CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

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GENERAL MEDICINE

COMPREHENSIVE ASSESSMENT OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION USING ADVANCED ECHOCARDIOGRAPHIC METHODS AND CORRELATION WITH THE INNATE IMMUNE RESPONSE FOR RISK STRATIFICATION AND THE DEVELOPMENT OF NOVEL THERAPEUTIC PERSPECTIVES

PhD THESIS ABSTRACT

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INTRODUCTION

Cardiovascular diseases represent the leading cause of morbidity and mortality worldwide, generating a substantial socio-economic burden. In this context, prevention, early diagnosis, and effective therapeutic interventions are essential pillars in reducing both the individual burden on patients and the pressure on healthcare systems. Among cardiovascular conditions, acute myocardial infarction (AMI) holds a central position due to its potentially fatal course and subsequent complications. Beyond the importance of early identification and guideline-recommended management of patients with acute coronary syndromes (ACS), a major ongoing challenge is accurate risk stratification and prognostic assessment.

The main hypotheses of this thesis are based on the role of novel echocardiographic parameters such as myocardial work (derived from speckle tracking) in identifying patients at risk of developing heart failure, as well as their correlation with systemic inflammation and innate immune response. Another key hypothesis refers to the reproducibility of these methods across operators with different levels of echocardiographic experience, compared to the current reference standard—three-dimensional echocardiography (3-DE).

Based on these hypotheses, the following objectives were formulated: to assess the reproducibility of myocardial work measurements when performed by beginner, intermediate, and advanced echocardiographers; to confirm the correlation between myocardial work and systemic inflammatory response represented by serum C-reactive protein levels; and to identify associations between novel immune-related biomarkers and myocardial performance assessed by 3-DE.

This thesis is structured into two main parts: the general part, composed of three chapters, and the original research section, structured into four chapters. The general section aims to provide an overview of the current state of knowledge regarding the pathophysiology, diagnosis, and management of acute coronary syndromes, the echocardiographic methods used in the evaluation of ischemic heart disease (2-DE, 3-DE, and myocardial work), as well as current evidence supporting the involvement of inflammation and the immune system in post-infarction evolution.

The special section presents the results of a clinical study that evaluated 90 patients with acute myocardial infarction admitted to the Cardiology Department of the University Emergency Hospital of Bucharest, diagnosed with ST-elevation or non-ST-elevation ACS. All patients underwent two-dimensional echocardiography (2-DE), three-dimensional

echocardiography (3-DE), speckle tracking analysis, and myocardial work assessment. Image acquisition was performed within the first 24 hours from admission and repeated during a follow-up visit 6 to 8 weeks after the acute event. Both visits also included a comprehensive clinical evaluation and the collection of an extended panel of biological markers, including routine laboratory tests, high-sensitivity cardiac troponin, C-reactive protein, and biomarkers of innate immunity and inflammation: VCAM-1, NCAM-1, PRCP, NRP1, FETUB, AOC3, and CST3.

This thesis demonstrates that myocardial work, a novel parameter derived from speckle tracking, is reproducible across operators with different levels of echocardiographic experience, showing a reproducibility comparable to that of 3-DE and superior to 2-DE. The study also reveals correlations between C-reactive protein levels at admission and myocardial work parameters, potentially aiding the identification of patients with active inflammatory syndrome at higher risk of post-infarction cardiac remodeling. Additionally, it identifies novel associations between pro-inflammatory proteins related to innate immunity and 3-DE-derived measures of function, supporting the future development of targeted anti-inflammatory and immunomodulatory therapies.

This research was supported by public funding through a European-financed UEFISCDI grant (project number 5/2018) within the project entitled *INNATE-IM: Targeting innate immune mechanisms for improved risk stratification and the identification of novel therapeutic options in acute myocardial infarction*.

I express my sincere gratitude to all those who contributed directly or indirectly to this work, with special thanks to Professor Dragoș Vinereanu for his guidance, support, and trust throughout the research process.

GENERAL PART

The general part of this thesis addresses essential aspects related to acute myocardial infarction (AMI), advanced echocardiographic assessment of ventricular dysfunction, and the implications of inflammation and innate immunity in post-infarction evolution. This section is structured into three chapters.

The first chapter presents general concepts regarding myocardial infarction, including its definition, classification, and epidemiological impact as a major cardiovascular pathology. This chapter also details the complex pathophysiology of AMI, hemodynamic imbalances, and the most frequent complications. In addition, it summarizes current therapeutic directions, including early revascularization, antiplatelet and anticoagulant therapies, pharmacological strategies, and secondary prevention measures [1].

The second chapter focuses on modern imaging techniques used to assess left ventricular function. It highlights the advantages of three-dimensional echocardiography (3-DE) as well as novel speckle tracking-derived methods, particularly myocardial work. This chapter also discusses the clinical relevance of integrating myocardial work parameters in the assessment of patients with acute coronary syndrome. Myocardial work is an innovative technique that incorporates estimated left ventricular pressure into the strain analysis derived from echocardiography. By accounting for afterload (estimated non-invasively via cuff-measured blood pressure), this method allows a more comprehensive evaluation of left ventricular performance. Compared to global longitudinal strain (GLS), myocardial work is less dependent on loading conditions and more accessible in routine practice than 3-DE [2].

The third chapter analyzes the role of inflammation and innate immunity in the pathogenesis of AMI and subsequent cardiac remodeling. Special emphasis is placed on C-reactive protein (CRP), a widely used marker in the evaluation of patients with ischemic heart disease. Furthermore, recent data on biomarkers such as VCAM-1, NCAM-1, PRCP, NRP1, Fetuin-B, AOC3/VAP-1, and Cystatin C are discussed, underlining their potential roles at the intersection of inflammation, metabolic stress, and ventricular remodeling, suggesting their utility both as risk markers and as emerging therapeutic targets.

SPECIAL PART – PERSONAL CONTRIBUTIONS

1. Working Hypothesis and General Objectives

In the current context of cardiovascular research, the integration of advanced echocardiographic parameters with inflammatory and immunological biomarkers has gained major importance in evaluating patients with acute myocardial infarction (AMI).

The scientific premise of this thesis is based on the hypothesis that advanced echocardiographic parameters, such as myocardial work, calculated through modern speckle tracking techniques, may contribute to a more accurate identification of patients at high risk of progressing toward heart failure. Furthermore, the research assumes that significant interactions exist between these functional parameters and systemic inflammatory markers, as well as specific elements of the innate immune response. Evaluating these interrelations could offer novel perspectives in risk stratification and the personalization of therapeutic strategies.

The main specific objectives pursued in this thesis are:

- To evaluate the reproducibility of echocardiographic measurements of left ventricular performance, with a focus on myocardial work parameters (notably global work efficiency – GWE) and left ventricular ejection fraction (LVEF), using both two-dimensional and three-dimensional echocardiography, in inter-observer comparisons among operators with varying levels of expertise, as well as intra-observer consistency;
- To analyze the correlation between systemic inflammation, represented by C-reactive protein (CRP) measured within the first 24 hours of admission, and changes in myocardial work parameters observed at follow-up;
- To identify a clinically applicable CRP threshold value associated with increased risk of ventricular remodeling and myocardial dysfunction, usable for post-ACS risk stratification;
- To determine the relationship between serum levels of immunological biomarkers—VCAM-1 (vascular cell adhesion molecule-1), NCAM-1 (neural cell adhesion molecule-1), PRCP (prolylcarboxypeptidase), NRP1 (neuropilin-1), FETUB (fetuin B), AOC3 (also known as VAP-1 – vascular adhesion protein-1), and CST3 (cystatin C)—in the acute phase, and the evolution of left ventricular end-diastolic volume and ejection fraction at 6–8 weeks following acute coronary syndrome.

2. General Research Methodology

This thesis was based on a prospective, observational, single-center clinical study aimed at the detailed evaluation of patients diagnosed with acute coronary syndrome (ACS), regardless of the presence of ST-segment elevation. The study included patients admitted to the Cardiology Department of the University Emergency Hospital of Bucharest, all of whom underwent coronary angiography and received treatment in accordance with current European guidelines. The study protocol was approved by the local ethics committee, and participation required signed informed consent.

Inclusion criteria:

- Age over 18 years;
- Diagnosis of acute coronary syndrome according to the most recent clinical guidelines;
- Adequate echocardiographic windows for acquisition of high-quality images.

Exclusion criteria:

- Presence of an irregular heart rhythm, preventing the acquisition of three-dimensional images;
- Presence of severe valvular heart disease;
- Severe systemic pathology or inflammatory syndromes;
- Hemodynamic instability;
- Inability to perform speckle-tracking echocardiography (STE) or 3D echocardiographic imaging due to technical limitations.

A total of 90 patients were included in the analysis.

Echocardiographic evaluation was performed within the first 24 hours of hospital admission and repeated at 6–8 weeks using two-dimensional (2D), three-dimensional (3D), and speckle-tracking (STE) techniques, in accordance with current recommendations. Imaging was performed using high-performance ultrasound systems (Vivid E9/E95, GE Healthcare), and data were processed offline using EchoPAC™ software (version 203, GE Healthcare). Measurements included 2D and 3D left ventricular ejection fraction (LVEF), global longitudinal strain (GLS), and advanced myocardial work parameters (GWI, GCW, GWW, GWE), integrated with non-invasively estimated systolic blood pressure and echocardiographically determined valvular event timings.

Biological samples, including high-sensitivity troponin, C-reactive protein levels, and immunological biomarkers (VCAM-1, NCAM-1, PRCP, NRP1, Fetuin-B, AOC3/VAP-1,

Cystatin C), were collected both during the acute phase (within 24 hours) and at the 6–8-week follow-up, following the same standardized protocols.

Statistical analysis was conducted using IBM SPSS Statistics, employing appropriate methods based on variable distribution, with a significance threshold set at $p < 0.05$. Bland–Altman plots were used to assess reproducibility, and the predictive performance of echocardiographic and biological parameters was analyzed using ROC curves, with determination of the area under the curve (AUC), sensitivity, and specificity.

3. Comparative Reproducibility of Myocardial Work and Left Ventricular Ejection Fraction in Patients with Acute Coronary Syndrome

3.1. Introduction

Cardiovascular imaging is one of the most important tools for assessing left ventricular (LV) function in patients who have experienced an acute coronary syndrome (ACS), due to its accuracy, accessibility, and non-invasive nature [3]. In recent years, international guidelines for echocardiographic quantification of cardiac chambers have recommended the use of three-dimensional echocardiography (3-DE) for assessing LV volumes and ejection fraction, as this method provides a more accurate representation of ventricular morphology and superior reproducibility by eliminating geometric assumptions [4].

Alongside imaging advancements, the concept of myocardial work (MW) has emerged—a non-invasive echocardiographic parameter derived from two-dimensional speckle tracking echocardiography (2D-STE), designed to assess both global and regional myocardial performance.

However, for myocardial work to be fully integrated into routine clinical practice, its reproducibility must be rigorously demonstrated through dedicated studies. In this context, the main objective of our study was to assess the reproducibility of myocardial work parameters in evaluating ventricular function in ACS patients, and to compare them with the reproducibility of parameters obtained by two-dimensional and three-dimensional echocardiography, when measurements are performed by operators with different levels of experience.

3.2. Patients and Methods

This prospective study included all patients diagnosed with acute coronary syndrome enrolled in the present thesis. The inclusion criteria for the reproducibility analysis were the same as those previously stated.

Two-dimensional (2-DE) and three-dimensional (3-DE) echocardiographic image acquisitions followed the protocol detailed in the General Research Methodology chapter and were performed by an advanced operator with five years of experience in 2-DE and three years in 3-DE and STE analysis.

Echocardiographic data analysis was performed offline using the EchoPac™ workstation (version 203, GE Healthcare). Three echocardiographers participated in this study, each representing a different level of professional experience: an advanced operator designated as Reader 1 (R1), who also performed the image acquisition, with five years of experience in 2-DE and three years in 3-DE and STE; an intermediate operator, Reader 2 (R2), with three years of experience in 2-DE and one year in 3-DE and STE; and a beginner operator, Reader 3 (R3), with one year of experience in 2-DE and three months in 3-DE and STE.

All three readers performed measurements independently using the same echocardiographic images. For each patient, measurements were taken sequentially in the same order: Reader 1, followed by Reader 2, and then Reader 3. For intra-observer reproducibility, each operator performed two independent measurements on the same echocardiographic datasets with a one-month washout period to minimize memory bias. All measurements for the analyzed parameters (2D LVEF, 3D LVEF, and GWE) were repeated under identical conditions.

In a randomly selected subpopulation of 41 patients from the initial cohort, the echocardiographic analysis was extended by involving an additional group of operators. In addition to the initial three operators (one advanced, one intermediate, and one beginner), two additional operators were added for each experience level, resulting in three evaluators per category: advanced (R1a, R1b, R1c), intermediate (R2a, R2b, R2c), and beginner (R3a, R3b, R3c). All operators independently performed measurements under the same conditions, using the same echocardiographic datasets and software versions.

Statistical analysis followed the methodology previously described.

3.3.Results

3.3.1.Study Population

The study population included 90 patients diagnosed with acute coronary syndrome, admitted and evaluated according to standardized protocols. The main characteristics of the study population are presented in Table 3.1.

Table 3.1. Characteristics of study population

Population Characteristics	All patients (N=90)
Age, years	54 ± 9
Sex, male	75 (83.3)
BMI > 25 kg/m²	76 (84.4)
Hypertension	59 (65.6)
Diabetes	23(25.6)
Dyslipidemia	52 (92.8)
ST-segment elevation ACS	71 (78.9)
One-vessel disease	53 (58.9)
Two-vessel disease	27(30.0)
Three-vessel disease	10 (11.1)
2-DE LVEF (%)	46.8 ± 8
3-DE LVEF (%)	47.1 ± 7
GWE	85.9 ± 8
3-DE LVEF ≥ 50%	42 (46.7)
3-DE LVEF 41-49%	35 (38.9)
3-DE LVEF ≤ 40%)	13 (14.4)

Data are presented as mean ± SD, median [interquartile range], or n (%)
2-DE – 2D echocardiography, 3-DE – 3D echocardiography, ACS – acute coronary syndrome, BMI – body mass index, GWE – Global Work Efficiency, LVEF – left ventricular ejection fraction,

3.3.2. Comparison of 2-DE Measurements Performed by Operators with Different Levels of Experience

The analysis of Bland–Altman plots revealed low variability between measurements performed by operators with varying levels of experience. A bias of -0.9 was observed between the advanced and intermediate operators, with limits of agreement (LOA) ranging from -5 to 4. Between the intermediate and beginner operators, the bias was -4, with LOA from -10 to 4, indicating a slight increase in variability as experience level decreases. When directly

comparing the advanced and beginner operators, a bias of -5 was noted, with LOA between -8 and 4, reflecting moderate but still clinically acceptable variability (Figure 3.1).

All correlations between measurements performed by the different operators were excellent, with Pearson correlation coefficients above 0.90 and high statistical significance ($p < 0.001$), confirming the strong consistency of independent assessments regardless of operator experience (Figure 3.2).

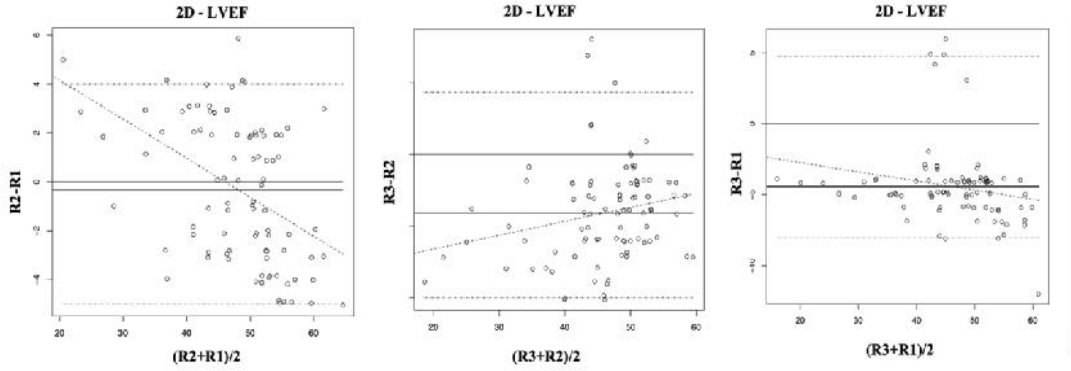

Figure 3.1. Bland–Altman plots for left ventricular ejection fraction (LVEF) measurements performed using two-dimensional echocardiography (2-DE) by the advanced operator (R1), intermediate operator (R2), and beginner operator (R3).

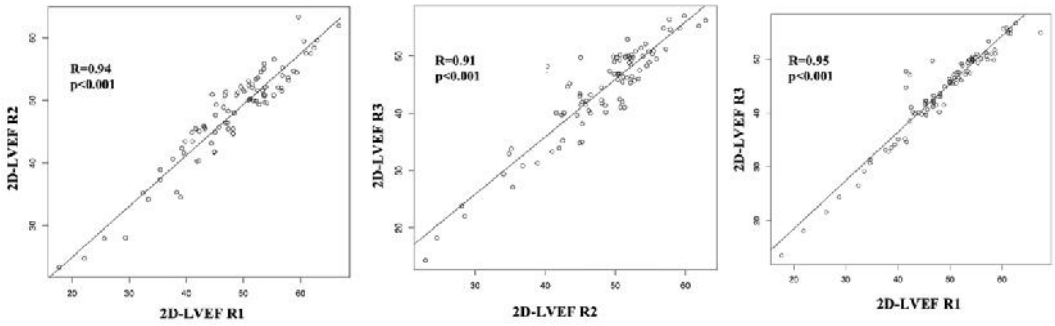

Figure 3.2. Correlations between left ventricular ejection fraction (LVEF) measurements performed using two-dimensional echocardiography (2-DE) by the advanced (R1), intermediate (R2), and beginner (R3) operators.

3.3.3. Comparison of 3-DE Measurements Performed by Operators with Different Levels of Experience

The Bland–Altman plot analysis for three-dimensional echocardiographic (3-DE) measurements revealed very low variability among operators with different levels of experience. A bias of -0.6 was observed between the advanced and intermediate operators, with

limits of agreement (LOA) ranging from -3 to 4. Comparing the intermediate and beginner operators, the bias was -2, with LOA between -7 and 4. Between the advanced and beginner operators, the bias was -1.4, with LOA between -6 and 4 (Figure 3.3).

Compared to 2-DE measurements, 3-DE measurements demonstrated lower systematic bias values and narrower limits of agreement, indicating superior reproducibility of the three-dimensional method. Additionally, all correlations between measurements performed by operators of different experience levels were excellent, with Pearson correlation coefficients exceeding 0.90 and p-values < 0.001, indicating a very high degree of agreement (Figure 3.4).

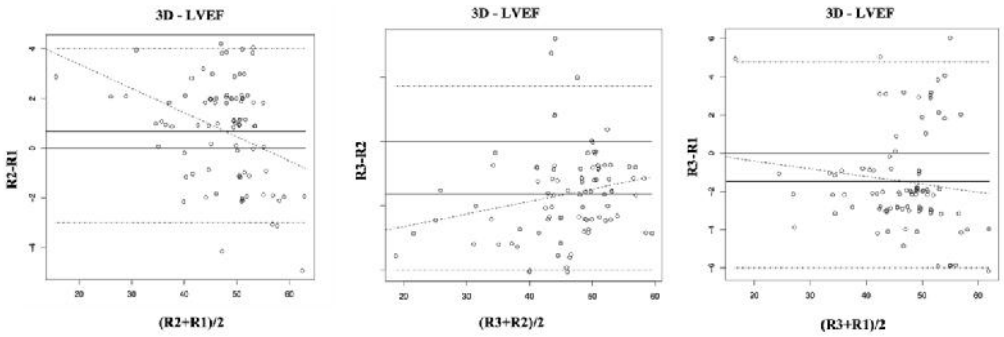

Figure 3.3. Bland–Altman plots for left ventricular ejection fraction (LVEF) measurements performed using three-dimensional echocardiography (3-DE) by the advanced (R1), intermediate (R2), and beginner (R3) operators.

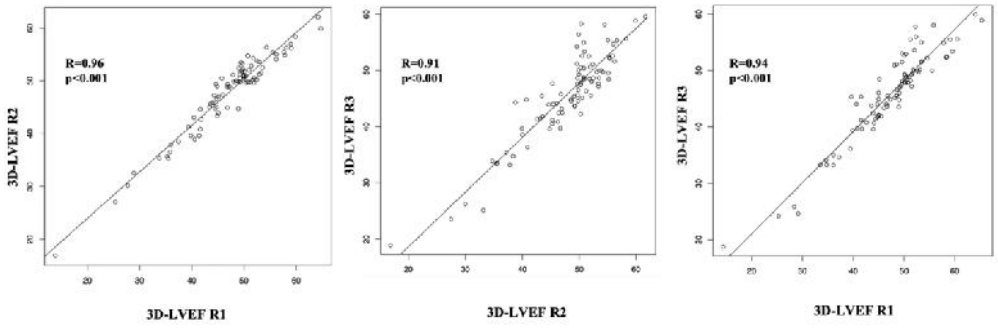

Figure 3.4. Correlations between left ventricular ejection fraction (LVEF) measurements performed using three-dimensional echocardiography (3-DE) by the advanced (R1), intermediate (R2), and beginner (R3) operators.

3.3.4. Comparison of Global Work Efficiency (GWE) Measurements Performed by Operators with Different Levels of Experience

The Bland–Altman plot analysis for global work efficiency (GWE) demonstrated very low variability among operators with different levels of experience. Between the advanced and

intermediate operators, a bias of -0.6 was observed, with limits of agreement (LOA) between -3 and 2. Between the intermediate and beginner operators, the bias was -0.3, with LOA between -3 and 5. Comparing the advanced and beginner operators, a bias of -1.0 was found, with LOA ranging from -5 to 4 (Figure 3.5).

All correlations for GWE measurements were very strong, with Pearson correlation coefficients above 0.94 and p-values < 0.001, indicating excellent agreement between measurements performed by operators with varying levels of experience (Figure 3.6).

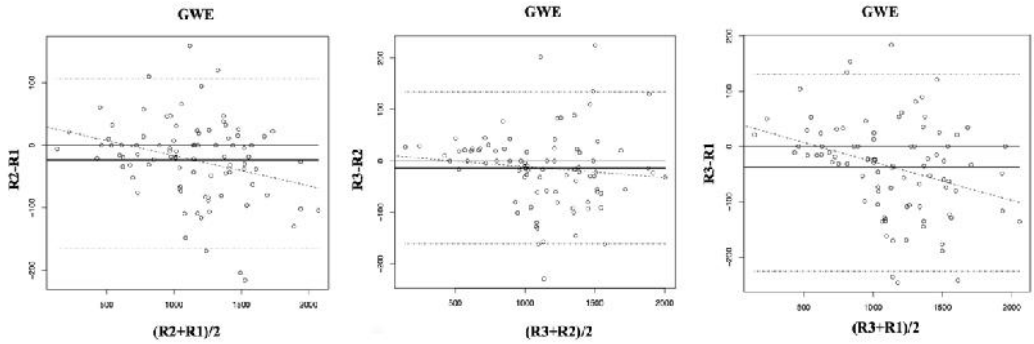

Figure 3.5. Bland–Altman plots for global work efficiency (GWE) measurements performed by the advanced (R1), intermediate (R2), and beginner (R3) operators.

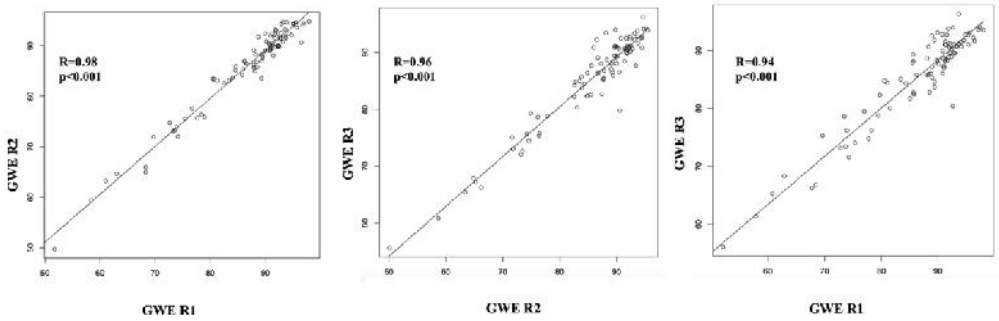

Figura 3.6. Corelațiile dintre măsurătorile eficienței globale a muncii miocardice (GWE), realizate de către operatorii avansat (R1), intermediart (R2) și începătort (R3).

3.3.5. Comparison of Inter-Operator Variability and Agreement Among Echocardiographers with Different Levels of Experience

The comparison of inter-operator variability and agreement for global work efficiency (GWE) and left ventricular ejection fraction (LVEF) measurements obtained by 2-DE and 3-DE is summarized in Table 3.2. Bias values were close to zero for both GWE and 3-DE-derived LVEF across all operator pairings, suggesting excellent inter-operator consistency—superior to that observed for 2-DE-derived LVEF.

Similarly, the limits of agreement (LOA) were consistently narrower for both GWE and 3-DE LVEF, indicating a very high level of agreement among operators. In contrast, 2-DE showed the widest LOA intervals in all comparisons, reflecting greater measurement variability.

Table 3.2. Inter-operator variability and agreement among echocardiographers with different levels of experience for global work efficiency (GWE) and left ventricular ejection fraction (LVEF) measurements performed using two-dimensional (2-DE) and three-dimensional echocardiography (3-DE).

R1 vs R2	GWE	FEVS - 3DE	FEVS - 2DE
Bias	-0.6	-0.6	-0.9
LOA	-3;2	-3;4	-5;4

R2 vs R3	GWE	FEVS - 3DE	FEVS - 2DE
Bias	-0.3	-2	-4
LOA	-3;5	-7;4	-10;4

R1 vs R3	GWE	FEVS – 3DE	FEVS - 2DE
Bias	-1	-0.4	-5
LOA	-5;4	-6.4	-8;4

3.3.6. Reproducibility Analysis According to Categories of Left Ventricular Dysfunction

Figure 3.7 summarizes the reproducibility of the three echocardiographic parameters assessed, based on operator experience level, in **patients with reduced left ventricular ejection fraction (LVEF ≤ 40%).** GWE exhibited the highest reproducibility among all param. eters evaluated. All inter-class comparisons showed very small bias values (below 2 units), narrow limits of agreement, and extremely high Pearson correlation coefficients (R = 0.98–0.99).

The reproducibility analysis in patients with moderate left ventricular dysfunction **(LVEF between 41–49%)** is presented in Figure 3.8. GWE (global work efficiency) demonstrated excellent reproducibility regardless of the evaluator's experience level, suggesting superior robustness compared to LVEF.

Figure 3.9 presents Bland-Altman plots comparing readers with advanced (R1), intermediate (R2), and beginner (R3) experience levels among patients with preserved systolic

function (LVEF ≥ 50%). GWE demonstrated excellent reproducibility across all reader combinations, with notable consistency regardless of experience level.

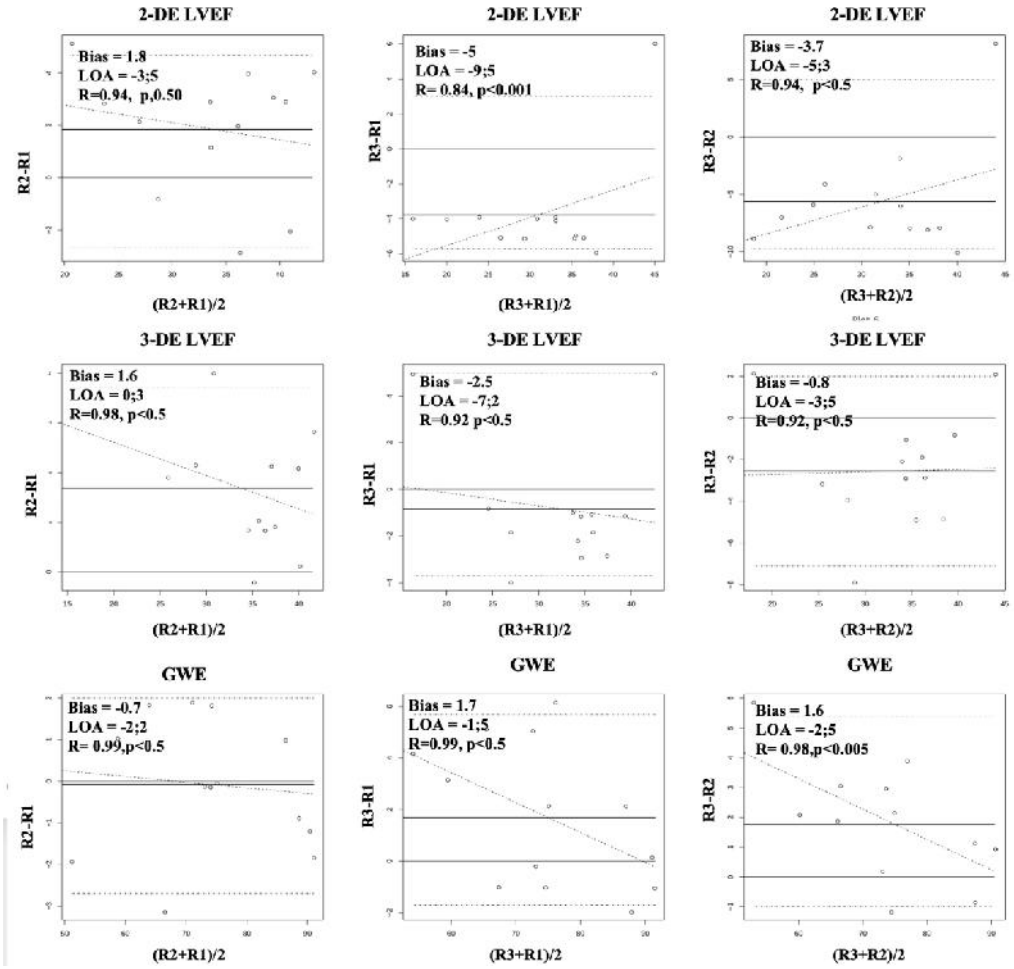

Figure 3.7. Reproducibility of LVEF assessed by 2-DE and 3-DE, and GWE measurements among advanced (R1), intermediate (R2), and beginner (R3) readers in patients with LVEF ≤ 40%. LVEF – left ventricular ejection fraction.

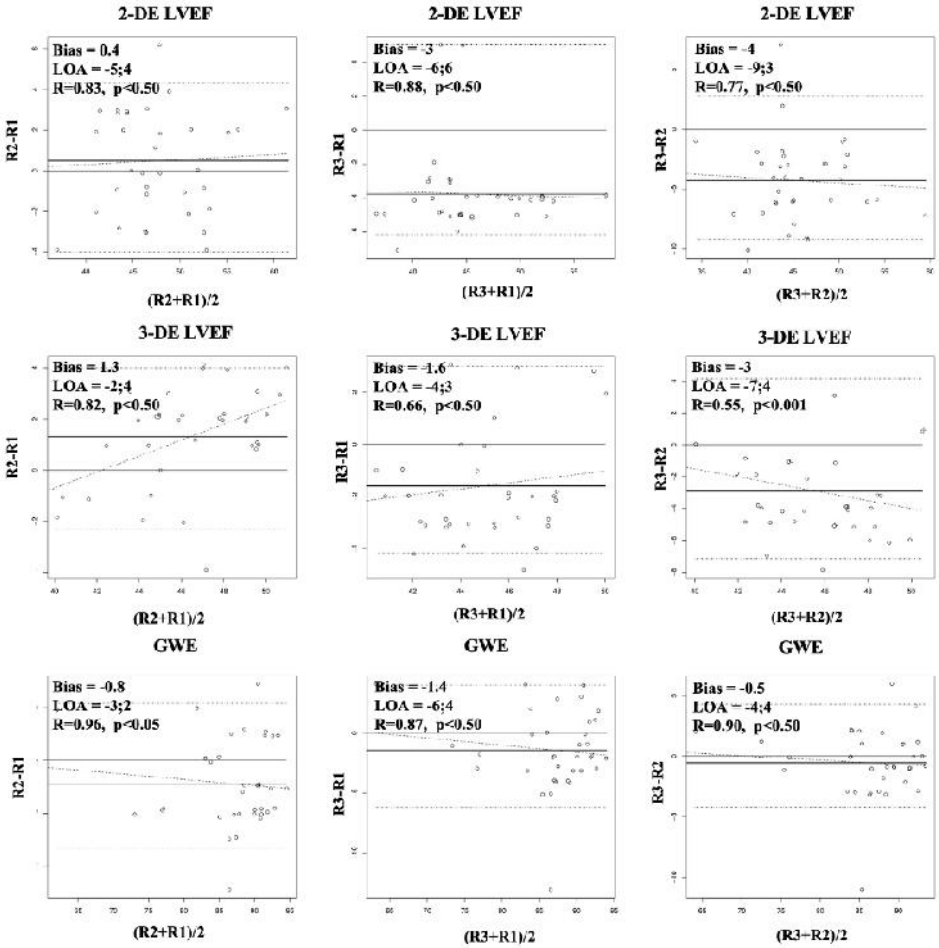

Figure 3.8. Reproducibility of LVEF assessed by 2-DE, 3-DE, and GWE among advanced (R1), intermediate (R2), and beginner (R3) readers in patients with LVEF between 41–49%. LVEF – Left Ventricular Ejection Fraction.

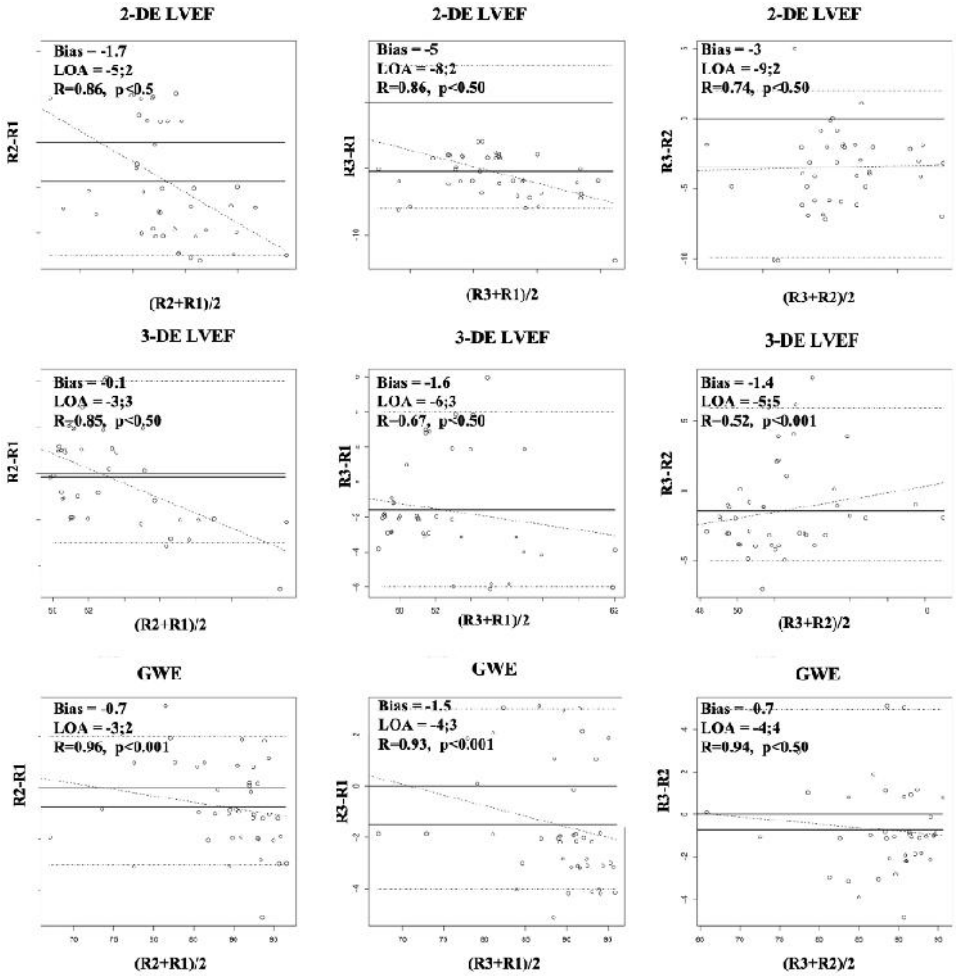

Figure 3.9. Reproducibility of left ventricular ejection fraction (LVEF) measured by 2D, 3D echocardiography, and Global Work Efficiency (GWE) among advanced (R1), intermediate (R2), and beginner (R3) readers in patients with preserved LVEF (≥ 50%). LVEF – left ventricular ejection fraction.

3.3.7. Comparison of Intra-Operator Variability and Agreement Among Echocardiographers with Different Levels of Experience for GWE and LVEF Measured by 2-DE and 3-DE

The results of the intra-observer reproducibility analysis are summarized in Table 3.3. These findings indicate minimal variability in repeated measurements performed by the same observer.

Table 3.3. Intra-observer variability for echocardiographers with different levels of experience for GWE, as well as for LVEF measurements obtained via 2-DE and 3-DE

Parameter	Reader 1	Reader 2	Reader 3
2-DE LVEF (r)	0.89	0.87	0.89
2-DE LVEF (Bias, %)	-0.3	-0.4	-0.5
2-DE LVEF (LOA, %)	-6; 7	-7;6	-7;7
3-DE LVEF (r)	0.96	0.94	0.95
3-DE LVEF (Bias, %)	-0.2	-0.3	-0.5
3-DE LVEF (LOA, %)	-4;4	-4;4	-5;4
GWE (r)	0.97	0.97	0.96
GWE (Bias, %)	-0.4	-0.3	-0.2
GWE (LOA, %)	-4;3	-3;4	-3;3

3.3.8. Extended assessment of reproducibility based on operator experience level

The reproducibility analysis conducted in a dedicated subpopulation, involving readers from all levels of experience (advanced, intermediate, and beginner), confirmed the general findings of the study . Global Work Efficiency (GWE) stood out due to its excellent reproducibility in both intra- and inter-class assessments, appearing less influenced by the reader's level of expertise. These findings support the notion that advanced parameters such as GWE and 3D techniques exhibit superior robustness in the context of inter-observer variability.

3.4. Discussions

In the present study, we demonstrated that the reproducibility of global work efficiency (GWE) in patients with acute coronary syndrome (ACS) is independent of operator experience and comparable to the reproducibility of left ventricular ejection fraction (LVEF) measured by three-dimensional echocardiography (3-DE). In contrast, LVEF assessment by two-dimensional echocardiography (2-DE) showed greater inter-operator variability and lower agreement. Stratification of patients according to ventricular function confirmed the consistency of GWE reproducibility across all functional categories, including patients with preserved, moderately impaired, or severely reduced left ventricular function. The analysis of a dedicated subgroup of 41 patients, involving additional evaluators with varying levels of

experience, added significant value to the study objectives and further confirmed the excellent reproducibility of GWE across a wide spectrum of operators.

To our knowledge, this is the first study to comparatively assess the reproducibility of left ventricular functional parameters measured using myocardial work (GWE) and ejection fraction via 2-DE and 3-DE across users with different levels of experience. In this study, GWE was selected as the primary myocardial work parameter, as it is considered the most relevant functional indicator and has been shown to be an independent prognostic factor in patients with ACS [5].

Several limitations of this study should be acknowledged. First, because accurate assessment of myocardial work parameters requires high-quality echocardiographic images, patients with atrial fibrillation, severe valvular disease, and suboptimal imaging windows were excluded. Additionally, all image acquisitions were performed by a single operator, so extrapolation of results to images acquired by multiple operators should be done with caution. Finally, the results were not compared to those obtained by cardiac magnetic resonance imaging, which is considered the gold standard for evaluating left ventricular function.

3.5. Conclusions

This study demonstrates that myocardial work analysis is a highly reproducible method for evaluating left ventricular performance in patients with acute coronary syndrome, providing results comparable to those obtained by three-dimensional echocardiographic assessment of ejection fraction, regardless of the operator's level of experience. Therefore, integrating myocardial work into standard echocardiographic evaluation could contribute to optimizing the management of patients with ACS and improving long-term cardiovascular outcomes.

4. The Utility of Non-Invasive Myocardial Work Assessment and Its Correlation with Systemic Inflammation in Patients with Acute Coronary Syndrome

5.

4.1. Introduction

New tools, such as non-invasive assessment of myocardial work (MW) derived from speckle tracking echocardiography (STE), have recently been used to evaluate both global and

regional myocardial performance while accounting for loading conditions. Based on these results, identifying correlations between MW and conventional biomarkers is essential to recognize patients at risk for myocardial dysfunction and poor prognosis after an ischemic event. Previous studies have shown that C-reactive protein (CRP) significantly correlates with parameters of left ventricular remodeling when measured two months after percutaneous coronary intervention (PCI). Although local and systemic inflammation following myocardial infarction is expected to resolve within four weeks, a prolonged inflammatory phase may persist in post-ACS patients. Most studies on the inflammatory response in ACS have focused on myocardial evaluation within the first one to three months after the index event [5-7].

Given the recently documented prognostic value of STE and MW [8-10], our study aimed to evaluate myocardial work alongside conventional systolic function parameters in the acute phase of ACS, and its relationship with high-sensitivity troponin and systemic inflammation, assessed by CRP.

4.2. Patients and Methods

This prospective study included patients from the main study cohort diagnosed with acute coronary syndrome (with or without ST-segment elevation), according to current European guidelines. All patients underwent coronary angiography and standard optimal treatment. Inclusion and exclusion criteria were consistent with those in the previous study, ensuring a homogeneous patient profile—sinus rhythm, no significant valvular pathology, no systemic illness, and hemodynamic stability. Patients in whom STE analysis was not technically feasible were excluded.

4.2.1. Baseline and Follow-Up Evaluation

Patient demographics, clinical characteristics, laboratory results, two-dimensional LVEF (2D LVEF), GLS, MW parameters, angiographic data, and administered treatments were recorded. The first evaluation was performed within 24 hours of hospital admission. A second follow-up visit, including the same clinical, laboratory, and echocardiographic investigations, occurred 6–8 weeks later.

4.2.2. Laboratory Analyses

Laboratory workup included complete blood count, ESR, coagulation profile, fasting glucose, HbA1c, liver enzymes, renal function parameters (creatinine, urea, uric acid), electrolytes, total and fractional cholesterol, triglycerides, and other relevant parameters (e.g.,

iron, ferritin when necessary). Key biomarkers were high-sensitivity cardiac troponin I (hs-cTnI, Pathfast; LSI Medience), measured at emergency admission, and CRP, assessed within the first 24 hours of hospitalization.

4.2.3. Echocardiography

All patients underwent comprehensive transthoracic echocardiography both acutely (within 24 hours) and at 8 weeks using standardized equipment. Main echocardiographic parameters, including 2D LVEF and MW indices, were analyzed offline according to validated protocols, with manual contour adjustments as needed. Myocardial work quantification was based on 2D longitudinal strain and non-invasively estimated peak systolic pressure using brachial cuff measurements before each echo study. The parameters recorded were:

- **GWI (Global Work Index):** Total mechanical work done by the LV myocardium during the cardiac cycle, measured between mitral valve closure and opening, analyzed through pressure-strain loops.
- **GCW (Global Constructive Work):** Effective myocardial work, including systolic contraction and physiologic lengthening during isovolumic relaxation, contributing to LV ejection.
- **GWW (Global Wasted Work):** Ineffective myocardial energy expenditure, associated with myocardial lengthening during systole and shortening during isovolumic relaxation.
- **GWE (Global Work Efficiency):** Ratio of constructive to total (constructive + wasted) work, indicating the LV's functional efficiency [83].

The same parameters were collected at the 6–8 week follow-up.

4.2.4. Statistical Analysis

Statistical analysis was conducted using SPSS v25.0 (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean ± SD. Categorical variables were expressed as count and percentage. Comparisons used chi-square (χ^2) tests. Non-parametric methods were applied for AUC, sensitivity, and specificity analyses. Pearson correlations were used to evaluate linear relationships between continuous variables. A correlation coefficient (R) > 0.40 was considered indicative of a good correlation, and p-values < 0.05 were considered statistically significant.

4.3. Results

4.3.1. Study Population

A total of 56 patients (mean age 53 ± 10 years, 45 male) admitted with a diagnosis of acute coronary syndrome (ACS) were included in the analysis. The essential characteristics of the cohort, including comorbidities and angiographic profiles, are summarized in **Table 4.1**

Table 4.1. Characteristics of the study population

Clinical characteristics	All patients (N=56)
Age, years	53 ± 10
Sex, male	45 (80.6)
Hypertension	34 (60.7)
Diabetes	26(45.6)
Dyslipidemia	52 (92.8)
Smoking	39(69.6)
Systolic blood pressure	127.5 ± 17.4
Dyastolic blood pressure	77.5 [70-80]
AMI type – ST- segment elevation	50 (89.2)
Thrombolysis	14 (25)
One-vessel disease	34 (61.8)
Two-vessel disease	17(30.9)
Three-vessel disease	4 (7.2)
CRP within 24 hours (lab range 1-5 mg/dl)	16.75 [5.5-29.4]
hs-cTn I at admission (ug/L)	3311 [486-10990]

Data are presented as mean ± standard deviation (SD), median [interquartile range], or absolute number (n) and percentage (%).

4.3.2. Echocardiographic Characteristics and Myocardial Work Indices

Values for speckle tracking echocardiography (STE) and myocardial work indices were recorded both at baseline and at the follow-up visit, as presented in **Table 4.2**. As expected, the parameters recorded within 24 hours of hospitalization were significantly lower compared to those obtained at follow-up. All initial values were below the reference limits described in the literature for healthy subjects.

Table 4.2. Myocardial work indices and global longitudinal strain (GLS).

Myocardial work indices	Baseline*	Follow-up*
GWI, mmHg%	1112 ± 372.6	1521 ± 467
GCW, mm Hg%	1248 ± 408.8	1666 ± 517
GWW, mmHg%	117 [77.2-138.8]	91 [59.7-140]
GWE, %	89 [82.7-92]	93 [89.7-95]
GLS	-10 ± 3.8	-14.2 ± 4.11

Data are expressed as mean ± SD or median [interquartile range].

LV GLS = left ventricular global longitudinal strain;
GWI = Global Work Index; GCW = Global Constructive Work;
GWW = Global Wasted Work; GWE = Global Work Efficiency.

4.3.3. Correlation Between Systemic Inflammation and Myocardial Work (MW)

Systemic inflammation, assessed via CRP levels, showed a significant correlation with changes in myocardial work parameters between the two visits. A **negative correlation** was observed between CRP levels and changes in **GWE** ($r = –0.53$, $p < 0.001$; see **Figure 4.1**), and a **positive correlation** with changes in **GWW** ($r = 0.48$, $p < 0.001$; see **Figure 4.2**), suggesting a detrimental effect of inflammation on myocardial efficiency.

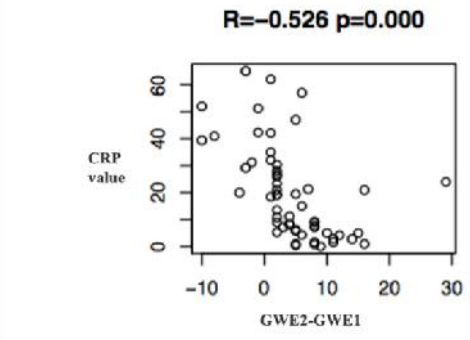

Figure 4.1. Correlation between CRP and GWE changes between visits.
GWE – Global Work Efficiency; GWE1 – GWE at baseline ; GWE2 – GWE at follow up

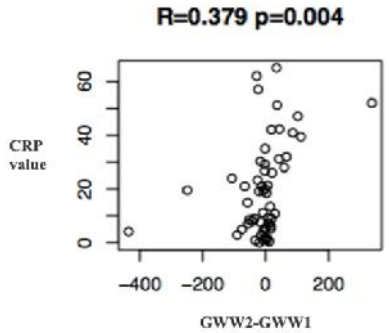

Figure 4.2. Correlation between CRP and GWW changes between visits.
GWW – Global Wasted Work; GWW1 – GWW at baseline ; GWW2 – GWW at follow up

A CRP level above **28 mg/L** (lab reference range: 1–5 mg/dL) predicted a **decline in GWE** between baseline and follow-up, supporting the use of CRP as a potential marker of left ventricular dysfunction (**Figure 4.3**).

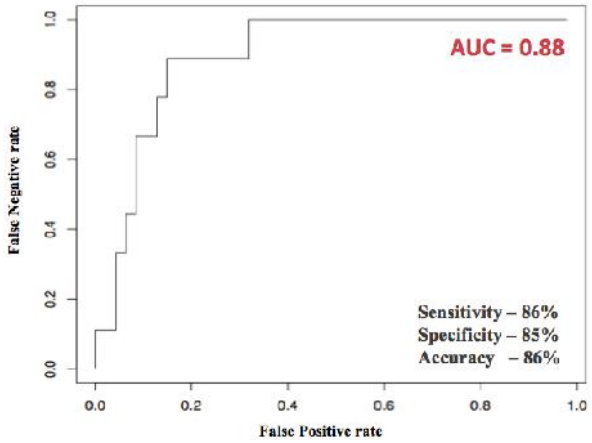

Figure 4.3.A C-reactive protein (CRP) level greater than 28 mg/L was able to predict a decrease in global myocardial work efficiency (GWE) between the baseline and the follow-up visit (visit 2).

"Bull's-eye" maps of myocardial work in patients with low CRP levels clearly illustrated the role of MW in subclinical LV dysfunction, showing improved GWE parameters at follow-up. Conversely, images from patients with a high inflammatory response (elevated CRP) revealed a decline in GWE (**Figure 4.4**).

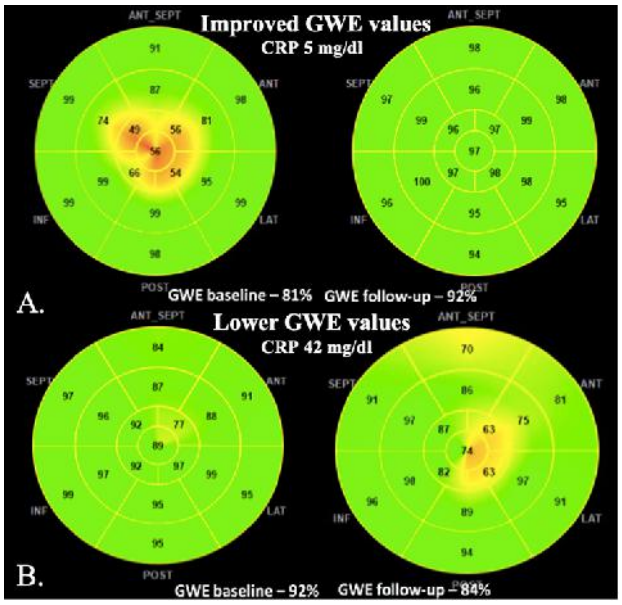

Figure 4.4. Changes in global myocardial work efficiency (GWE) parameters between visits: an improvement in GWE values was observed in patients with low levels of C-reactive protein (CRP) (A), whereas in patients with elevated CRP levels (B), global work efficiency was lower.

4.3.4. Correlation Between High-Sensitivity Troponin, 2D LVEF, GLS, and Myocardial Work (MW)

In our study, myocardial necrosis assessed by **high-sensitivity troponin I (hs-cTnI)** at admission showed no significant correlation with **2D LVEF**. However, there was a significant **negative correlation** with **GLS** (r = –0.43, p = 0.001) and with MW parameters measured at admission: **GWI** (r = –0.43, p = 0.001) and **GCW** (r = –0.40, p = 0.002) (**Figure 4.5**)

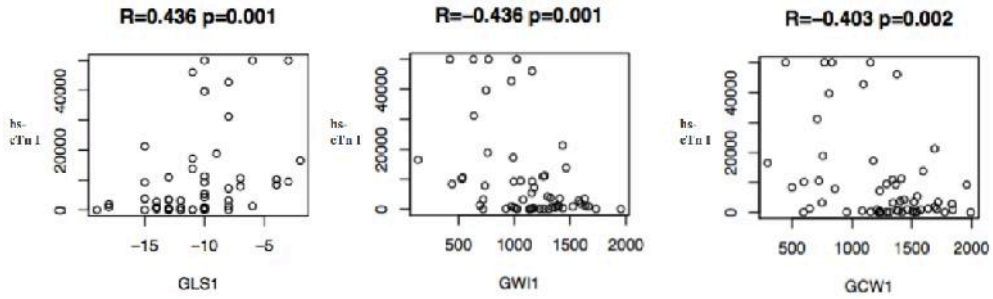

Figure 4.5. Correlation between high-sensitivity cardiac troponin I (hs-cTn I) and global longitudinal strain (GLS), global work index (GWI), and global constructive work (GCW) at admission

4.4.Discussions

In our study, CRP levels correlated with left ventricular dysfunction, as assessed by changes in GWE from 24 hours post-admission to two months post-ACS. Patients with lower initial CRP showed improved MW parameters (increased GWE), whereas those with higher CRP had increased GWW, indicating inefficient myocardial contraction.

Given the key role of systemic inflammation in the pathophysiology of ACS, the CRP cutoff associated with LV dysfunction progression was identified as **28 mg/dL** (reference range: 0–5 mg/dL). A 5–6 fold increase in CRP may thus indicate patients at risk of adverse remodeling or dysfunction post-ACS. These findings align with previous studies showing that CRP levels two days after PCI in STEMI patients correlate with infarct size and ventricular remodeling [10-12].

Regarding the relationship between hs-cTnI and echocardiographic parameters, our data showed no correlation between hs-cTnI and 2D LVEF but did reveal a significant association with baseline GLS and MW parameters (GCW and GWI). However, hs-cTnI levels did not correlate with changes in these parameters over time, raising concerns about its prognostic value in assessing post-ACS remodeling. These findings are consistent with recent literature, which indicates that while elevated troponin levels may predict adverse events post-ACS [13], their correlation with LV dysfunction is inconsistent [14].

While GWI has been supported as a predictive indicator of mid-term cardiovascular events after ACS, the role of GCW remains under debate [15]. Our data do not support the use of hs-cTnI as a marker for the progression of LV dysfunction.

Study limitations include the relatively small sample size and variability in angiographic profiles. The short follow-up duration and absence of a third assessment visit limit the evaluation of long-term remodeling. Although high-sensitivity CRP (hs-CRP) is preferred for assessing inflammation, limited availability in many centers justifies the use of standard CRP, which our results suggest is a reliable marker of acute myocardial injury**.**

4.5. Conclusions

This prospective study in patients with acute coronary syndrome shows that CRP levels measured within 24 hours of admission significantly correlate with changes in myocardial work parameters, particularly GWE and GWW**.** These findings support the early use of CRP as a prognostic biomarker to identify patients at risk for myocardial dysfunction.

6. The Impact of the Adhesion Molecule VCAM-1 on Myocardial Remodeling in Patients with Acute Coronary Syndrome

5.1. Introduction

In recent years, numerous immunological biomarkers have been investigated for their potential association with cardiovascular pathology, given their possible contribution to improving prognostic assessment. Among these, vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin superfamily, has emerged as a key mediator in the inflammatory response. Its soluble form (sVCAM-1) is predominantly upregulated under the influence of proinflammatory cytokines at the vascular endothelium, where it facilitates the adhesion, recruitment, and transmigration of immune cells, including monocytes, lymphocytes,

basophils, and eosinophils [16]. Sustained elevation of this biomarker in patients with acute coronary syndrome (ACS) has been associated with a higher incidence of major adverse cardiovascular events over a six-month period [17]. In patients diagnosed with acute myocardial infarction (AMI), elevated serum concentrations of sVCAM-1 have shown a significant association with the subsequent development of heart failure, thereby reinforcing its prognostic value in this context [18].

The primary objective of this study was to analyze the association between serum levels of sVCAM-1, left ventricular end-diastolic volume (LVEDV), and left ventricular ejection fraction (LVEF), as measured by three-dimensional echocardiography, in patients diagnosed with acute coronary syndromes.

5.2. Patients and Methods

5.2.1. Study Population

This prospective study included patients diagnosed with acute coronary syndrome (ACS), both with and without ST-segment elevation. Inclusion criteria were aligned with those previously described for the study population. The initial evaluation was performed within the first 24 hours of admission and included collection of clinical and demographic data, laboratory parameters, and three-dimensional echocardiographic imaging (for the assessment of ventricular volumes and ejection fraction). A follow-up evaluation was conducted 6–8 weeks later, using the same clinical, biological, and imaging parameters.

5.2.2. Biological Assessment

As part of the study protocol, all patients underwent a comprehensive panel of laboratory tests, including routine hematological and biochemical investigations. Serum levels of soluble VCAM-1 (sVCAM-1) were measured using a commercially available sandwich-type ELISA kit (Quantikine® Human VCAM-1/CD106 ELISA, R&D Systems, Minneapolis, MN, USA), in accordance with the manufacturer's instructions. Sample collection for sVCAM-1 analysis was repeated at the follow-up visit.

5.2.3. Echocardiographic Assessment

All participants underwent a complete transthoracic echocardiographic examination, performed according to current guideline standards using advanced imaging equipment (Vivid E9 or Vivid E95, GE Healthcare). Three-dimensional (3D) acquisitions were obtained using

the 4V probe, as detailed in the Research Methodology section. The images were subsequently analyzed offline using EchoPac™ software (version 203, GE Healthcare).

5.2.4. Statistical Analysis

Statistical processing of the data was conducted using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as absolute numbers and percentages of the study population. Linear correlations between continuous variables were assessed using Pearson's correlation coefficient. A correlation coefficient (r) greater than 0.40 was considered indicative of a moderate to strong association. The threshold for statistical significance was set at $p < 0.05$.

5.3. Results

5.3.1. Study Population

A total of 90 patients were included in the analysis, with a mean age of 54 ± 9 years, of whom 75 were male, all hospitalized with a diagnosis of acute coronary syndrome (ACS). The mean level of soluble VCAM-1 (sVCAM-1) measured within the first 24 hours of admission was 4.65 ± 0.33, while the mean value at follow-up was 4.72 ± 0.29, the difference being statistically significant ($p = 0.04$). The initial left ventricular ejection fraction (LVEF), assessed using three-dimensional echocardiography (3DE), was 47 ± 8%. All patients underwent coronary angiography and received guideline-directed medical therapy in accordance with current European clinical practice recommendations [1].

5.3.2. Correlation Between Initial sVCAM-1 Serum Levels and Changes in Left Ventricular End-Diastolic Volume and Ejection Fraction Assessed by 3D Echocardiography

Statistical analysis revealed a significant negative correlation between sVCAM-1 levels measured at admission and the change in left ventricular end-diastolic volume (LVEDV) between the initial and follow-up evaluations ($r = –0.42$; $p < 0.05$) (Figure 5.1). This relationship suggests that elevated baseline levels of sVCAM-1 are associated with a greater increase in ventricular volume during the post-event period.

Furthermore, a significant negative correlation was observed between baseline sVCAM-1 concentrations and the change in left ventricular ejection fraction (LVEF) between admission and the 6–8 week follow-up ($r = –0.43$; $p < 0.05$), indicating that higher sVCAM-1

values at presentation are linked to a more pronounced decline in left ventricular systolic function, as reflected by the reduced LVEF measured by 3DE. (Figure 5.2

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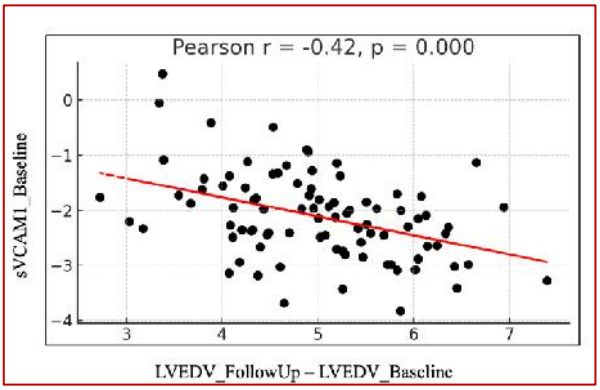

Figure 5.1. Correlation between baseline sVCAM-1 levels and changes in left ventricular end-diastolic volume (LVEDV) between the two evaluations

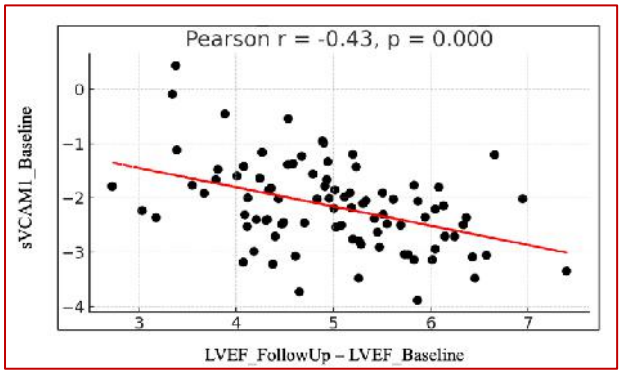

Figura 5.2. Correlation between baseline sVCAM-1 levels and changes in left ventricular ejection fraction (LVEF) between the two evaluations

5.3.3. Correlation Between Changes in Serum VCAM-1 Levels Between Baseline and Follow-Up and Changes in 3D Echocardiographic Parameters: LVEDV and LVEF

A statistically significant positive correlation was observed between the change in left ventricular end-diastolic volume (LVEDV), assessed via three-dimensional echocardiography (3DE), and the increase in serum sVCAM-1 levels from hospital admission to follow-up ($r = 0.41$; $p < 0.05$). This association suggests that patients who experienced a more pronounced rise in sVCAM-1 concentrations over the follow-up period also demonstrated more substantial ventricular dilation, as evidenced by increased LVEDV at reevaluation (Figure 5.3).

Furthermore, a significant negative correlation was identified between changes in left ventricular ejection fraction (LVEF) and changes in sVCAM-1 levels during the follow-up interval ($r = –0.46$; $p < 0.05$). These findings indicate that patients with greater increases in sVCAM-1 concentrations between the initial and follow-up assessments experienced a more marked decline in left ventricular systolic function, as reflected by reduced LVEF (Figure 5.4).

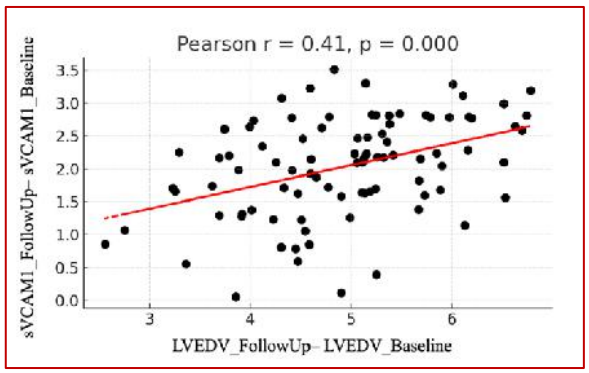

Figura 5.3. Correlation between changes in sVCAM-1 levels and changes in left ventricular end-diastolic volume (LVEDV) between baseline and follow-up

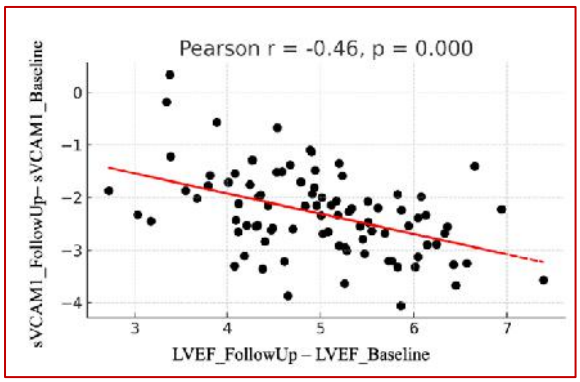

Figura 5.4. Correlation between changes in sVCAM-1 levels and changes in left ventricular ejection fraction (LVEF) between baseline and follow-up

5.4. Discussions

In the present study, patients diagnosed with acute coronary syndrome (ACS) who exhibited elevated VCAM-1 levels at the time of hospital admission demonstrated a significant increase in left ventricular end-diastolic volume (LVEDV) at the 6–8-week follow-up, as assessed by three-dimensional echocardiography (3DE). This finding suggests a trend toward adverse ventricular remodeling. Moreover, higher baseline VCAM-1 levels were associated with a progressive reduction in left ventricular ejection fraction (LVEF) during the follow-up period. This inverse relationship supports the hypothesis that a heightened inflammatory status during the acute phase of ACS may contribute to the progressive deterioration of left ventricular systolic function.

To the best of our knowledge, this is the first study to investigate the relationship between sVCAM-1 levels and left ventricular functional performance using three-dimensional echocardiographic techniques. Our findings support the potential role of VCAM-1 as a predictive biomarker for adverse remodeling and functional decline during the subacute phase following myocardial infarction. Existing literature has already established that serum VCAM-1 levels in patients with myocardial infarction are a sensitive indicator of post-ACS heart failure risk, being significantly higher in patients with ST-segment elevation who develop heart failure symptoms upon discharge [17].

Beyond the analysis of baseline values, our study also included an assessment of dynamic changes in sVCAM-1 levels at two months following the acute coronary event. A significant increase in sVCAM-1 between the initial and follow-up assessments was directly correlated with markers of adverse remodeling, namely, an increase in LVEDV and a decrease in LVEF, indicating progressive systolic dysfunction. These findings support the hypothesis that persistent inflammatory activation, reflected by a sustained rise in VCAM-1, may play a pathogenic role in structural and functional myocardial deterioration after ACS. Our data are consistent with previously published observations. Elevated sVCAM-1 levels have been correlated with infarct size, ventricular remodeling, and the occurrence of adverse clinical events at one-year follow-up [19]. Although challenges remain in terms of targeted delivery and clinical implementation, advances in molecular engineering and drug delivery technologies offer promising perspectives for the development of clinically viable and effective therapeutic interventions [20].

Among the limitations of this study, it should be noted that while the data suggest a potential association between sVCAM-1 levels and left ventricular remodeling, further studies with larger patient cohorts and extended follow-up durations are necessary to validate these findings and better elucidate the relationship between VCAM-1 expression, adverse remodeling, and the progression to heart failure.

5.5. Conclusions

This study highlights the prognostic potential of VCAM-1, a biomarker of inflammation and immune activation, as an indicator of left ventricular dysfunction, as reflected by changes in ejection fraction (LVEF) and end-diastolic volume (LVEDV) during the post-acute phase of acute coronary syndrome (ACS).

The findings highlight the crucial role of inflammatory mechanisms in the cardiac remodeling process and underscore the need for further research into the utility of VCAM-1 for both risk stratification and as a potential therapeutic target in managing patients with ACS.

6. Association Between Serum Inflammatory Biomarkers, Ejection Fraction, and Left Ventricular Volume Assessed by 3D Echocardiography in Patients with Acute Myocardial Infarction

6.1. Introduction

Inflammation and immune activation play a central role in the pathogenesis and progression of acute myocardial infarction (AMI), directly influencing post-ischemic myocardial remodeling and left ventricular dysfunction. Several molecules described in the context of AMI and ventricular remodeling have a significant role in modulating the innate immune response.

NCAM-1, by maintaining cellular integrity and reactivating embryonic signaling pathways, may modulate the innate immune response by limiting excessive inflammatory cell activation and promoting a protective microenvironment in the early phases of ischemic injury [20,21]. PRCP (prolylcarboxypeptidase), a serine protease involved in vasopeptide metabolism, has been shown to be essential in regulating cardiac mitophagy and reducing reperfusion injury, with a positive impact on ventricular function [22,23]. NRP1 (neuropilin-1) is a multifunctional coreceptor involved in vascular permeability and endothelial barrier integrity. Through its effects on vascular permeability and endothelial stability, it plays a crucial role in leukocyte trafficking, thus influencing the activation and recruitment of innate immune cells (monocytes, macrophages, neutrophils) and contributing to the regulation of the balance between inflammation and post-ischemic repair [24,25].

Conversely, Fetuin-B, an emerging hepatokine, is associated with metabolic dysfunction, inflammation, and atherosclerotic plaque instability, and has been identified as a predictive marker for AMI [26,27]. AOC3/VAP-1, which has both adhesive and enzymatic functions, facilitates leukocyte recruitment into ischemic tissue and contributes to the amplification of vascular inflammation, having been proposed as a therapeutic target for preventing ventricular remodeling [28]. Fetuin-B and AOC3/VAP-1 are linked to exacerbated activation of the innate immune response, promoting leukocyte recruitment, oxidative stress, and a proinflammatory profile that contributes to plaque instability and adverse remodeling.

Finally, Cystatin C, an endogenous inhibitor of cysteine proteases and a sensitive marker of renal function, has demonstrated significant predictive value for cardiovascular events, heart failure, and mortality in patients with AMI [29–31]. These proteins appear to play a central role in orchestrating the interactions between metabolic and ischemic stress, early inflammatory response, and innate immune activation, representing potential therapeutic targets for

modulating these pathways in order to prevent ventricular dysfunction and progression to heart failure post-AMI.

The aim of this study was to investigate the correlation between serum levels of these biomarkers (NCAM-1, PRCP, NRP1, Fetuin-B, AOC3, and Cystatin C) and left ventricular ejection fraction (LVEF) assessed by 3D echocardiography in patients with acute myocardial infarction, in order to highlight their potential prognostic value for identifying post-ischemic ventricular dysfunction and guiding future therapeutic strategies.

6.2. Patients and Methods

6.2.1. Study Population

This prospective study was conducted on the same cohort of patients diagnosed with acute coronary syndrome (ACS), regardless of the presence or absence of ST-segment elevation. Patient selection for this analytical subgroup followed the same methodology as the original study and involved patients from the same cohort. The initial clinical, biological, and imaging evaluations were performed within the first 24 hours of admission, and follow-up was conducted at 6–8 weeks using the same standardized clinical and investigational protocols as those applied in the main study.

6.2.2. Laboratory Analyses

Biological assessment for this subgroup followed the protocol established in the primary study. All participants underwent a complete panel of routine hematological and biochemical tests. For this analysis, serum levels of proteins involved in inflammation, cardiac remodeling, and ventricular dysfunction with potential prognostic roles in AMI were determined. These included NCAM-1, PRCP, NRP1, Fetuin-B, AOC3/VAP-1, and Cystatin C. Except for Cystatin C, which was determined either by automated immunoturbidimetric methods (e.g., using the Roche Cobas C system, Roche Diagnostics) or ELISA kits, all other proteins were measured using commercially available sandwich-type ELISA kits, according to the manufacturers' instructions. Blood samples were collected both at admission and at the 6–8-week follow-up visit, allowing for the evaluation of biomarker dynamics and correlation with changes in left ventricular function.

6.2.3. Echocardiographic Evaluation

In this subgroup analysis, left ventricular function was assessed using the same echocardiographic methodology as described in the main study and in previous publications, in accordance with standard protocols of the European Society of Cardiology and the European Association of Cardiovascular Imaging.

6.2.4. Statistical Analysis

Data were processed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as absolute frequency and percentage. The chi-square test was used to analyze differences between categorical variables. Correlations between continuous variables were assessed using Pearson's correlation coefficient. A correlation coefficient (r) > 0.40 was interpreted as indicative of a moderate to strong association. The level of statistical significance was set at $p < 0.05$.

6.3. Results

6.3.1. Study Population

The clinical and demographic characteristics of the 90 patients included in this subgroup were detailed in previous sections of this thesis, as they are part of the same cohort initially analyzed. Regarding the biological profile of the evaluated biomarkers, mean values along with standard deviations, minimum, and maximum values at hospital admission (V1) and at the 6–8-week follow-up (V2) are summarized in Table 6.1. Significant inter-individual variability was observed for each protein, with some markers showing upward trends during the monitoring interval.

Table 6.1. Statistical summary of serum biomarker levels at baseline (V1) and at follow-up (V2, 6–8 weeks later).

Biomarker	Dosing Moment	Median	SD	Minimal Value	Maximal Value
AOC3	V1	3.3866	0.3985	2.2351	4.2549
AOC3	V2	3.5135	0.4068	2.3277	4.5791
CST3	V1	6.2933	0.4793	5.2701	7.7965
CST3	V2	6.2808	0.5158	4.9170	7.3935
FETUB	V1	1.0551	0.5279	-0.1574	2.1792
FETUB	V2	1.3748	0.4412	0.4898	2.3284
NCAM1	V1	3.0484	0.4385	2.0509	4.3947

NCAM1	V2	3.0351	0.3565	2.3201	3.9699
NRP1	V1	0.7484	0.4311	-0.4135	1.7188
NRP1	V2	1.1524	0.3415	0.0396	1.7593
PRCP	V1	0.6572	0.4116	-0.3174	1.6629
PRCP	V2	0.7616	0.3664	0.0956	1.5648

6.3.2. Correlation Between NCAM-1 and Left Ventricular Remodeling

Pearson correlation analysis revealed a statistically significant negative association between the change in left ventricular ejection fraction (LVEF) and the change in serum NCAM-1 concentration, with a correlation coefficient of r = –0.40, p = 0.018 (Figure 6.1). Regarding cavity remodeling, a statistically significant positive correlation was identified between the difference in three-dimensional end-diastolic volume (3D-EDV difference) and the change in NCAM-1 levels, with a Pearson coefficient of r = 0.50, p = 0.012. This finding indicates that patients who experienced an increase in NCAM-1 levels also exhibited an increase in left ventricular end-diastolic volume, reflecting progressive cavity dilation (Figure 6.2). Although NCAM-1 is considered to have a protective role during the acute phase of myocardial infarction, this evolution suggests that persistently elevated NCAM-1 levels may reflect inefficient reparative processes, chronic inflammation, or progressive fibrosis, thereby contributing to long-term ventricular dysfunction.

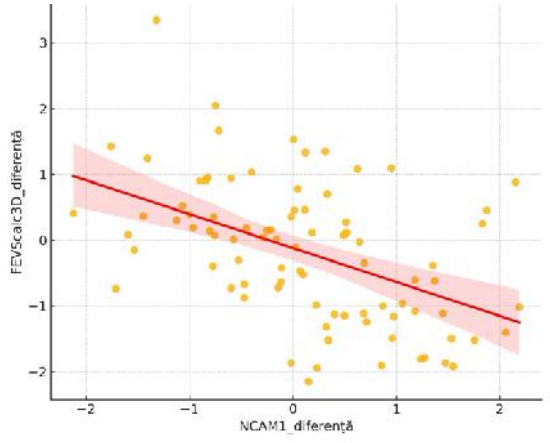

Figura 6.1. Correlation between the change in three-dimensional left ventricular ejection fraction (LVEF_3D difference) and the change in serum NCAM-1 concentration (r = –0.42)

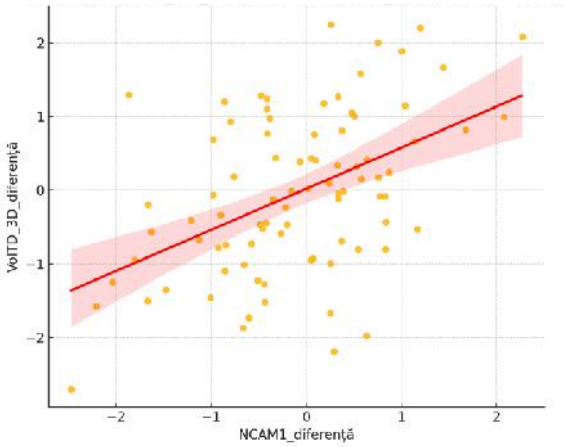

Figura 6.2. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and the change in serum NCAM-1 concentration (r = 0.50)

6.3.3. Correlation Between PRCP and Left Ventricular Remodeling

A statistically significant positive correlation was observed between the change in three-dimensional end-diastolic volume (LVEDV_3D difference) and the change in PRCP levels, with a Pearson correlation coefficient of r = 0.45 and a p-value of 0.026. This suggests that an increase in PRCP levels over time is associated with progressive left ventricular dilation (Figure 6.3).

Within the same cohort, a statistically significant negative correlation was identified between the change in three-dimensional ejection fraction (LVEF_3D difference) and the change in PRCP levels (r = –0.42, p = 0.018), indicating that rising PRCP levels during follow-up are associated with a decline in left ventricular systolic function, suggesting a potential role of PRCP in ventricular dysfunction (Figure 6.4).

In a separate analysis conducted on a sample of 90 patients, the relationship between baseline PRCP values (PRCP_V1) and subsequent changes in LVEDV (LVEDV_3D difference) was assessed. A significant negative correlation was found (r = –0.47, p = 0.026), indicating that higher initial PRCP values may be associated with more favorable structural outcomes (Figure 6.5).

These results suggest a possible biphasic or context-dependent role of PRCP, wherein its longitudinal elevation may reflect adverse cardiac remodeling, while elevated baseline levels might exert a protective or favorable predictive effect on ventricular evolution.

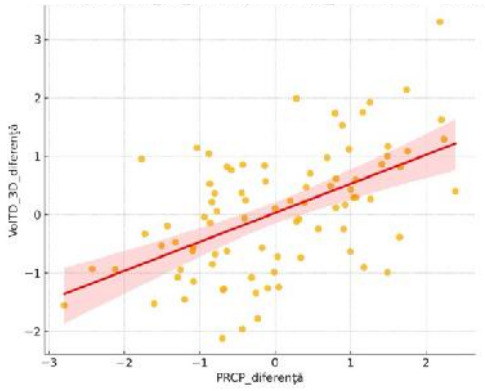

Figura 6.3 . Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and the change in serum PRCP concentration (r = 0.45)

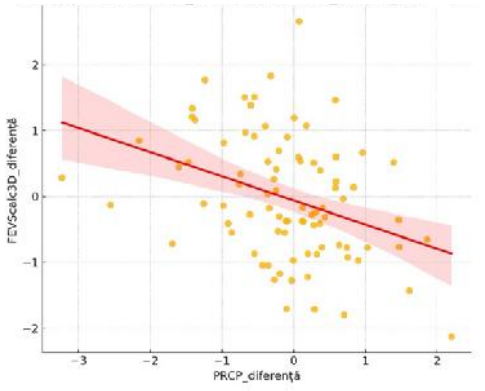

Figura 6.4. Correlation between the change in three-dimensional left ventricular ejection fraction (LVEF_3D difference) and the change in serum PRCP concentration (r = –0.42)

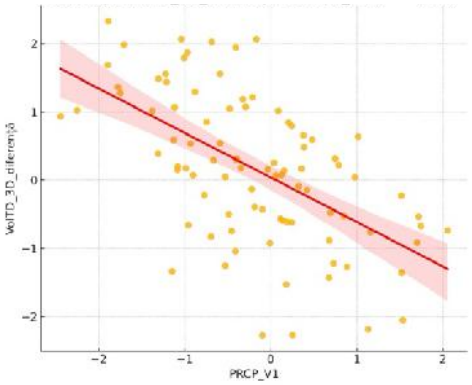

Figura 6.5. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and baseline serum PRCP level (PRCP_V1) (r = –0.47)

6.3.6. Correlation Between AOC3 and Left Ventricular Remodeling

A statistically significant negative correlation was observed between baseline serum levels of AOC3 (AOC3_V1) and the change in three-dimensional end-diastolic volume (LVEDV_3D difference), indicating that patients with higher AOC3 levels at the first visit experienced a reduction in LVEDV during follow-up. This relationship suggests a potential protective role of elevated baseline AOC3 levels in early stages, particularly with respect to left ventricular cavity remodeling (r = –0.47, p = 0.012) (Figure 7.8).

Conversely, analysis of longitudinal changes revealed a statistically significant positive correlation between LVEDV_3D difference and AOC3 difference (r = 0.50, p < 0.001), indicating that an increase in AOC3 levels over time is associated with progressive left ventricular dilation (Figure 7.9).

Regarding systolic function, a significant negative correlation was identified between the change in three-dimensional ejection fraction (LVEF_3D difference) and the change in AOC3 levels (r = –0.45, p = 0.049). This association suggests that rising AOC3 levels are accompanied by a mild deterioration in left ventricular systolic function, supporting a potential role of this molecule in adverse cardiac remodeling—both structural and functional (Figure 7.10).

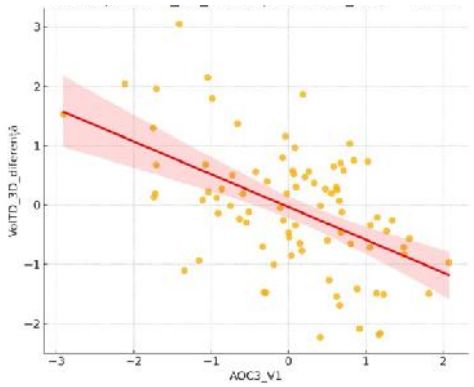

Figura 6.8. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and baseline serum AOC3 concentration (AOC3_V1) (r = –0.47)

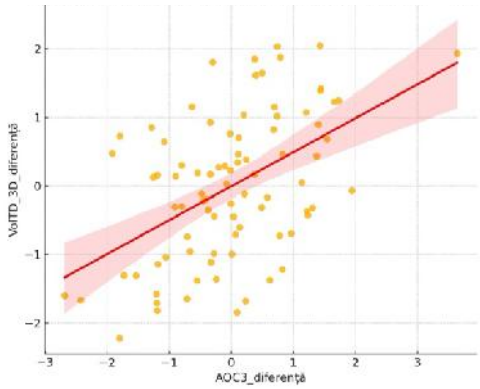

Figura 6.9. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and the change in serum AOC3 concentration (AOC3 difference) (r = 0.50)

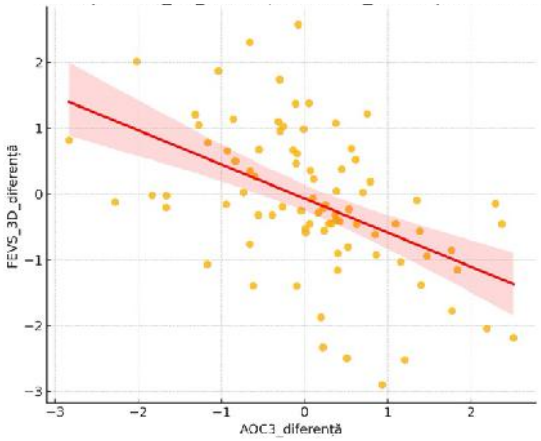

Figura 6.10. Correlation between the change in three-dimensional left ventricular ejection fraction (LVEF_3D difference) and the change in serum AOC3 concentration (AOC3 difference) (r = –0.45)

6.3.7. Correlation Between CST3 and Left Ventricular Remodeling

A statistically significant positive correlation was identified between the change in three-dimensional end-diastolic volume (LVEDV_3D difference) and the change in CST3 levels (CST3 difference) (r = 0.58, p = 0.018), suggesting that an increase in CST3 during the follow-up period is associated with progressive left ventricular cavity dilation (Figure 6.11).

In addition, a significant negative correlation was found between LVEDV_3D difference and baseline CST3 levels (CST3_V1) (r = –0.60, p = 0.014), which may suggest a protective

effect of higher initial CST3 values or a complex time-dependent behavior of this biomarker depending on the timing of evaluation (Figure 6.12).

Regarding systolic function, a statistically significant negative correlation was observed between the change in three-dimensional ejection fraction (LVEF_3D difference) and the change in CST3 levels (r = –0.52, p = 0.011), indicating that rising CST3 concentrations over time are associated with a deterioration in left ventricular systolic function (Figure 6.13).

These findings support a potential involvement of CST3 in pathological ventricular remodeling, with implications for both myocardial structure and function.

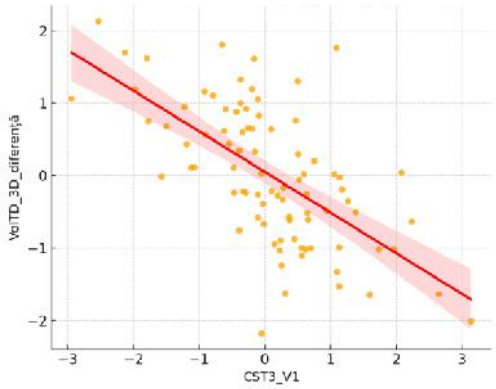

Figura 6.11. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and baseline serum CST3 concentration (CST3_V1) (r = –0.60)

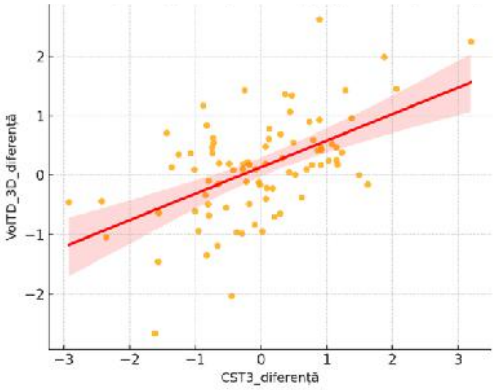

Figura 6.12. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and the change in serum CST3 concentration (CST3 difference) (r = 0.58)

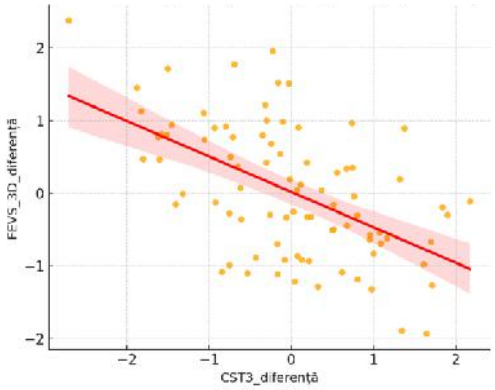

Figura 6.13. Figure 6.13. Correlation between the change in three-dimensional left ventricular ejection fraction (LVEF_3D difference) and the change in serum CST3 concentration (CST3 difference) (r = –0.52)

6.3.8. Correlation Between FETUB and Left Ventricular Remodeling

The results revealed a statistically significant positive correlation between the change in FETUB levels and the change in three-dimensional end-diastolic volume (LVEDV_3D difference) during the follow-up period ($r = 0.52$, $p < 0.05$) (Figure 7.14). This association indicates that patients with an increase in serum FETUB levels over time also exhibited a tendency toward increased LVEDV, an echocardiographic marker of cavity dilation. Thus, FETUB difference may represent a potential indicator of an adverse remodeling process, possibly reflecting subclinical activation of inflammatory or fibroproliferative mechanisms.

Conversely, analysis of baseline FETUB levels (FETUB_V1) showed a statistically significant negative correlation with LVEDV_3D difference ($r = –0.55$, $p = 0.049$) (Figure 7.15). This relationship suggests that higher initial FETUB concentrations may be associated with a less pronounced progression of ventricular dilation over time, potentially reflecting a transient compensatory or protective effect in the early stages of cardiac remodeling.

Therefore, the findings support a bidirectional dynamic behavior of FETUB depending on the timing of evaluation: while elevated baseline values might reflect a relatively favorable compensatory state, a sustained increase in FETUB over time appears to be associated with progressive structural deterioration.

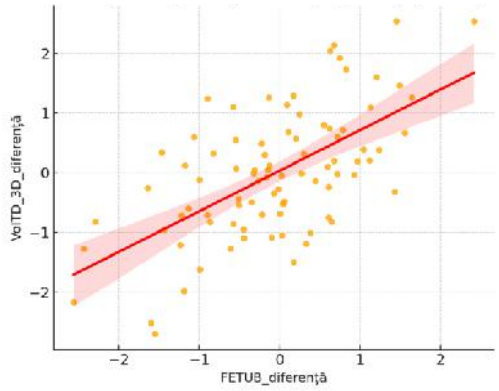

Figura 6.14. Correlation between the change in three-dimensional left ventricular ejection fraction (LVEF_3D difference) and the change in serum FETUB concentration (FETUB difference) (r = –0.52)

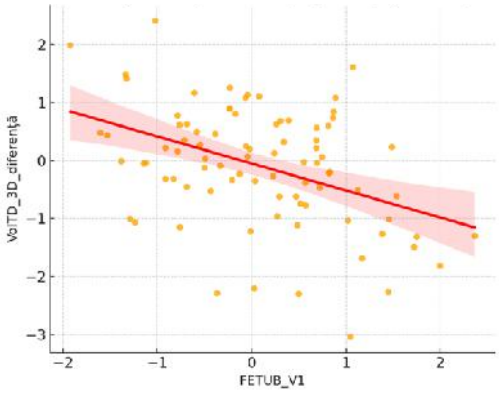

Figura 6.15. Correlation between the change in three-dimensional left ventricular end-diastolic volume (LVEDV_3D difference) and baseline serum FETUB concentration (FETUB_V1) (r = –0.55)

6.4. Discussions

The findings of this study highlight the significant potential of several circulating biomarkers—belonging to the families of cell adhesion molecules, proteases, and their inhibitors—in monitoring and understanding the process of left ventricular remodeling following acute myocardial infarction. Correlation analyses demonstrated significant associations between changes in these biomarkers and key echocardiographic parameters, both volumetric (3D-LVEDV) and functional (3D-LVEF), providing an expanded perspective on the biological processes involved in the progression of patients with ischemic cardiac injury.

The results revealed that several serum biomarkers are associated with echocardiographic parameters of post-infarction left ventricular remodeling. NCAM1 was negatively correlated with systolic function and positively correlated with cavity dilation, suggesting its involvement in adverse structural and functional remodeling. PRCP and NRP1 exhibited a biphasic behavior, with baseline levels favorably associated with clinical evolution, while subsequent increases were correlated with ventricular dysfunction and dilation—indicating a potential dual role: early protective and late maladaptive. AOC3 appeared to exert a protective effect during the acute phase, but was associated with inflammatory and adverse remodeling potential over time. CST3 and FETUB showed a similar pattern, with initial protective roles followed by positive correlations with cavity dilation and declining systolic function, suggesting involvement in progressive inflammation and fibrosis.

Overall, these findings support the concept that post-infarction remodeling is governed by a complex interplay between compensatory processes and progressive pathological mechanisms, mediated by molecular interactions detectable through circulating biomarkers. However, interpretation of these results should be approached with caution in light of certain limitations, including the observational nature of the study, the inability to establish direct causality, and the lack of stratification based on infarct severity, treatment type, or comorbidities.

6.5. Conclusions

This study provides a meaningful contribution to the understanding of the role of biomarkers involved in inflammation, cell adhesion, and tissue remodeling in ischemic heart disease. The results support the need for continued research in this area and the development of complex predictive models that integrate both biological markers and advanced

echocardiographic parameters, with the goal of personalizing therapeutic strategies and improving the prognosis of patients with acute myocardial infarction.

10. Conclusions and Personal Contributions

The present thesis aimed to address a fundamental objective in contemporary clinical practice: the identification of more sensitive and integrative methods for evaluating patients with acute myocardial infarction, with the goal of predicting adverse ventricular remodeling and improving long-term functional outcomes. The study was structured along four principal directions, each contributing meaningfully to the validation of the proposed hypotheses and to the formulation of clinically and scientifically relevant conclusions.

Firstly, it was demonstrated that the myocardial work quantification method—derived from global longitudinal strain and arterial blood pressure—is reproducible both intra- and inter-observer, including among echocardiographers with varying levels of experience (advanced, intermediate, and beginner). The consistent reproducibility across experience levels supports the standardization of this technique and its applicability in multicenter studies or in long-term monitoring of patients with ischemic heart disease.

Secondly, the impact of systemic inflammation on myocardial performance was explored using C-reactive protein (CRP) as a standardized biomarker. Elevated CRP levels were found to be associated with reduced myocardial work efficiency and increased ineffective energy consumption, suggesting a direct negative functional impact of inflammation on the myocardium. This relationship underscores the role of inflammation not merely as a secondary effect but as an active mechanism in the deterioration of myocardial contractility. These data support the integration of inflammatory markers into predictive models of ventricular dysfunction, alongside traditional imaging parameters.

The study also demonstrated the involvement of vascular cell adhesion molecule-1 (VCAM-1) in myocardial remodeling after acute myocardial infarction, identifying a significant association between its serum levels and echocardiographic parameters indicative of cavity dilation and reduced ejection fraction at 6–8 weeks post-infarction. These findings support the prognostic potential of VCAM-1 as a circulating biomarker for risk stratification of adverse ventricular remodeling in patients with acute coronary syndrome.

A major contribution of the thesis was the integration and analysis of several emerging serum biomarkers—including AOC3, CST3, NCAM-1, PRCP, FETUB, and NRP1—that have been scarcely studied in the context of post-infarction remodeling. Correlational analyses revealed dynamic and often biphasic behavior of these proteins: elevated baseline levels of some markers were associated with favorable echocardiographic progression, suggesting a potential protective role in the acute phase; in contrast, increases in these markers over time were associated with progressive cavity dilation and decreased ejection fraction, indicating a transition toward adverse remodeling. These findings support the notion that post-ischemic remodeling is influenced not only by ischemia and myocardial necrosis but also by immune, inflammatory, and tissue reorganization processes reflected at the molecular level.

Personal contributions include:

- Conducting the first Romanian clinical study to assess the interobserver reproducibility of myocardial work across different levels of echocardiographic experience, with methodological implications for validating and expanding this technique in current practice;
- Demonstrating for the first time in Romanian literature a direct relationship between CRP and global work efficiency (GWE), thereby adding clinical value to a recently introduced imaging parameter;
- Performing the first correlative assessment between VCAM-1 and left ventricular remodeling parameters assessed by three-dimensional echocardiography in patients with acute coronary syndrome, supporting its potential prognostic role in early identification of patients at high risk for adverse remodeling and its inclusion in future post-infarction risk stratification algorithms.
- Integrating novel biomarkers of inflammation and immune response (including AOC3, NCAM1, PRCP, FETUB, and CST3) into an advanced 3D echocardiographic analysis;
- Proposing a model for integrative interpretation of volumetric and functional changes of the left ventricle in correlation with biomarker dynamics;
- Participating in the standardization of the repeated echocardiographic acquisition protocol within the same center, with direct applications in clinical research and echocardiographic practice.

The study offers several technical and methodological strengths, including the use of advanced imaging technology (3D echocardiography), dynamic patient assessment post-infarction, and the integration of imaging data with serum biomarker profiles. Furthermore, this complex and multidimensional analytical approach enables a more comprehensive

understanding of the subtle pathological processes that precede overt dysfunction and may guide early and personalized therapeutic interventions.

Future research directions include validating these biomarkers in large and diverse cohorts, integrating them into composite clinical scores that incorporate imaging and serological data, exploring the therapeutic role of anti-inflammatory and immunomodulatory interventions, and developing predictive models for individualized risk stratification of adverse remodeling.

In conclusion, this doctoral thesis makes a relevant and original scientific contribution to the fields of cardiovascular imaging and circulating biomarkers. By demonstrating a significant relationship between systemic inflammation, molecular markers, and advanced echocardiographic parameters, the study provides a solid foundation for enhancing the understanding of cardiac remodeling and for developing personalized prevention and treatment strategies in acute myocardial infarction.

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