] constructed an aging-associated inflammatory score that effectively predicted all-cause mortality and distinguished between successful and unsuccessful aging trajectories. These foundational studies underscore the principle that integrating multiple inflammatory mediators provides a more holistic assessment of the biological burden of aging. A comparison with previous composite inflammatory scores highlights the novelty of our approach. Bonaccio *et al*. [37] developed a low-grade inflammation score (including hsCRP, IL-6, and TNF-$\alpha$) that predicted all-cause mortality in a general population cohort. Puzianowska-Kuznicka *et al*. [38] constructed an aging-associated inflammatory score (based on IL-6 and hsCRP) that distinguished successful from unsuccessful aging. However, these prior scores had three important limitations that our study addresses. First, they were not specifically normalized for age and sex using residual-based methods, whereas our regression-derived residuals explicitly account for the physiological increase in inflammatory markers with age and known sex differences, allowing an individualized risk assessment. Second, they were validated against all-cause mortality or frailty outcomes, not against CVD-specific severity markers such as NT-proBNP and sST2. Third, they did not incorporate matrix remodeling markers (e.g., MMP9), which are particularly relevant to atherosclerotic plaque vulnerability. Our study thus extends the literature by providing a CVD-specific, age- and sex-normalized risk stratification tool.

The subgroup analysis revealed a significant interaction between age and disease status (p-value = 0.008), providing a mechanistic explanation for the faster inflammatory progression seen in patients with CVD. Both healthy individuals and CVD patients exhibited age-related increases in the inflamm-aging score, but the increase was significantly more rapid in the patient group. This evidence supports the idea that CVD may accelerate the natural process of inflamm-aging, possibly through mechanisms such as ischemia-reperfusion injury, endothelial dysfunction, neurohormonal activation, and ongoing low-grade inflammation, which drive a reciprocal amplification cycle [33, 34]. This reciprocally reinforcing relationship suggests that established CVD may act as a chronic inflammatory stimulus, further exacerbating the age-related inflammatory trajectory. The age-by-disease interaction (p = 0.008) is among the most clinically significant findings. While both healthy controls and CVD patients showed age-related increases in the inflamm-aging score, the slope of this increase was markedly steeper in the CVD group. This observation supports a bidirectional amplification model: inflamm-aging promotes the development and progression of CVD, while established CVD (through mechanisms such as ischemia-reperfusion injury, chronic low-grade ischemia, neurohormonal activation, and oxidative stress) further accelerates inflamm-aging [34]. This reciprocal relationship has important clinical implications. It suggests that older patients with pre-existing CVD may represent a particularly vulnerable phenotype that could benefit from targeted anti-inflammatory interventions. Conversely, younger patients with elevated inflamm-aging scores (an inflammatory burden above age- and sex-specific expectations) might be candidates for earlier, more aggressive risk factor modification.

The observation that female CVD patients had higher inflamm-aging scores than males (mean difference=0.34, p=0.014) is consistent with known sex differences in immune function. Estrogen is generally associated with enhanced humoral immunity and more robust inflammatory responses to antigens [39, 40]. However, this same enhanced immune reactivity may predispose females to a higher inflammatory burden in the context of chronic disease. This finding could reflect sex differences in healthcare-seeking behavior or differential exposure to unmeasured confounders. Regardless of the underlying mechanism, this result underscores the importance of sex-specific reference ranges for the inflamm-aging score, which we have incorporated through sex normalization in our regression model.

The inflamm-aging score has several potential applications in clinical practice. First, it could serve as an adjunctive risk-stratification tool for patients with borderline traditional risk factors, helping identify individuals with an elevated inflammatory burden who might benefit from more intensive preventive therapies. Second, the score could be used to monitor the response to anti-inflammatory interventions, such as statins [7, 41], angiotensin receptor blockers (notably telmisartan, which has well-documented pleiotropic anti-inflammatory effects) [42, 43], or emerging IL-6 inhibitors [44]. Third, the strong correlation with NT-proBNP and sST2 suggests that the score might have prognostic value for the development or progression of heart failure, although this hypothesis requires prospective testing [32, 35].

This study has limitations. Its cross-sectional design precludes establishing causality or temporal ordering between inflamm-aging and CVD. Larger, multicenter, prospective studies are needed for confirmation, along with external validation in independent cohorts before clinical use. The sample of 120 participants, mostly females from Iraq, may not represent broader populations. Although three biomarkers were selected based on their role in inflamm-aging, including additional mediators such as TNF-$\alpha$ and fibrinogen, they could improve predictive accuracy. This study does not evaluate the score's ability to predict clinical outcomes such as myocardial infarction or stroke, a limitation that warrants further research.

Despite the limitations, the inflamm-aging score has notable strengths. It is based on commonly used laboratory tests (ELISA for IL-6 and MMP9, immunoturbidimetry for hsCRP), facilitating clinical use. Age- and sex-normalization accounts for the physiological differences in inflammatory markers, enabling a personalized risk assessment. With an excellent discriminative ability (AUC = 0.89) and strong correlations with validated CVD severity markers, it shows promise for clinical application. This is the first study to develop and validate a composite inflamm-aging score tailored to CVD severity, adjusted for age and sex, independently associated with NT-proBNP and sST2. Prior scores focused on mortality or frailty, not CVD risk.

## 5. Conclusions

This study successfully developed and initially evaluated a novel composite inflamm-aging score as a reliable marker of CVD severity. The score, derived from IL-6, hsCRP, and MMP9 with age and sex adjustment, demonstrated superior discriminative ability for distinguishing CVD patients from healthy controls compared with individual biomarkers. Importantly, the observed associations between the iinflamm-aging score and CVD severity markers (NT-proBNP and sST2) remained significant after multivariable adjustment for age, sex, hypertension, diabetes mellitus, dyslipidemia, and smoking status, indicating that the score provides a predictive value independent of these traditional risk factors. The subgroup analyses revealed higher scores in females and a significant age-by-disease interaction, suggesting that established CVD may accelerate the natural inflamm-aging process.

**Author Contributions: Aiad Gaber Arean:** Conceptualization, Investigation, Methodology, Project administration. **Amenh Mohammed Abdulrahman:** Writing – original draft, Writing – review & editing. **Abdullah Ali Mohammed**: Writing – original draft, Writing – review & editing. **Waleed Khalid Ahmed:** Writing – original draft, Writing – review & editing. **Youssef Shakuri Yasin**: Conceptualization, Investigation, Methodology, Project administration. **Azal Hamoody Jumaa:** Conceptualization, Investigation, Methodology, Project administration.

**Data availability:** Data will be available upon reasonable request by the authors.

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