„CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST

DOCTORAL SCHOOL
GENERAL MEDICINE

THE ROLE OF CIRCULATING MICRORNAS IN VASCULAR CELLS WITHIN COMPLICATED ATHEROSCLEROTIC PLAQUES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND TYPE 2 DIABETES MELLITUS

THESIS SUMMARY

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Published papers

Articles published in specialized journals

1. **Iacobescu L,** Ciobanu A, Corlatescu A, Simionescu M., Iacobescu G., Dragomir E., Vinereanu D., (July 08, 2024) The Role of Circulating MicroRNAs in Cardiovascular Diseases: A Novel Biomarker for Diagnosis and Potential Therapeutic Targets?. Cureus 16(7):e64100. DOI 10.7759/cureus.64100. FI 1.1. https://www.cureus.com/articles/270144-the-role-of-circulating-micrornas-in-cardiovascular-diseases-a-novel-biomarker-for-diagnosis-and-potential-therapeutic-targets?token=Hdh3xdfndPkszvH_XwjX&utm_medium=email&utm_source=transaction#!/ .
2. **Iacobescu, L.;** Ciobanu, A.O.; Macarie, R.; Vadana, M.; Ciortan, L.; Tucureanu, M.M.; Butoi, E.; Simionescu, M.; Vinereanu, D. Diagnostic and Prognostic Role of Circulating microRNAs in Patients with Coronary Artery Disease—Impact on Left Ventricle and Arterial Function. Curr. Issues Mol. Biol. 2024, 46, 8499–8511. https:// doi.org/10.3390/cimb46080500. FI 2.8. https://www.mdpi.com/1467-3045/46/8/500.

INTRODUCTION

Atherosclerosis is the leading cause of cardiovascular diseases, which include myocardial infarction, heart failure, stroke, and peripheral arterial disease. Increasing evidence shows that inflammation plays a crucial role in the atherosclerotic process, underlying the pathological changes that initiate and lead to the development of atherosclerosis. This occurs through the activation of endothelial cells, infiltration of monocytes and their differentiation into macrophages, as well as the formation of foam cells, which lead to endothelial dysfunction and proliferation of smooth muscle cells.[1] Endothelial dysfunction is an early event that disrupts vascular homeostasis and stimulates the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. These molecules contribute to vascular adhesion, infiltration, and lesion formation in the neointima.[2] In advanced stages of atherosclerosis, macrophages and inflammatory cytokines lead to vascular wall infiltration, secretion of matrix metalloproteinases (MMPs), which results in collagen fiber degradation, rupture of the atherosclerotic plaque, and consequently, thrombosis.[3]

Recent data highlight the importance of diabetes mellitus in the pathogenesis of atherosclerosis through involvement in oxidative stress and inflammation mechanisms associated with circulating microRNAs.[4] Diabetes mellitus causes dysfunction of the microbiota and the production of active metabolites, such as reactive oxygen species and lipopolysaccharides, leading ultimately to the expression of nuclear factor kB and acceleration of atherosclerosis.[5]

MicroRNAs are considered important regulators in cellular migration, differentiation, proliferation, and cytokine production, which lead to new molecular mechanisms of systemic atherosclerosis and may serve as new therapeutic targets.[6] Recent literature indicates that microRNAs are involved in the pathogenesis of multiple conditions, including cardiovascular diseases, diabetes mellitus, renal pathologies, rheumatoid arthritis, and neoplastic diseases.[6]

This research, titled *"The Role of Circulating MicroRNAs in Vascular Cells within Complicated Atherosclerotic Plaques in Patients with Acute Myocardial Infarction and Type 2 Diabetes Mellitus,"* presented as a doctoral thesis, aims to evaluate cardiac functional changes in patients with acute myocardial infarction, with or without diabetes mellitus, and the relationship of these changes with circulating microRNAs. The research is descriptive in nature, entirely original, and seeks to "in the addressed field, by completing the research domain with new aspects

that can contribute to the diagnosis, prognosis, and therapeutic approach of patients with acute myocardial infarction. The thesis is structured into two main parts: the general part with four chapters and the personal contributions part structured into five chapters. The general part of this doctoral research aims to identify the current state of knowledge and present the most recent concepts related to the pathophysiology, diagnosis, and treatment of acute coronary disease, as well as concepts related to the involvement of microRNAs and MMPs in the pathophysiology of atherosclerosis and vascular dysfunction in patients with acute myocardial infarction.

The special part of this research presents the results of a clinical study evaluating 60 patients with acute myocardial infarction (30 patients with diabetes mellitus, 30 patients without diabetes mellitus) and 30 patients with chronic coronary syndrome, focusing on left and right ventricular function, left and right atrial function, vascular function, and myocardial work function, and correlating the main parameters with circulating microRNAs and MMPs.

The thesis concludes with a chapter of conclusions, highlighting the main results of the study and the new research directions opened by it.

This research is part of a research project supported by the Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-0965, which was conducted interdisciplinary with the Cellular Biology and Pathology Institute 'Nicolae Simionescu' Bucharest. In closing, I would like to thank all those who, through their competence and goodwill, helped in the realization of this work.

I. General part

This first part of the thesis contains four chapters with information on acute myocardial infarction, the role of circulating microRNAs in cardiovascular diseases, the role of MMPs in cardiovascular diseases, and the mechanisms of vascular dysfunction in patients with myocardial infarction.

The first chapter, titled 'Acute Myocardial Infarction with ST-Segment Elevation,' highlights the most important concepts regarding the epidemiology, pathophysiological mechanisms, cardiovascular risk factors, and key diagnostic biomarkers of acute myocardial infarction. Acute myocardial infarction (AMI) remains one of the leading causes of mortality and morbidity worldwide. According to the American Heart Association, one in six patients experiences an acute myocardial infarction every six minutes. The monogenic expression of atherosclerotic disease leads to the occurrence of AMI at a young age and is associated with familial dyslipidemia, homocystinuria, antiphospholipid syndrome, fibromuscular dysplasia, and other rare syndromes. Myocardial ischemia and coronary reperfusion have significant effects, including an intense inflammatory response at the myocardial and vascular levels. The mechanisms by which cardiac cells and the immune system participate in the inflammatory response after a myocardial infarction are not fully understood. The ideal biomarker model for diagnosing acute myocardial infarction needs to be highly expressed in cardiac tissue, have high clinical specificity and sensitivity, and be detected in the blood quickly after the onset of symptoms, most commonly chest pain.[7] Numerous biomarkers are described in the literature, making it useful to classify them into various pathophysiological groups such as myocardial ischemia, myocardial necrosis, inflammation, hemodynamic markers, markers related to angiogenesis, atherosclerosis, or unstable plaques.

The next chapter synthesizes information regarding the implications of circulating microRNAs in cardiovascular pathology. MicroRNAs (miRs) are short, endogenous, non-coding RNAs that dictate gene expression at the post-transcriptional level by binding to the 3'-untranslated region of target mRNAs. Recent studies have shown that microRNAs play an essential role in certain biological processes, such as cellular proliferation or differentiation, as well as apoptosis. Additionally, they are associated with significant pathologies, including cancer and cardiovascular diseases.[8] According to some researchers, over 1,500 microRNAs have been identified in humans, some of which (miR1, miR133, miR206, miR208) are muscle-specific and

are termed myomiRs.[9] Recently, microRNAs have been described as regulators of important pathways, such as cellular adhesion, proliferation, and inflammation, with a central role in the development of atherosclerosis.

The third chapter outlines the main implications of circulating MMPs in cardiovascular diseases. Matrix metalloproteinases (MMPs) are morphologically composed of endopeptidases regulated by inflammatory signals intended to mediate changes in processes occurring at the extracellular matrix level, representing the main group of proteases involved in this biological process. The role of MMPs is extremely important at the pathophysiological level in processes such as embryonic development, morphogenesis, angiogenesis, bone remodeling, or cellular apoptosis. MMPs thus affect vascular remodeling, not only in the overall vascular architecture but also in the process of atherosclerotic plaque development.

In the final chapter of the general part, I addressed the topic of vascular dysfunction in patients with acute myocardial infarction, referring to the main mechanisms of vascular dysfunction and methods of diagnosing it, as well as the role of circulating microRNAs and MMPs in the progression of this pathology. Currently, several non-invasive imaging methods are being evaluated to determine if they can increase the accuracy of cardiovascular risk assessment. Among the most effective are endothelial dysfunction assessment, intima-media thickness, ankle-brachial index, and pulse pressure.[10] This intense inflammatory process involves immune cells, monocytes, and macrophages, endothelial cells that produce vascular remodeling through the contribution of fibroblasts in the adventitial layer, smooth muscle cells in the medial layer, and endothelial cells in the intimal layer, as well as macrophages in the bloodstream. Activation of endothelial cells leads to the release of endothelin-1 with a vasoconstrictor role, elastase which reduces cellular elasticity, and tenascin-C which induces vascular proliferation. The role of circulating microRNAs and MMPs in these vascular processes is not fully understood.[11]

II. Personal contributions

1. The implications of circulating microRNAs on biventricular function in patients with complicated atherosclerotic plaque in the context of acute myocardial infarction and type 2 diabetes mellitus

1.1.Introduction

Myocardial infarction and post-infarction myocardial remodeling pose a high risk of mortality and morbidity. Despite current treatment methods, a significant percentage of patients experience adverse myocardial remodeling and the development of heart failure. Recent studies have highlighted the potential regulatory role of microRNAs in the pathophysiological effects of myocardial infarction, as well as their role as therapeutic targets in many cardiovascular diseases. Currently, the most commonly used biomarkers in the diagnosis of myocardial ischemia are cardiac troponins; however, their detection in peripheral blood is possible only 3-6 hours after symptom onset, making early diagnosis challenging and necessitating repeated testing in the emergency room.[12] The role of the miR30 family, particularly miR30c, has been demonstrated in myocardial injury through the activation of nuclear factor-kB and the reduction of sirtuin-1 synthesis. Additionally, they have a protective role against myocardial ischemia, suggesting they could be potential therapeutic targets for ischemic heart diseases. miR126 is a biomarker of endothelial activation that maintains vascular integrity and endothelial homeostasis by reducing the synthesis of angiogenesis inhibitors, inhibiting the expression of VCAM1 at the endothelial level, and decreasing the production of monocyte chemoattractant protein-1, thereby inhibiting the infiltration of monocytes/macrophages.[8]

One of the main objectives of this study was to quantify the investigated microRNAs (miR30c, miR133, miR29a, miR126, miR146, miR21) as well as MMP1 and MMP9, which are known in the literature for their potential role in cardiovascular diseases, in the serum of patients with ST-segment elevation myocardial infarction (STEMI) and in the serum of patients with chronic coronary syndrome (CCS). Another objective was to correlate these circulating microRNAs and MMPs with key parameters of left and right ventricular function. The final objective was to identify biomarkers with predictive power for the improvement of ventricular function.

1.2. Materials and methods

In the doctoral research, a prospective cohort study was conducted, involving 90 patients at the Cardiology Clinic of the University Emergency Hospital Bucharest. The study was carried out over a period of four years, from 2017 to 2020. The study protocol and all experimental protocols were approved by the Ethics Committee of the University Emergency Hospital Bucharest, and informed consent was obtained from each participant.

At the time of enrollment, all patients underwent evaluations that included Clinical examination and medical history; Resting ECG; 2D conventional echocardiography, tissue Doppler, and 2D Speckle Tracking; 3D echocardiography; Biological tests.

One year later, the patients were re-evaluated using the same methods: Resting ECG; 2D conventional echocardiography, tissue Doppler, and 2D Speckle Tracking; 3D echocardiography. For the statistical analysis, the following software was used: SPSS version 26; Python 3; Microsoft Excel 365. The statistical methods applied included: T-test for two independent samples; Pearson's correlation coefficient; Logistic regression; ROC curves for prediction.

1.3.Results

The 90 enrolled patients were divided into two groups: one group with STEMI (Group 1) and another group with chronic coronary syndrome (CCS) (Group 2). Within Group 1, the patients were further categorized into those with STEMI and diabetes and those with STEMI without diabetes.

In the entire cohort of 90 patients, there was a predominant distribution of male cases, with 68% of the patients being men (n=61) and 32% being women (n=29). The average age of the patients across the entire cohort was 59 years, with a mean error of 1.3 years (standard deviation: 12.641) (Fig. 1.1).

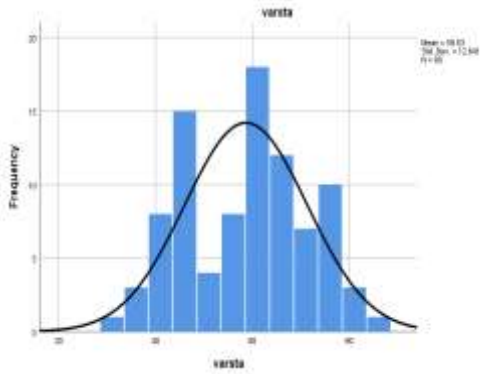


Fig. 1.1 . – The age distribution of the entire cohort analysis

In the cohort of patients with STEMI and diabetes mellitus, the average age was 60 years ± 11.0. In the STEMI cohort without diabetes, the average age was 54 years ± 14.8. Meanwhile, in the cohort with chronic coronary syndrome (CCS), the average age of the patients was 61 years ± 10.6 (Fig. 1.2).

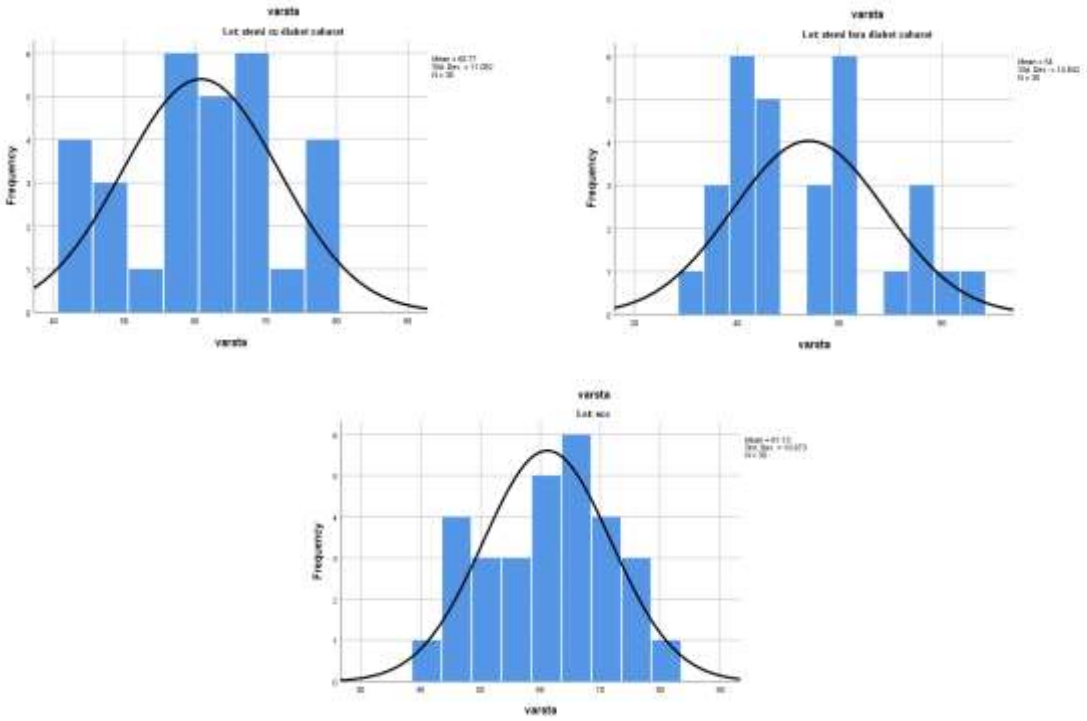


Fig. 1.2. – The distribution of patients by age in the STEMI with diabetes, STEMI without diabetes, and CCS groups

The analysis of the main risk factors revealed that 63% of the total patients were non-smokers, 81% had essential hypertension, 42% had type 2 diabetes, and 92% had dyslipidemia. Regarding the patients' baseline medication, 42% were not receiving any treatment before hospitalization, and only 29% of the total patients were on standard treatment with beta-blockers, statins, and ACE inhibitors for risk factor control (Fig. 1.3).

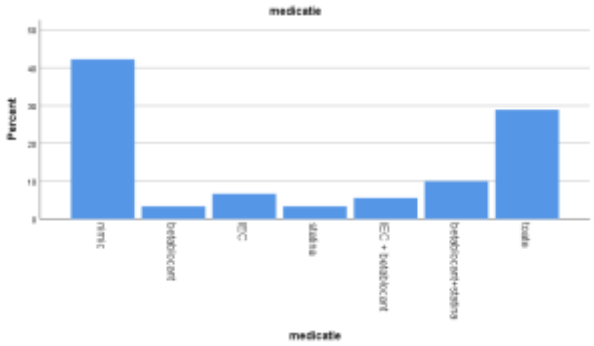


Fig. 1.3. – The distribution of patients by home treatment in the overall cohort

Regarding the evaluation of parameters at one year, statistical analysis revealed a uniform distribution of age and body mass index both in the overall cohort and in each studied subgroup, with patients remaining overweight in both the STEMI and CCS groups.

From the evaluation of standard parameters using the T-test, a statistically significant difference was found between ejection fraction (EF) measured by conventional echocardiography (p=0.0001), radial strain (p=0.01), longitudinal strain (p=0.0001), longitudinal strain rate (p=0.004), detorsion rate (p=0.03), systolic volume measured by 3D echocardiography (p=0.02), cardiac output determined by 3D echocardiography (p=0.001), and EF determined by 3D echocardiography (p=0.0001) between STEMI patients and CCS patients (Fig 1.4).

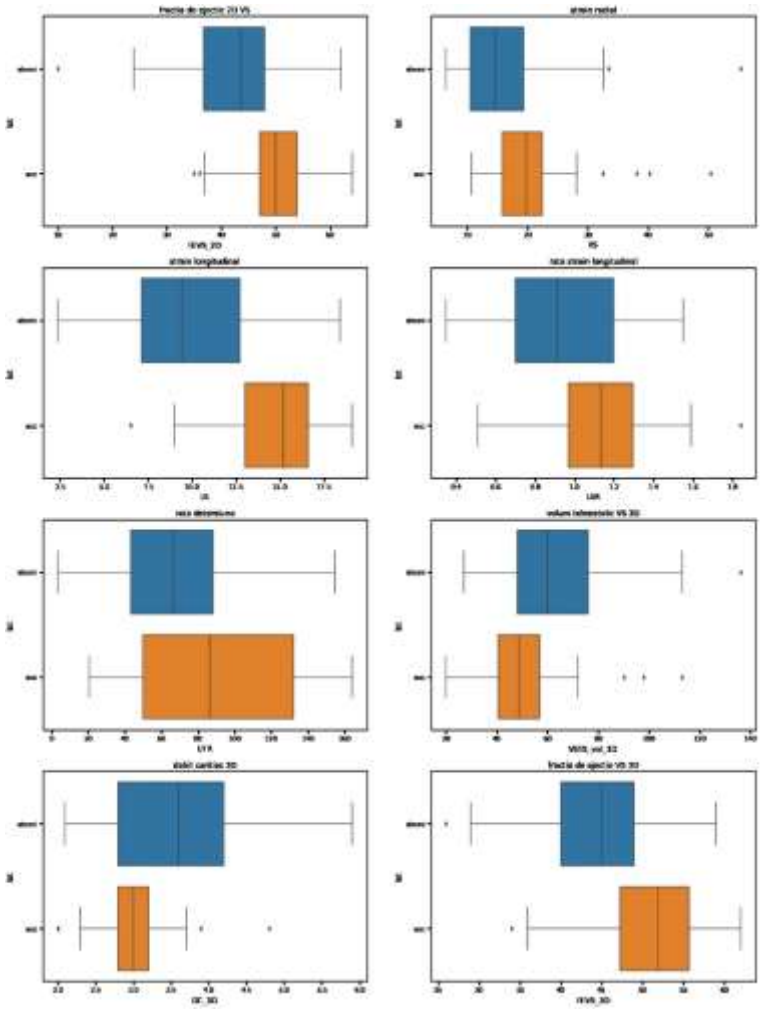


Fig. 1.4– Comparison of Statistically Significant Left Ventricular Function Parameters in STEMI and CCS Patients at Baseline

From the evaluation of standard parameters, using the Independent T-test and Levene's test, it was found that both the VD diameter and longitudinal VD strain are significantly lower in STEMI patients compared to those with CCS (t=-2.46, p=0.01, and t=-3.08, p=0.003, respectively) (Fig 1.5).

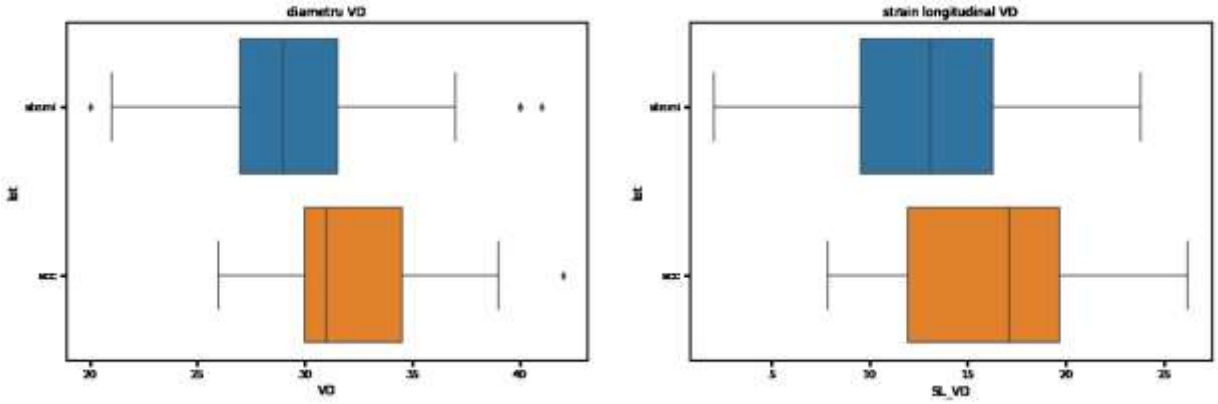


Fig. 1.5. Comparison of Right Ventricular Function Parameters in STEMI and CCS Patients at Baseline.

The assessment of circulating microRNAs and MMPs between the two groups using T-test and Levene's test revealed significantly higher differences in microRNAs in the STEMI group (Fig. 1.6). Furthermore, MMP1 and MMP9 levels were significantly higher in the STEMI group compared to the CCS group, with an average value for MMP1 of 3.8±3.4 versus 1.7±1.03 (t=4.3, p=0.0001), and for MMP9 an average value of 302.9±135.1 versus 240.4±89.4 (t=2.56, p=0.01). Comparative analysis of microRNAs did not show statistically significant differences for miR30c (t=1.37, p=0.17) and miR126 (t=1.49, p=0.14) between the two studied groups.

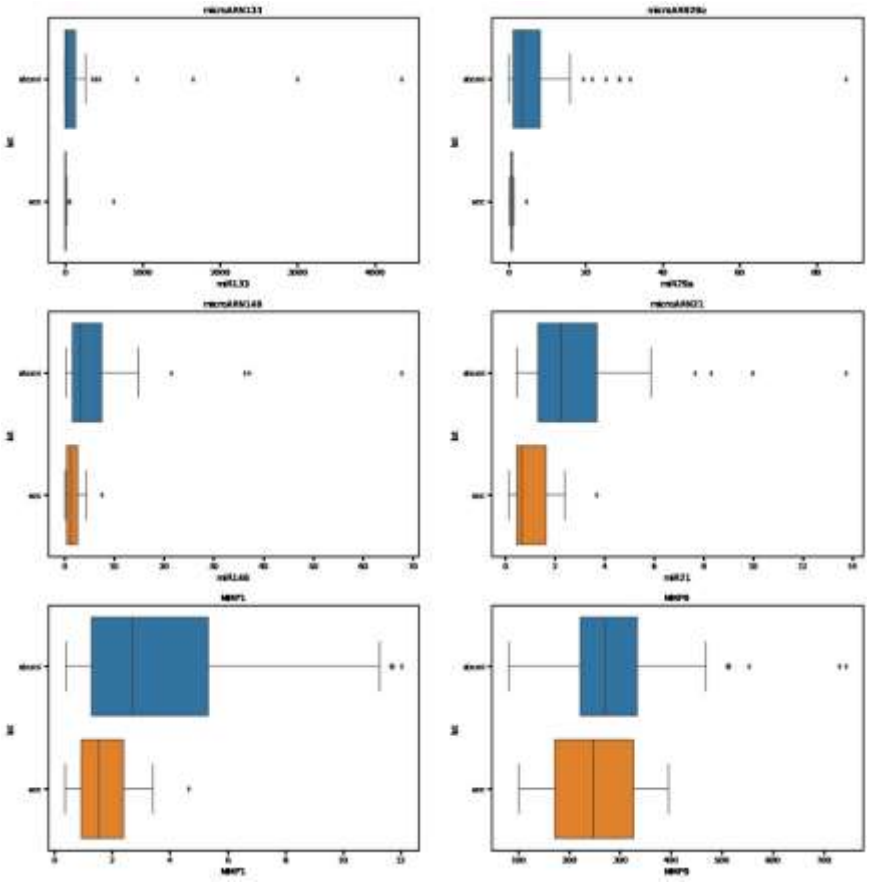


Fig. 1.6. Statistically Significant Comparisons of Circulating microRNAs and MMPs between STEMI and CCS Patients at Baseline

Using Pearson correlation tests, we evaluated the main parameters of left ventricular function and their correlation with circulating microRNAs and MMPs in the overall cohort. Both miR133, miR29, miR126, miR21, and MMP1 and MMP9 were found to be correlated with ventricular function parameters and myocardial deformation.

The extensive analysis of left ventricular function parameters and their correlation with circulating microRNAs and MMPs is illustrated in the heat map below, where stronger Pearson correlations are represented by more intense colors (Fig. 1.7).

Regarding the analysis of right ventricular function parameters at baseline, no statistically significant correlations were found for circulating microRNAs or MMPs, except for miR29a, which negatively correlated with TAPSE value (R=-0.29, p=0.02) (Fig.1.8).

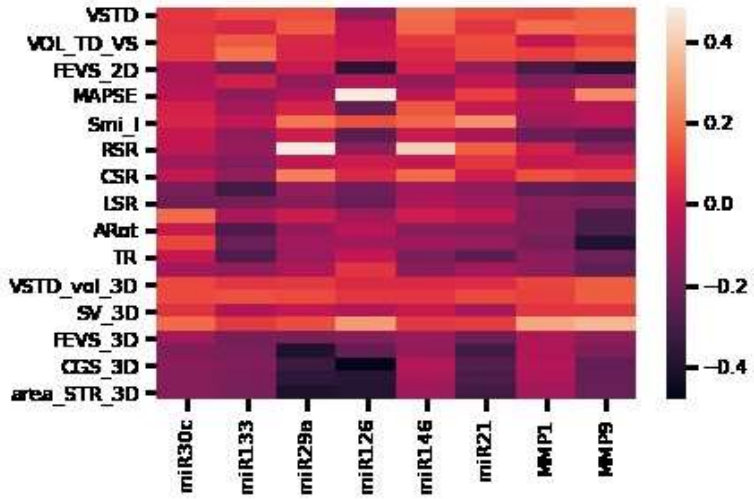


Fig. 1.7. Pearson Correlation Heat Map of Left Ventricular Function Parameters with Circulating MicroRNAs and MMPs in the Overall Cohort at Baseline

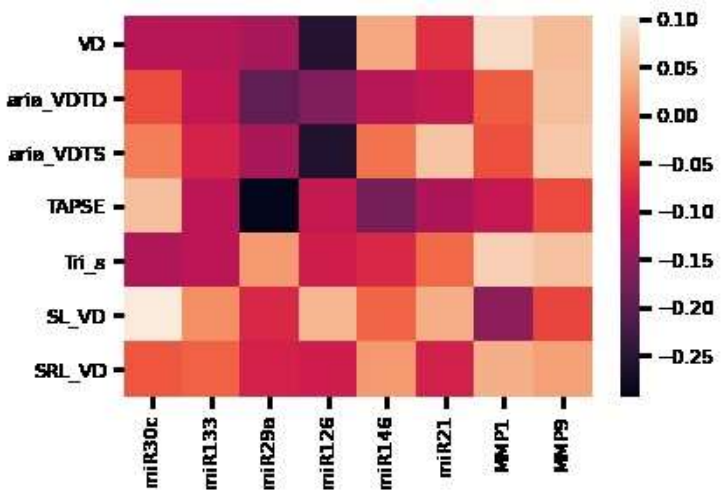


Fig. 1.8. Pearson Correlation Heat Map of Right Ventricular Function Parameters with Circulating MicroRNAs and MMPs in the Overall Cohort at Baseline

We then analyzed the correlation of circulating microRNAs and MMPs within each group using Pearson correlation tests, and obtained the following data. These results are shown in Figure 1.9.

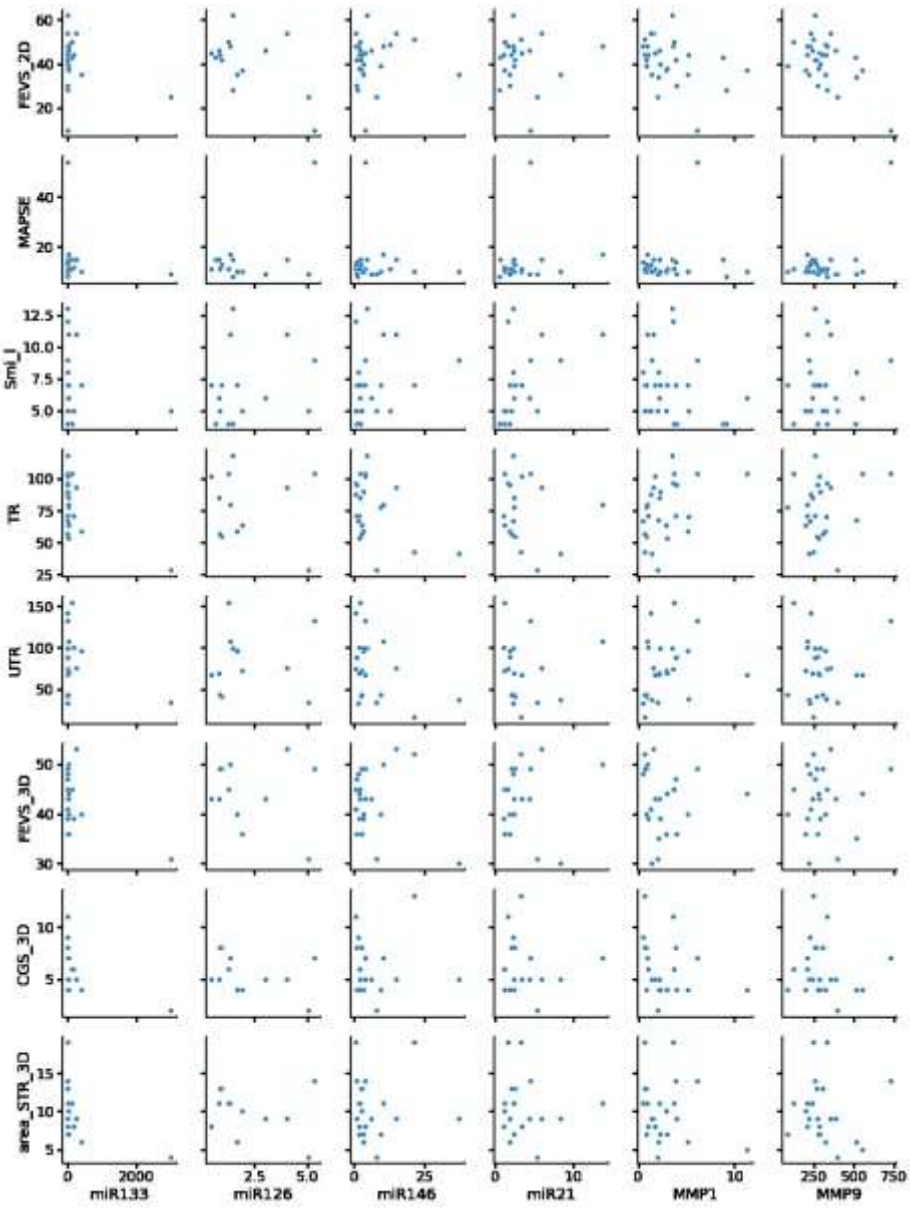


Fig 1.9. Statistically significant correlations of ventricular function parameters with microRNAs and MMPs in patients with STEMI and diabetes mellitus at baseline

The evaluation of ventricular function parameters at 1 year involved correlating circulating microRNAs and MMPs using Pearson correlation tests, yielding the following results. **Circulating microRNAs**: Significant correlations were observed with miR133 and miR146 for ventricular function and myocardial deformation parameters. No statistically significant correlations were found for microRNAs miR30c, miR126, and miR21 with left ventricular function parameters. **Circulating MMPs**: At 1 year, MMP1 showed a negative correlation with left ventricular short-

axis fraction (R=-0.28, p=0.02), while MMP9 showed a negative correlation with left ventricular ejection fraction (R=-0.28, p=0.01). An extensive analysis of the left ventricular function parameters and their correlation with circulating microRNAs and MMPs at 1 year is illustrated in the heat map below (Fig. 1.10).

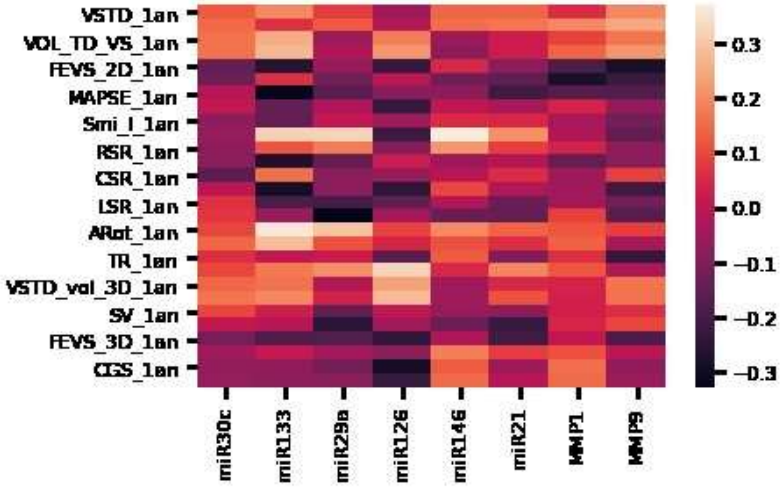


Fig. 1.10. Heat Map of Pearson Correlations of Left Ventricular Function Parameters with Circulating microRNAs and MMPs in the General Cohort at 1 Year

From the evaluation of right ventricular function parameters at 1 year, it was found that both miR29a and miR21 had negative correlations with TAPSE (R=-0.36, p=0.01 and R=-0.32, p=0.02, respectively). Additionally, miR21 showed a negative correlation with the right ventricular end-diastolic area (R=-0.32, p=0.03) (Fig. 1.11).

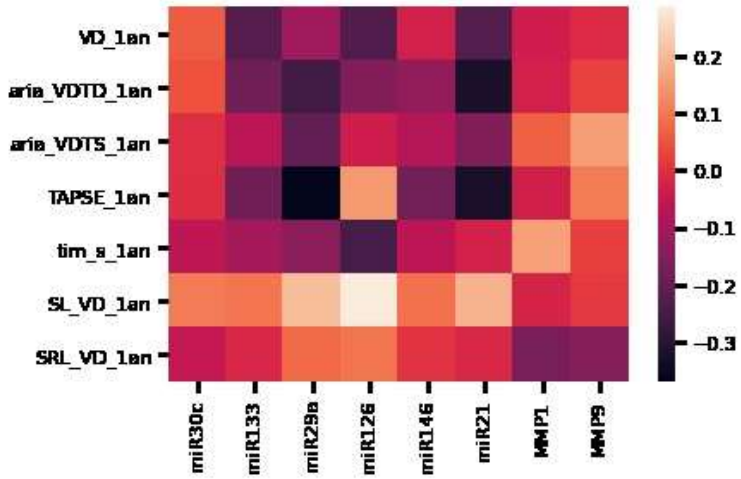


Fig. 1.11. Heat Map of Pearson Correlations of Right Ventricular Function Parameters with Circulating microRNAs and MMPs in the General Cohort at 1 Year

Using logistic regression models, including the Omnibus test for model coefficients, Nagelkerke R^2, and the Hosmer-Lemeshow test, the predictive power of each analyzed microRNA and MMP for myocardial deformation parameters was evaluated.

In the cohort analysis, miR133 showed high predictive value for increased radial strain measured by 3D echocardiography at 1 year in STEMI patients, with an AUC of 0.8, p=0.006, 95% CI 0.638 – 0.962, a threshold value of 3.22, sensitivity of 95%, and specificity of 90% (Fig. 1.12). However, in the SCC group, the predictive value of miR133 was not statistically significant for increased radial strain 3D (AUC=0.51, p=0.93, 95% CI 0.236-0.786) (Fig. 1.13).

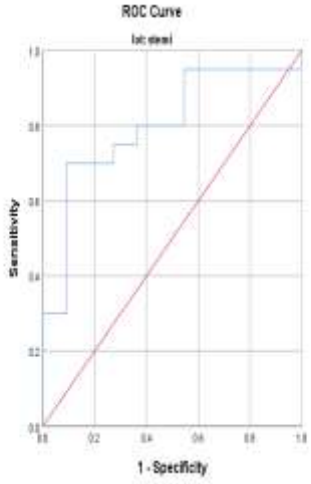


Fig. 1.12 – ROC curve of prediction the raise of SR 3D in STEMI by miR133

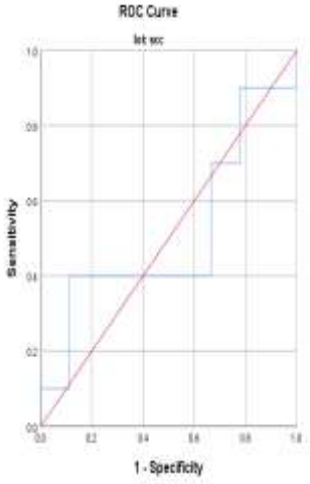


Fig. 1.13 – ROC curve of prediction the raise of SR 3D in CCS by miR133

1.4. Discussions

In this study, we demonstrated that ejection fraction (EF) measured both by 2D and 3D echocardiography, cardiac output, and myocardial deformation parameters (radial strain, longitudinal strain, longitudinal strain rate, and untwist rate) are significantly reduced in patients with STEMI compared to those with chronic coronary syndrome (CCS). This finding is also true for right ventricular function parameters, where both the diameter and longitudinal strain of the right ventricle were lower in patients with acute myocardial infarction.

Regarding the analysis of circulating microRNAs and MMPs, our results show that the expression levels of circulating miR133, miR146, miR29a, and miR21 are significantly higher in

patients with STEMI compared to those with CCS. Additionally, higher levels of miRNA126 and miR133 are associated with lower EF and reduced myocardial deformation parameters in STEMI patients compared to those with chronic atherosclerotic disease.[13][14][15]

The limitations of our study include the relatively small number of patients. The analysis was performed only at baseline and one year, necessitating further follow-up to establish the potential diagnostic and prognostic value of these biomarkers. We did not include a control group, as we did not anticipate any correlations in normal subjects at this stage; our aim was to establish the relationship of these biomarkers with ventricular function parameters.

This research highlights the significance of elevated levels of miR21, miR126, miR146, miR30c, miR29a, and miR133, as well as MMP1 and MMP9, which could be considered potential candidate biomarkers for the early diagnosis of acute myocardial infarction and for assessing left and right ventricular systolic dysfunction, as well as plaque vulnerability.

1.5.Conclusions

Circulating microRNAs, as well as MMP1 and MMP9, are potential biomarkers of ventricular function in patients with STEMI, correlating with myocardial deformation parameters and showing potential predictive roles. Circulating microRNAs are promising biomarkers for the early diagnosis and prognosis of acute myocardial infarction. However, further studies are needed to establish their role in cardiovascular diseases.

2. The implications of circulating microRNAs on atrial function in patients with complicated atherosclerotic plaque in the context of acute myocardial infarction and type 2 diabetes mellitus

2.1.Introduction

As previously outlined, the molecular and cellular mechanisms leading to cardiac remodeling following an acute myocardial infarction involve changes in cardiac myocyte function and the extracellular matrix (ECM).[16][17] ECM is a dynamic network of structural fibers and proteins, including fibrillar collagen, proteoglycans, and glycosaminoglycans, which regulates cellular morphology, differentiation, migration, and proliferation.[18] Matrix metalloproteinases (MMPs) are proteolytic enzymes found in the myocardium that are closely associated with myocardial remodeling and ECM metabolism regulation.[19] They are regulated at the

transcriptional level by endogenous physiological inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs).[20] Several studies have highlighted the importance of MMPs, particularly MMP-1 and MMP-9, in left ventricular remodeling and scar progression after myocardial infarction, and in the development of heart failure.[21] In this study, by examining the expression of microRNAs and MMPs in the left atrial myocardial structure in coronary artery disease, we aim to determine the relationship between miR30c, miR133, miR29a, miR126, miR146, and miR21, as well as MMP-1 and MMP-9, with structural remodeling and atrial function.

2.2. Materials and methods

For this research project, we prospectively followed 90 patients at the Cardiology Clinic of the Bucharest Emergency University Hospital over a period of 4 years, from 2017 to 2020. The patients were divided into 2 groups: 60 patients in the STEMI group (Group 1) and 30 patients in the chronic coronary syndrome (CCS) group (Group 2).

The study protocol and all experimental protocols were approved by the Ethics Committee of the Bucharest Emergency University Hospital, and informed consent was obtained from each participant. All patients were evaluated using conventional 2D echocardiography, tissue Doppler, 2D Speckle Tracking, 3D echocardiography, and biological samples.

Statistical analysis was performed using SPSS version 26, Python 3, and Microsoft Excel 365. To test differences between the means of two independent samples, it is necessary to test the homogeneity of variances a priori, as the value of the t-test is calculated differently depending on whether the assumption of equal variances is accepted or not. The test used for this purpose is Levene's test. If the significance value for this test is greater than 0.05, then the variances are considered equal and the result for the t-test is interpreted using the line corresponding to the conclusion of Levene's test.

2.3.Results

The descriptive analysis of the standard parameters of left and right atrial function shows a uniform distribution of patients based on diameter in both the general cohort at baseline (mean diameter for LA: 32.3±4.5, and for RA: 33.4±4.5) and at 1 year (mean diameter for LA: 33.8±4.7,

and for RA: 34.2±4.1) (Fig. 2.1 and Fig. 2.2), as well as in the subgroup analysis.

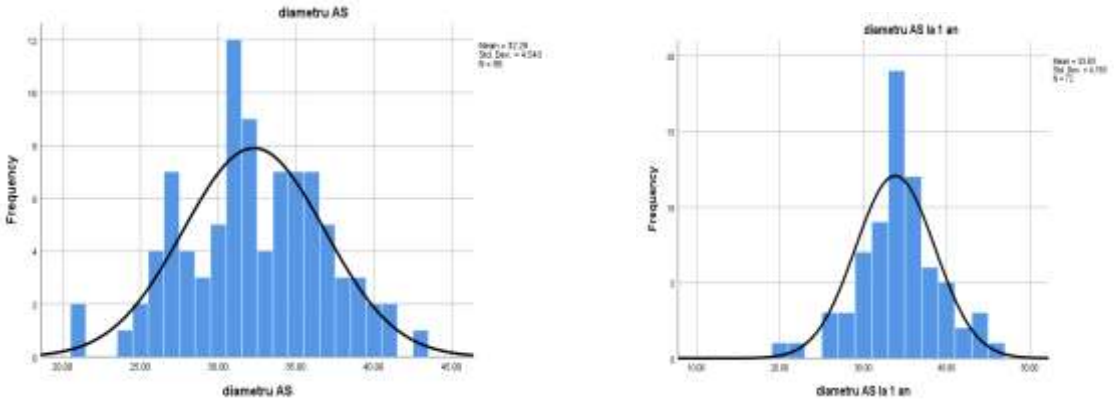


Fig. 2.1 Distribution of patients based on LA diameter at baseline and at 1 year

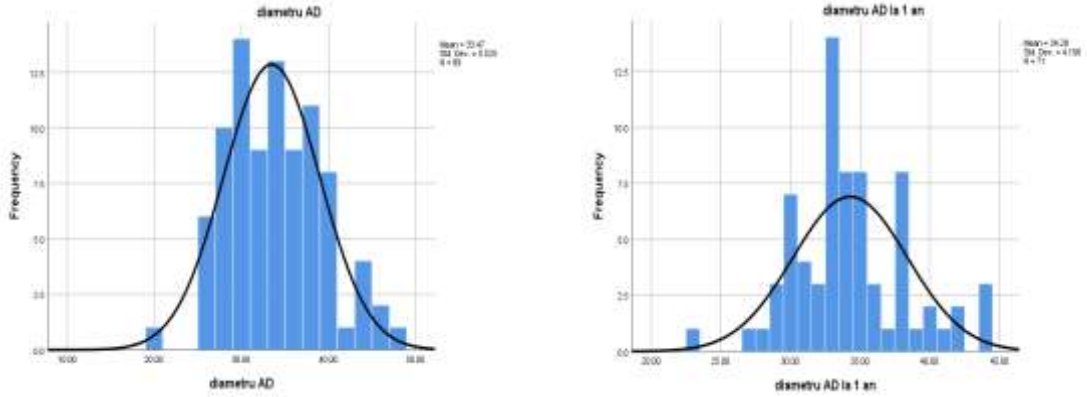


Fig. 2.2. Distribution of patients based on RA diameter at baseline and at 1 year

Using T-tests and Levene's test, we obtained the following statistically significant results between the two groups at baseline (Fig. 2.3). For the standard parameters of left atrial function assessment, there were significant differences between the expansion index, which was lower in the STEMI group compared to the SCC group (t=-2.55, p=0.01), active ejection fraction, which was also lower in the STEMI patients compared to those with SCC (t=-2.63, p=0.01), and total ejection fraction, which was reduced in the STEMI group (t=-2.50, p=0.01). The analysis of right atrial function revealed that positive RA strain, global RA strain, as well as negative strain rate and positive strain rate had significantly higher values in the STEMI group when assessed at baseline.

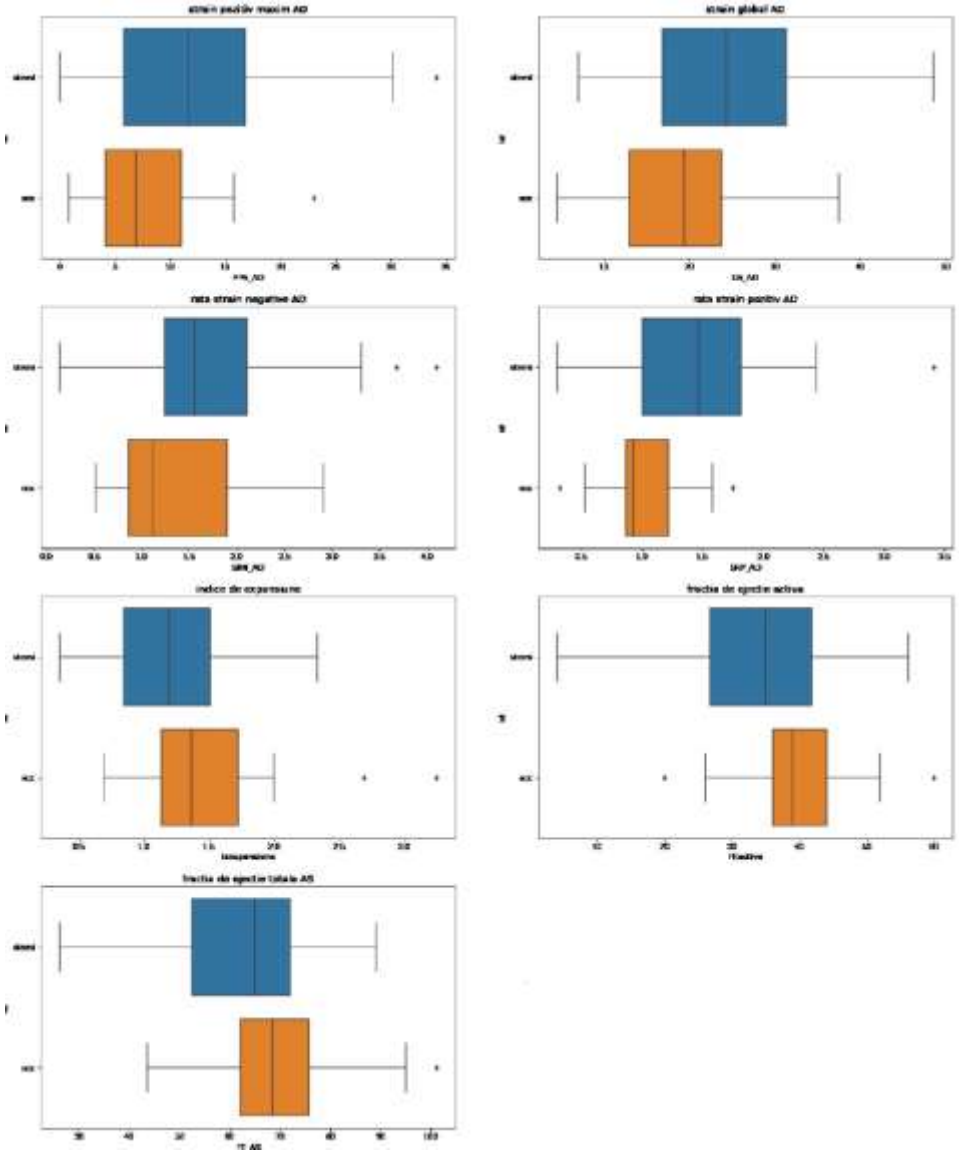


Fig. 2.3. – Comparison of Statistically Significant Left and Right Atrial Function Parameters between STEMI and SCC Patients at Baseline

Using Pearson Correlation Tests, the evaluation of the left atrium at baseline revealed a correlation between miR126 and the maximum positive strain of the left atrium (R=-0.40, p=0.01), with no other miRs showing correlations with left atrial function parameters. Regarding circulating MMPs, significant correlations were observed for both MMP1 and MMP9. The extensive analysis of left atrial function parameters and their correlation with microRNAs and circulating MMPs is illustrated in the heat map below, where stronger Pearson correlations are indicated by more intense shading (Fig. 2.4).

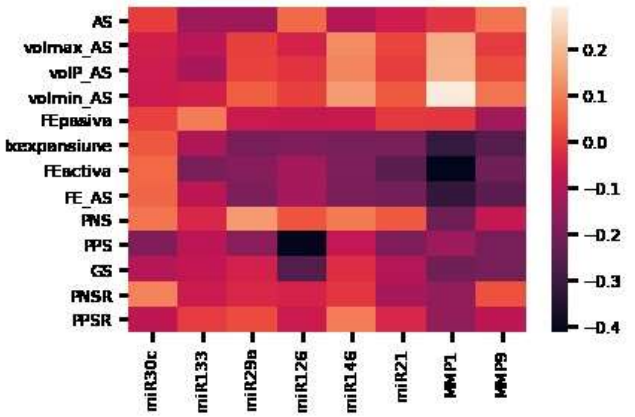


Figura 2.4. Pearson Correlation Heat Map of Left Atrial Function Parameters with Circulating MicroRNAs and MMPs in the General Cohort at Baseline

The analysis of right atrial function parameters at baseline revealed statistically significant correlations of microRNAs with atrial deformation parameters. Specifically, miR133, miR30c, miR146, and miR126 were found to correlate with atrial deformation parameters. Additionally, MMP9 exhibited significantly higher values associated with global strain and maximal negative strain rate (R=0.22, p=0.03, and R=0.26, p=0.01, respectively) (Fig. 2.5).

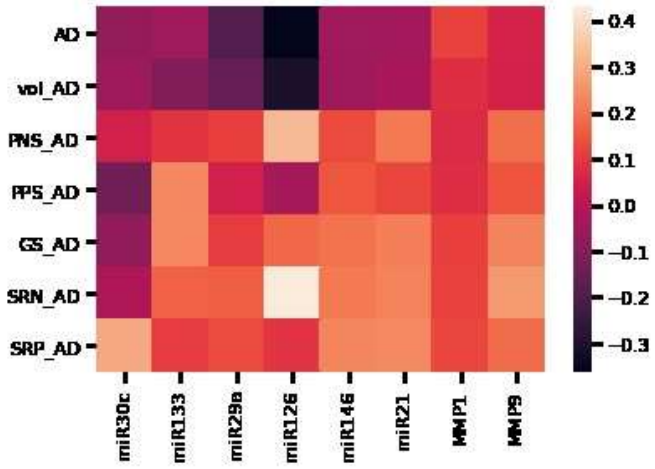


Figura 2.5. Heat Map of Pearson Correlations for Right Atrial Function Parameters with Circulating MicroRNAs and MMPs in the General Cohort at Baseline

We subsequently analyzed the correlation of circulating microRNAs and MMPs in each group using Pearson correlation tests and obtained the following results.

In the group of STEMI patients with diabetes, **miR30c** was positively correlated with the maximum positive strain rate of the right atrium (R=0.40, p=0.04). No other microRNAs showed statistically significant correlations with the left or right atrial function parameters. On the other hand, **MMP1** exhibited positive correlations with the diameter of the left atrium (R=0.37, p=0.04) and the minimum volume of the left atrium (R=0.38, p=0.04). Additionally, it was negatively correlated with the maximum positive strain rate of the left atrium (R=-0.42, p=0.02), and positively correlated with both the diameter (R=0.37, p=0.04) and volume (R=0.44, p=0.01) of the right atrium (Fig. 2.6).

In the group of STEMI patients without diabetes, the only microRNA associated with atrial function was **miR21**, which was negatively correlated with the active ejection fraction of the left atrium (R=-0.48, p=0.01). This microRNA did not show correlations with right atrial function parameters. Regarding MMPs, **MMP1** had higher values in patients with a lower ejection fraction of the left atrium (R=-0.43, p=0.02). **MMP9** was negatively correlated with the expansion index of the left atrium (R=-0.50, p=0.005) and the total ejection fraction of the left atrium (R=-0.50, p=0.005). Conversely, it was positively correlated with the maximum negative strain of the right atrium (R=0.50, p=0.005) and the rate of maximum negative strain of the right atrium (R=0.38, p=0.03) (Fig. 2.7).

Extensive evaluation of atrial function parameters at 1 year across groups revealed that, in the group of STEMI patients with diabetes, **miR133** was negatively correlated with the maximum negative strain rate of the left atrium (R=-0.46, p=0.05). Both **miR29a** and **miR126** had higher values in those with lower maximum positive strain of the left atrium (R=-0.53, p=0.01 and R=-0.60, p=0.04, respectively). Furthermore, **MMP1** showed a significant correlation with the diameter of the right atrium (R=0.45, p=0.04), with no other significant associations with atrial function parameters (Fig. 2.8).

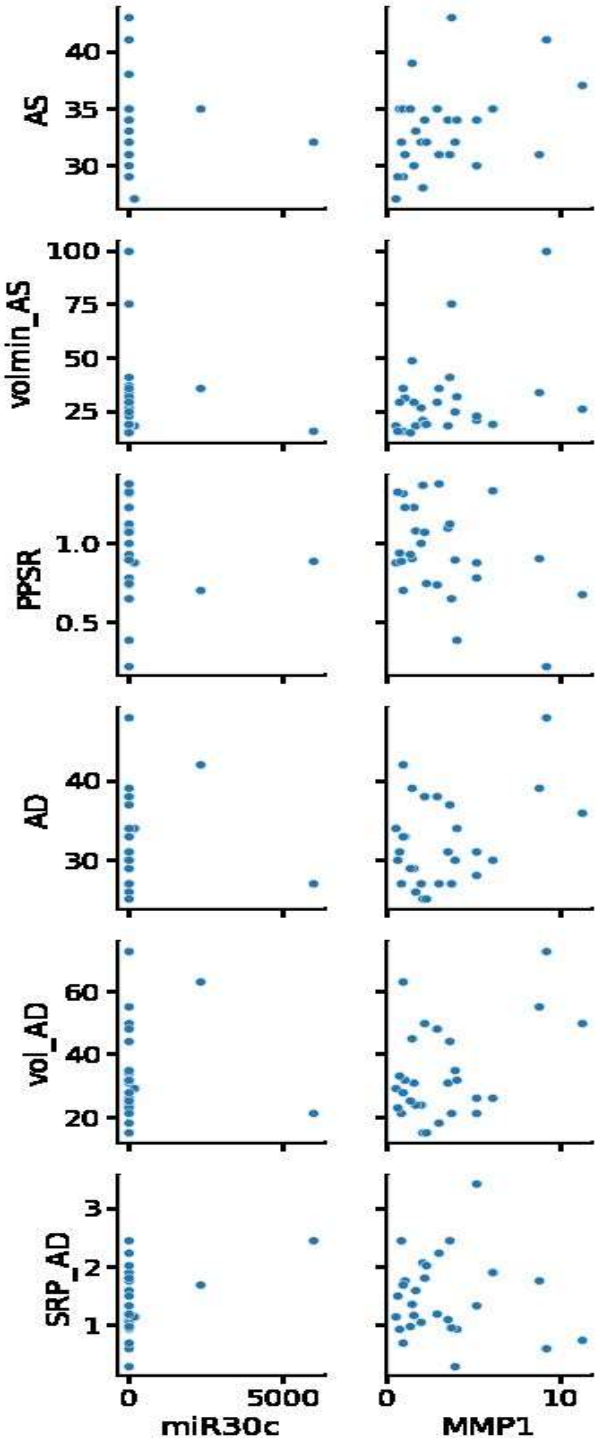


Fig 2.6. Statistically significant correlations of atrial function parameters with microRNAs and MMPs in patients with STEMI and diabetes mellitus at baseline

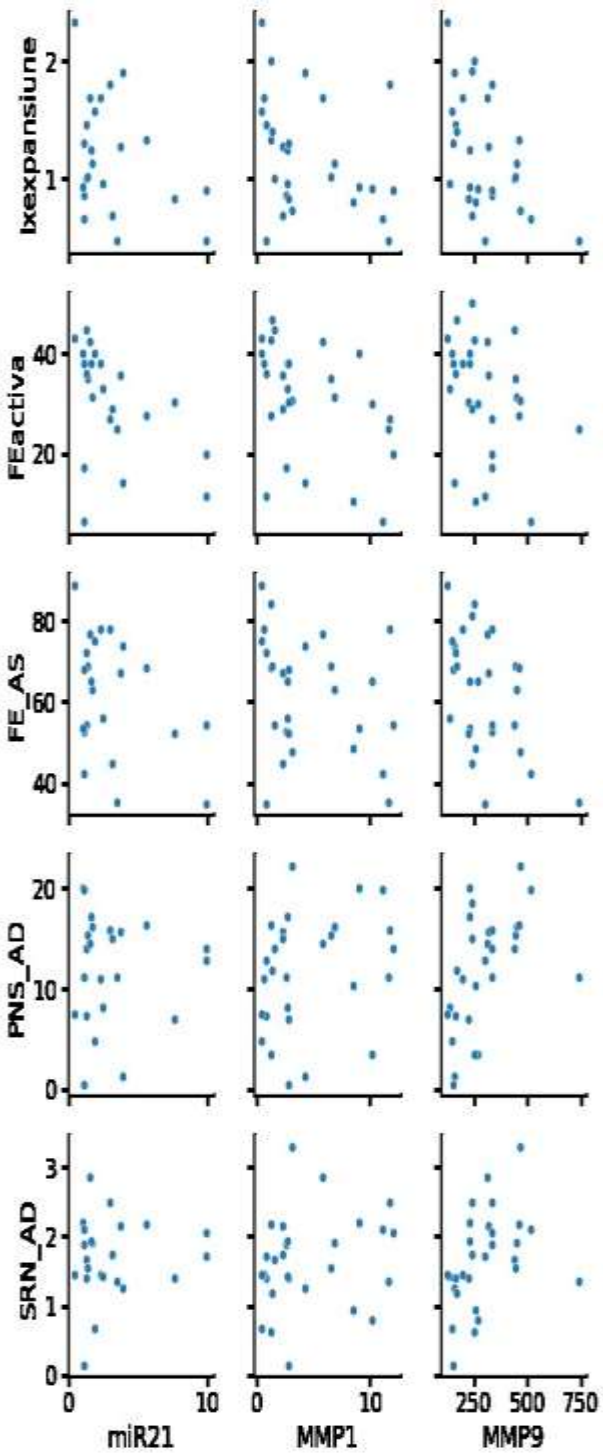


Fig 2.7. Statistically significant correlations of atrial function parameters with microRNAs and MMPs in patients with STEMI without diabetes mellitus at baseline

In the analysis of STEMI patients without diabetes mellitus, the assessment of left atrial function revealed the following results. Reduced values of the active ejection fraction of the left atrium at one year were correlated with higher levels of miR30c (R=-0.60, p=0.006), miR126 (R=-0.68, p=0.02), as well as MMP1 (R=-0.45, p=0.02) and MMP9 (R=-0.43, p=0.03). Additionally, MMP1 was negatively correlated with the total ejection fraction of the left atrium (R=-0.46, p=0.02), while MMP9 was negatively correlated with the expansion index of the left atrium (R=-0.50, p=0.01) and the total ejection fraction of the left atrium (R=-0.54, p=0.005), and positively correlated with the minimum volume of the left atrium (R=0.49, p=0.01). Regarding right atrial function, only a negative correlation of miR133 with the right atrial volume at one year was identified (R=-0.67, p=0.001) (Fig. 2.9).

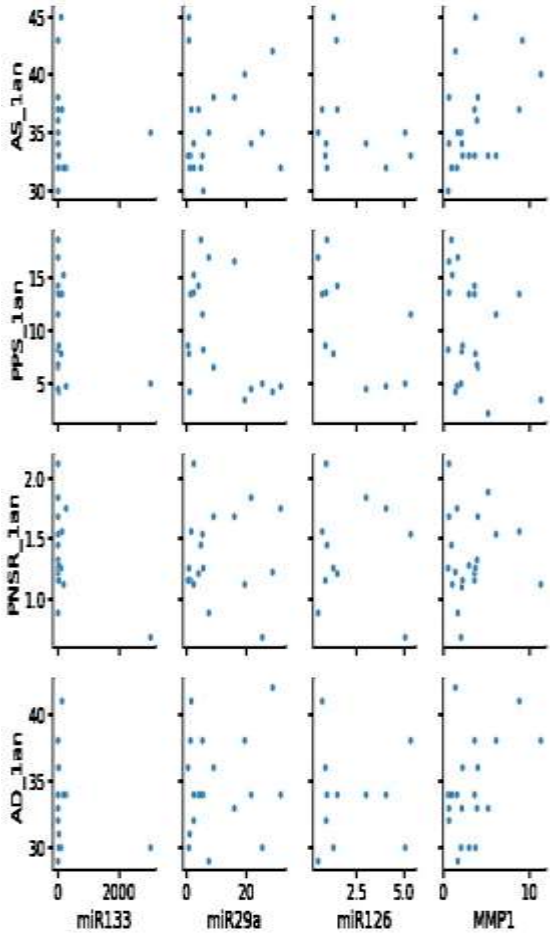


Fig 2.8. Statistically significant correlations of atrial function parameters with microRNAs and MMPs in STEMI patients with diabetes mellitus at one year

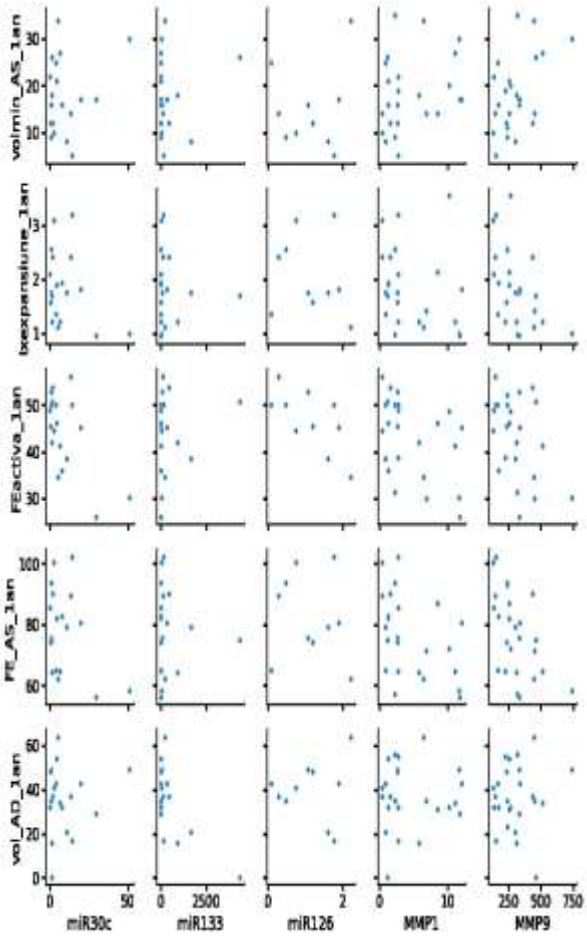


Fig 2.9. Statistically significant correlations of atrial function parameters with microRNAs and MMPs in STEMI patients without diabetes mellitus at one year

Using logistic regression models, including the Omnibus test for model coefficients, Nagelkerke R^2, and the Hosmer-Lemeshow test, the predictive power of each analyzed microRNA and MMP for atrial function parameters was evaluated.

In the general cohort, there were no statistically significant predictors for the improvement of atrial function parameters at one year, neither among the microRNAs nor the MMPs for STEMI patients. However, for patients with SCC, MMP9 emerged as a predictor for increased right atrial volume at one year (AUC 0.902, p=0.03, 95% CI 0.761-1.000), with a cutoff value of 158.65, sensitivity 90%, and specificity 87% (Fig. 2.10). MMP9 did not serve as a predictor for increased right atrial volume in STEMI patients (Fig. 2.11).

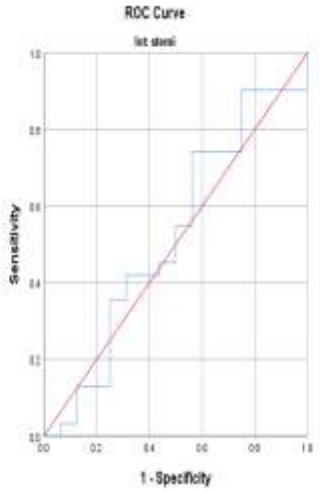


Fig 2.10. The ROC curve for increased right atrial volume at one year in CCS patients by MMP9

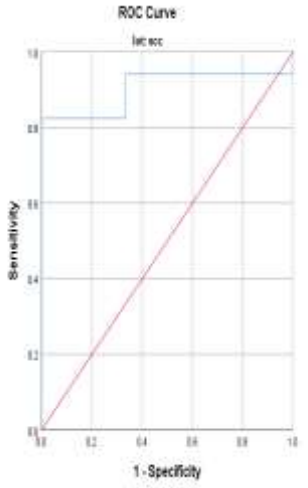


Fig 2.11. ROC Curve for Predicting the Increase in Right Atrial Volume at One Year in STEMI Patients by MMP9

2.4.Discussions

Current studies on the expression of circulating MMPs and microRNAs and their role in cardiovascular diseases focus on their expression levels in serum or using animal models to reproduce MMP and TIMP expression in human myocardium with cardiac diseases.[22] Research has shown that MMPs have been extensively studied as potential markers to predict cardiovascular disease development, particularly post-MI remodeling and heart failure.[23]

It has been demonstrated that MMPs, especially MMP1 and MMP9, are involved in matrix remodeling and atherosclerotic processes following myocardial infarction.[24] MMP1 has the highest affinity for fibrillar collagen, initiating the degradation of collagen fibers in the left atrium (LA) and left ventricle (LV). MMP9 is present in a variety of cell types, including cardiomyocytes, macrophages, endothelial cells, and fibroblasts, and its level increases on day 1, remaining elevated until day 7 after myocardial infarction in mice.[25]

In our study, we demonstrated that the expression levels of microRNAs as well as circulating MMP1 and MMP9 are elevated in the plasma of patients with STEMI compared to those with stable coronary artery disease (CAD). We found that both miR126 levels and serum levels of these MMPs, known for their involvement in remodeling processes, are significantly associated with LA function parameters, including maximum and minimum LA volume, preatrial contraction volume, active and total ejection fraction, as well as deformation parameters such as

positive strain, negative strain, and global strain in patients with STEMI. Regarding the right atrium (RA), miR30c, miR133, miR126, and miR146 were associated with atrial deformation parameters in the general cohort and in patients with CAD. However, no correlations were found between MMPs and LA function parameters in patients with CAD, indicating their important role in the atherosclerotic and inflammatory processes active in acute myocardial infarction, leading to atrial fibrosis and progression to heart failure.

The study has some limitations. The number of patients is relatively small, and the analysis was performed only for baseline parameters and at one year. Long-term follow-up is needed to establish the diagnostic and prognostic potential of these biomarkers, and MMPs should be detected at different time points due to their changes with myocardial infarction progression.

2.5.Conclusions

Circulating microRNAs and MMPs are promising biomarkers involved in atrial remodeling, directly affecting collagen metabolism changes, and are correlated with some of the most important parameters of atrial function and deformation. It is crucial to understand the pathophysiological processes and biological functions in which microRNAs and MMPs are involved to better comprehend their role in developing new therapeutic strategies to limit the progression of heart failure.

3. The role of circulating microRNAs in vascular cells within complicated atherosclerotic plaques in patients with acute myocardial infarction and type 2 diabetes mellitus

3.1.Introduction

Vascular remodeling can lead to endothelial cell dysfunction, activation of fibroblasts and smooth muscle cells, and the production of cytokines and inflammatory substances. These processes are ultimately involved in atherosclerosis through the activation of circulating immune cells and dysregulation of lipid metabolism.[26] The role of circulating microRNAs in these cells is not yet fully understood, but studies suggest their involvement in regulating smooth muscle cells, which are implicated in cell proliferation leading to vascular remodeling, as well as endothelial cells in repair processes or the inflammatory or anti-inflammatory responses of macrophages.[27] However, there are many challenges in modulating these microRNAs and in the

development of antimiR-based therapeutic strategies, such as the loss of microRNA functions through antagomirs or the enhancement of microRNA functions through stimulators.[28]

Matrix metalloproteinases (MMPs) also play a crucial role in vascular remodeling, being involved in various cellular processes, including proliferation, migration, cell differentiation, as well as tissue invasion and vascularization.[29] The stimulation of MMP activity leads to extracellular matrix degradation and activation of cellular and intracellular signaling pathways, which, together with the proliferation of vascular smooth muscle cells, contribute to endothelial injury and lipid accumulation, ultimately leading to atherosclerotic plaque formation.[30]

The objectives of this study, by examining the expression of microRNAs and MMPs in the vascular structure in atherosclerotic disease, were to determine the relationship between miR30C, miR133, miR29a, miR126, miR146, and miR21, as well as MMP-1 and MMP-9, and vascular remodeling and vascular function parameters, and also to identify potential predictors for post-myocardial infarction vascular function.

3.2.Materials and methods

In this research project, 90 patients were prospectively followed at the Cardiology Clinic of the Bucharest Emergency University Hospital. The patients were divided into three groups: those with ST-elevation myocardial infarction (STEMI) with type 2 diabetes mellitus, those with STEMI without type 2 diabetes mellitus, and those with chronic coronary syndrome (CCS) with or without diabetes. They were monitored over a period of three years.

The study protocol and all experimental protocols were approved by the Ethics Committee of the Bucharest Emergency University Hospital, and informed consent was obtained from each research participant, with all determinations carried out in accordance with current guidelines and regulations.[31]

Biological markers were collected within the first 24 hours of admission for the coronary event and included measurements of miR30c, miR133, miR29, miR126, miR146, miR21, MMP1, MMP9, as well as conventional myocardial injury markers such as troponin I. Sample collection and RNA extraction were performed for each patient within 24 hours of admission using approximately 5 ml of EDTA blood.

High-resolution vascular ultrasound is the standard method for evaluating both morphological and functional changes associated with vascular remodeling through intima-media

thickness and arterial stiffness parameters.[32] For assessing arterial stiffness parameters, an Aloka SSD 5500/Prosound α10 device (Tokyo, Japan) was used. Measurements were taken as the average of 5 cardiac cycles. The following parameters were obtained: intima-media thickness (IMT) measured according to current standards, PWV-E (pulse wave velocity "single point"), β (beta stiffness index), Ep (Young's modulus of stiffness), arterial compliance (AC), and arterial expansion index (AI-E, %). [32] Additionally, arterial function was evaluated through the cardio-ankle vascular index (CAVI) and PWV for subclinical atherosclerosis, and the ankle-brachial index (ABI) for peripheral arterial disease. These were obtained for all patients after 5 minutes of rest in the supine position using the VaSera CAVI device (Fukuda Denshi, Tokyo, Japan), which includes electrocardiography, phonocardiography, and mechanocardiography functions. CAVI is a new index that defines the stiffness of the aorta, femoral artery, and tibial artery, independent of blood pressure.[33]

Statistical data analysis was performed using SPSS version 26 (SPSS, Inc, Chicago, Illinois), Python 3, and Microsoft Excel 365. Correlations between two parameters were conducted using univariate Pearson correlation and multiple linear regression analysis using the Hosmer-Lemeshow test. Similar to the t-test for two populations, a Sig. value below 0.05 indicates statistical significance.

3.3.Results

The mean value of CAVI at baseline in the group of patients with STEMI and diabetes mellitus was 9.85 ± 1.92 for CAVI-R, and 10 ± 2.1 for CAVI-L (Fig. 3.1). In patients with STEMI without diabetes mellitus, the values were 8.75 ± 1.8 for CAVI-R and 8.7 ± 1.4 for CAVI-L (Fig. 3.2).

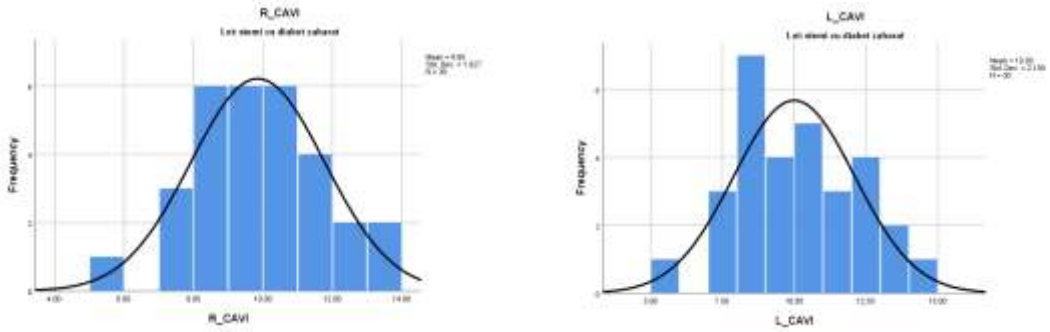


Fig. 3.1. Distribution of patients based on RCAVI and LCAVI in the STEMI group with diabetes mellitus

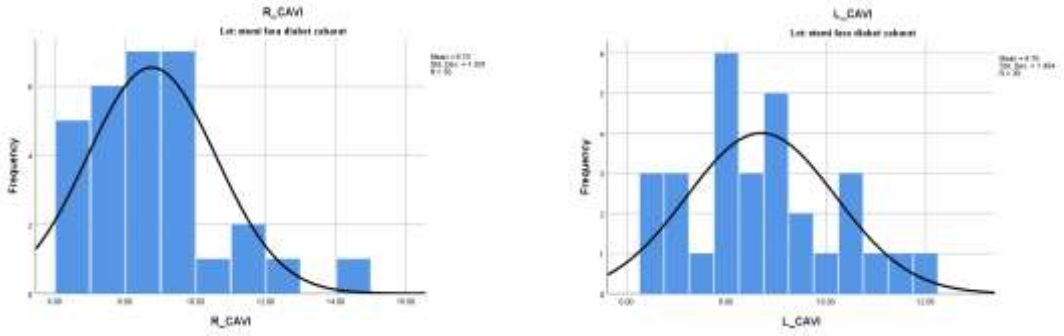


Fig. 3.2. Distribution of patients based on RCAVI and LCAVI in the STEMI group without diabetes mellitus

Using T-tests and Levene's tests, we obtained the following statistically significant results between the two groups at baseline: the values for secondary vascular peak and LIGB were lower in the STEMI group compared to the CCS group (t=-2.61, p=0.01, and t=4.68, p=0.03, respectively) (Fig. 3.3).

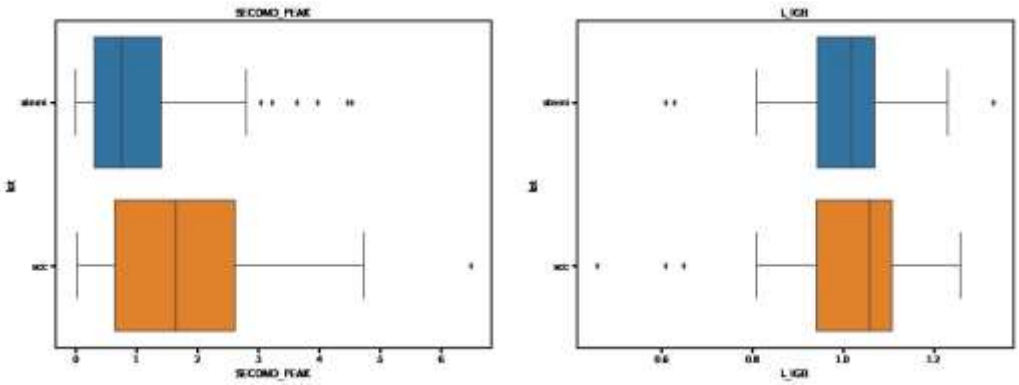


Fig. 3.3. – Comparison of Statistically Significant Vascular Function Parameters Between STEMI and CCS Patients at Baseline

Using Pearson correlation tests, we evaluated the primary vascular function parameters and their correlation with circulating microRNAs and MMPs in the general cohort. Thus, there were statistically significant correlations of the augmentation index with microRNA30c, a positive correlation (R=-0.25, p=0.04), and additionally, there were positive correlations in the general cohort between RCAVI and MMP9 (R=0.21, p=0.05) and between LCAVI and microRNA126 (R=0.32, p=0.04). An extensive analysis of vascular function parameters and their correlation with

microRNAs and circulating MMPs can be found in the heat map below, where the stronger the Pearson correlation, the more intense the shading of the graph (Fig. 3.4).

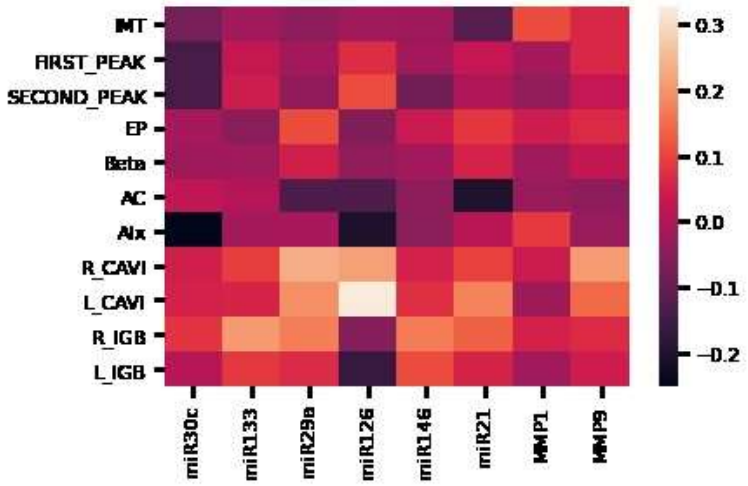


Figura 3.4. Heat map of Pearson correlations of vascular function parameters with circulating microRNAs and MMPs in the general cohort at baseline

We subsequently analyzed the correlation of circulating microRNAs and MMPs in each cohort using Pearson correlation tests, yielding the following results. In the cohort of patients with STEMI associated with diabetes mellitus, only miR29a showed a significant positive correlation with RCAVI (R=0.56, p=0.004), with no significant correlations for other microRNAs or circulating MMPs (Fig. 3.5).

In the cohort of patients with STEMI without diabetes mellitus, evaluated at baseline, higher values of miR126 were associated with increased values of EP (Young's modulus of rigidity) and beta (beta index of rigidity), with significant results R=0.82, p=0.002, and R=0.86, p=0.001, respectively. Among the circulating MMPs, MMP9 had a positive correlation with Young's modulus of rigidity (R=0.37, p=0.04) (Fig. 3.6).

The assessment of vascular function parameters at 1 year was conducted by correlating circulating microRNAs and MMPs using Pearson correlation tests, yielding the following results. In the general cohort, no statistically significant correlations were found between microRNAs or circulating MMPs and CAVI or ABI parameters, as shown in the heat map below (Fig. 3.7).

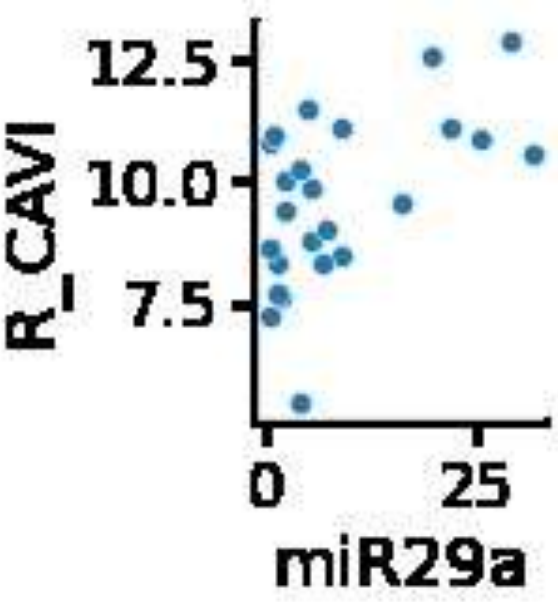


Fig 3.5. Statistically significant correlation of miR29a with RCAVI in patients with STEMI with diabetes mellitus at baseline

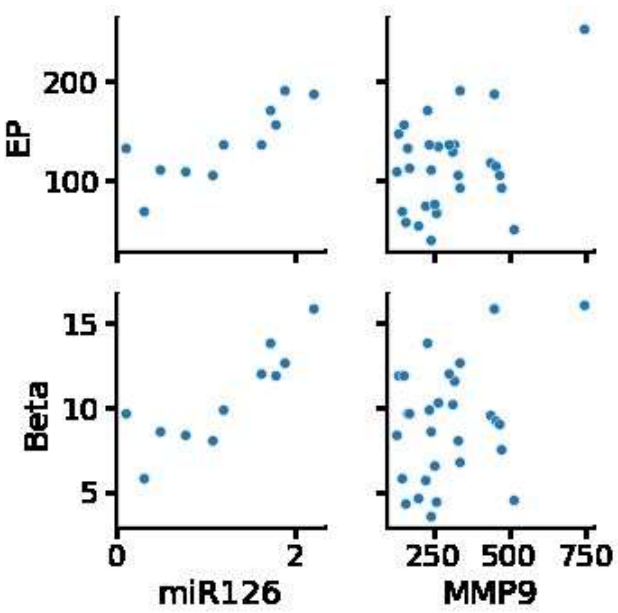


Fig 3.6. Statistically significant correlations of miR126 and MMP with beta and EP indices in patients with STEMI without diabetes mellitus at baseline

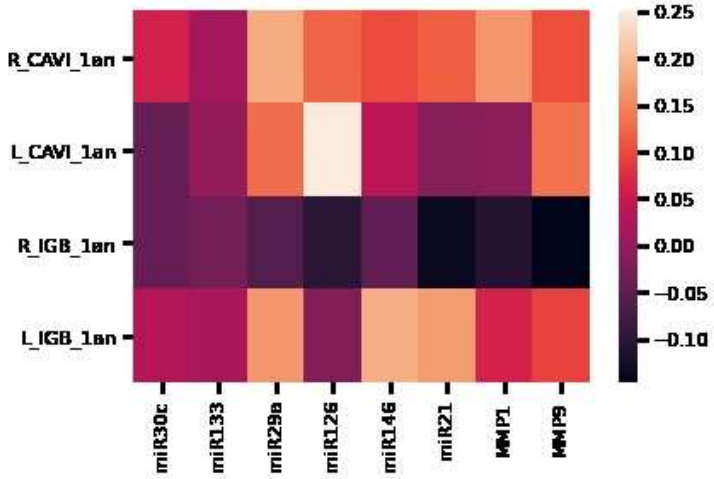


Figure 3.7. Heat map of Pearson correlations between vascular function parameters and circulating microRNAs and MMPs in the general cohort at one year

As with the ventricular and atrial function parameters analyzed in the previous chapters, we used logistic regression models, including the Omnibus test for model coefficients, Nagelkerke R^2, and the Hosmer-Lemeshow test, to assess the predictive power of each analyzed microRNA and MMP for vascular function parameters. In the general cohort analysis, miR30c emerged as a relatively statistically significant predictor for the decrease in RCAVI at one year in patients with chronic coronary syndrome (AUC 0.738, p=0.05, CI 95% 0.531-0.946), with a cutoff value of 2.62, sensitivity 70%, specificity 70% (Fig. 3.8), but not for the decrease in RCAVI in patients with STEMI (AUC 0.363, p=0.18, CI 95% 0.183-0.543) (Fig. 3.9). Additionally, there were no statistically significant predictors for LCAVI in either the chronic coronary syndrome or STEMI groups.

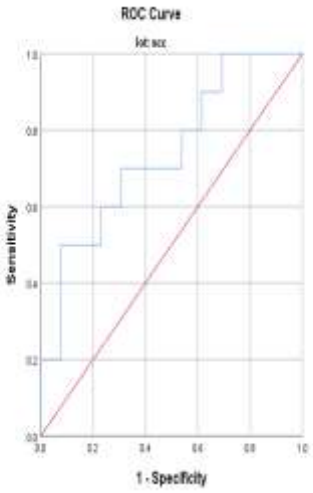


Fig 3.8. ROC Curve for Predicting Decrease in RCAVI at One Year in CCS by miR30c

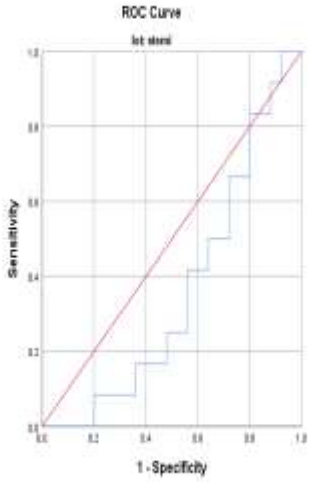


Fig 3.9. ROC Curve for Predicting Decrease in RCAVI at One Year in STEMI by miR30c

3.4.Discussions

As previously shown, circulating MMPs are secreted by various cells, including fibroblasts, smooth muscle cells, and leukocytes. Their action is regulated at the microRNA level through the activation and removal of the propeptide domains from their latent zymogen form. MMPs are most often secreted in an inactive proMMP form, which is subsequently cleaved to the active form by proteases or other MMPs.[34]

Recent studies have also demonstrated that circulating microRNAs play a significant role in vascular remodeling by acting on mechanisms such as smooth muscle cell proliferation, intimal endothelial cell activation, adventitial fibroblast activation, macrophage inflammation, and extracellular matrix protein involvement, with microRNAs being expressed in all these cell types.[35] Studies highlight that miR29 may be involved in the degradation of fibrosis-related genes such as those for collagen, elastin, or fibrillin, and reducing miR29 synthesis can increase the activity of these genes, leading to extensive cardiac fibrosis.[36] Therefore, miR29, and particularly miR29a and miR29b, have a protective effect and represent a potential therapeutic target against vascular and cardiac fibrosis, improving cardiac dysfunction and progression to heart failure. Decreased miR29 synthesis might also have a cardioprotective effect against ischemia/reperfusion injury.[37] Thus, determining the need to increase or decrease miR29 synthesis for cardiovascular protection remains a challenge, as inhibiting miR29 protects cardiomyocytes from ischemic injury but may simultaneously worsen vascular fibrosis.[38] This supports our study's findings, which show a positive correlation of miR29 with CAVI vascular function parameters in STEMI patients with diabetes and negative correlations with CAVI and ABI in patients with chronic coronary syndrome (CCS).

It has been previously shown that MMP1 and MMP9 are present in large quantities in active atherosclerotic plaques and contribute to plaque inflammation and instability.[39] In our study, we found that MMP9 was positively correlated with CAVI values in CCS patients, indicating an active atherosclerotic process and high arterial stiffness in these patients. Meanwhile, in STEMI patients with diabetes, MMP1 was positively associated with vascular dysfunction parameters such as CAVI.

3.5.Conclusions

In conclusion, both circulating microRNAs miR126, miR21, and miR29a, as well as MMP1 and MMP9, are strong regulators of the expression of transcription factors, signaling molecules, and contractile proteins, and play an important role in post-myocardial infarction vascular remodeling. Since tissue remodeling is a dynamic process, an increase in MMPs in one region may be accompanied by a decrease in another region due to differences in the proteolytic activity of MMP enzymes. Therefore, it is crucial to examine MMPs and TIMPs in different tissue locations and stages of disease.

4. The implications of circulating microRNAs on myocardial work in patients with complicated atherosclerotic plaque in the context of acute myocardial infarction and type 2 diabetes mellitus

4.1.Introduction

The assessment of left ventricular (LV) systolic function using ejection fraction (EF) is a fundamental component of clinical cardiology, being the most validated method and the most commonly used parameter for evaluating LV systolic function.[40] However, the main limitations of EF include its inability to assess subclinical and regional function, dependence on volume load, which can lead to loss of reproducibility, susceptibility to changes in geometry in cases of hypertrophied or dilated ventricles, and its failure to reflect true ventricular contractility.

In recent years, global longitudinal strain (GLS) obtained through speckle tracking echocardiography has emerged as a viable method for evaluating systolic function parameters, particularly global and regional LV function, including longitudinal, circumferential, and radial strain, as well as ventricular torsion. GLS is known for its precision, high sensitivity, and reproducibility.[41]

Myocardial work (MW), a new parameter derived from GLS, is used for functional assessment of the LV. It has the advantage of considering both LV deformation and hemodynamic changes by integrating GLS with non-invasive measurement of LV systolic pressure.[42] A recent study by Russell et al. introduced a new non-invasive method for assessing regional myocardial work by determining the pressure-strain loop (PSL) using brachial arterial pressure measurement and generating it from a baseline ventricular pressure curve, in conjunction with the duration of the ejection and isovolumic phases defined by mitral and aortic valve closure and opening, as measured through echocardiography.[43]

The total work of each contracted segment under physiological conditions is positive and termed global constructive work (GCW).[44] In the presence of myocardial impairment, there is a systolic elongation during LV ejection, with myocardial segments generating global wasted work (GWW) necessary for this systolic elongation, which does not contribute to ventricular ejection. Myocardial work efficiency (GWE) is calculated as the ratio of constructive work to the sum of wasted and constructive work, expressed as a percentage, while the global work index (GWI) represents the total myocardial work performed during systole, i.e., the area under the pressure-strain curve from mitral valve closure to mitral valve opening.[45]

Decreased values of myocardial work parameters (GWI, GWE, GCW) and increased values of GWW are observed in patients with acute ST-segment elevation myocardial infarction (STEMI) who develop ventricular remodeling 3 months after the acute ischemic event or in those with heart failure and reduced EF. These results reflect predominantly anaerobic altered metabolism occurring in remodeled, scarred myocardium.[47]

The objectives of our study were to evaluate the main myocardial work parameters and their association with key microRNAs and circulating MMPs, as well as to identify potential predictors of myocardial work parameters in patients with acute myocardial infarction or chronic coronary syndrome.

4.2.Materials and Methods

This research project was conducted through the prospective follow-up of 90 patients at the Cardiology Clinic of the Bucharest Emergency University Hospital. As mentioned in previous chapters, patients were divided into two groups: those with acute ST-segment elevation myocardial infarction (STEMI) and those with chronic coronary syndrome (CCS), followed for a period of 3 years from 2017 to 2020.

The study protocol and all experimental protocols were approved by the Ethics Committee of the Bucharest Emergency University Hospital, and informed consent was obtained from each research participant. Patient evaluation was performed in accordance with current guidelines and included measurement of systolic and diastolic blood pressure and heart rate after a 10-minute rest period, collection of biological samples, and advanced speckle tracking echocardiography to determine longitudinal global strain and myocardial work parameters.

All patients had their blood pressure measured in the same position as the transthoracic echocardiography, in the left lateral decubitus position, to estimate hemodynamic conditions as a non-invasive method of determining ventricular filling.

Biological markers were collected within the first 24 hours of admission and measured through serum microRNA quantification (miR30c, miR133, miR29, miR126, miR146, and miR21) using TaqMan PCR analysis, as well as MMP-1 and MMP-9 in serum using ELISA kits. Additionally, conventional myocardial injury markers, such as troponin I, were collected.

Two-dimensional speckle tracking echocardiography (STE) was used to determine LV longitudinal deformation (in apical 2, 3, and 4-chamber views), with all recordings and

measurements performed according to current guidelines. Myocardial work parameters were determined through offline analysis using dedicated software.

4.3.Results

As mentioned earlier, the 90 patients were enrolled in two groups: one with STEMI (Group 1) and one with chronic coronary syndrome (CCS) (Group 2). The STEMI patients were further divided into those with and without diabetes mellitus.

The distribution of patients based on myocardial work parameters is shown in Figure 4.1 and Figure 4.2 for STEMI patients without diabetes mellitus, in Figures 4.3 and 4.4 for STEMI patients with diabetes mellitus, and in Figures 4.5 and 4.6 for CCS patients.

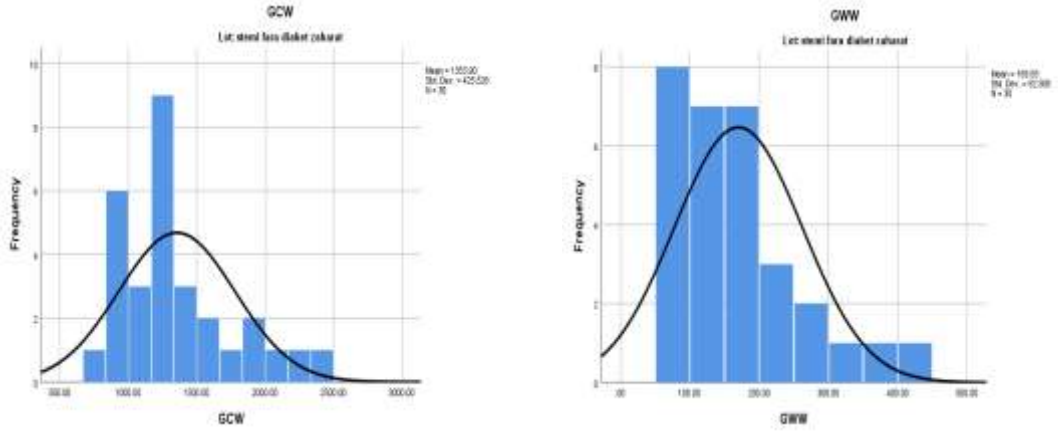


Fig. 4.1. Distribution of Patients Based on GCW and GWW in the STEMI Group Without Diabetes Mellitus

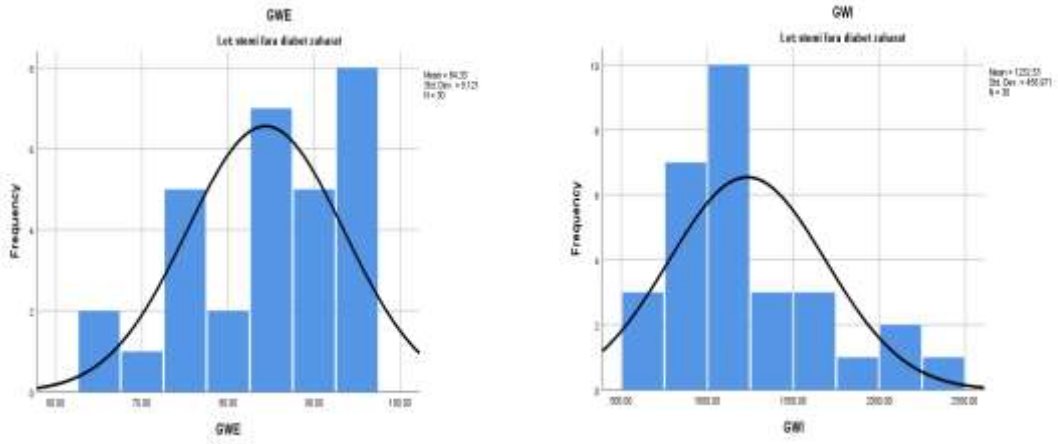


Fig. 4.2. Distribution of Patients Based on GWE and GWI in the STEMI Group Without Diabetes Mellitus

In the group of patients with STEMI and diabetes mellitus, the mean baseline value of GCW was 1158.1 ± 414.5, the mean value of GWW was 203.04 ± 124.7, the mean value of GWE was 79.8 ± 13.0, and the mean value of GWI was 1022 ± 430.1.

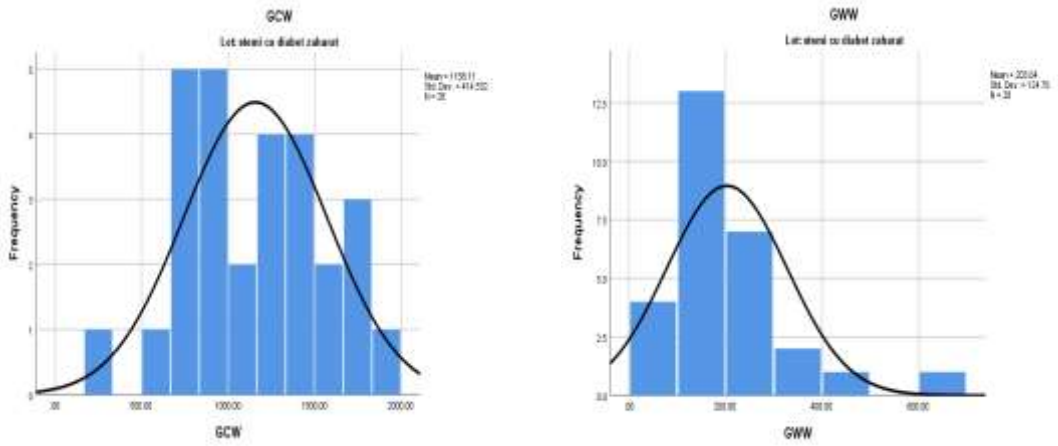


Fig. 4.3. Distribution of patients based on GCW and GWW in the STEMI with diabetes mellitus group

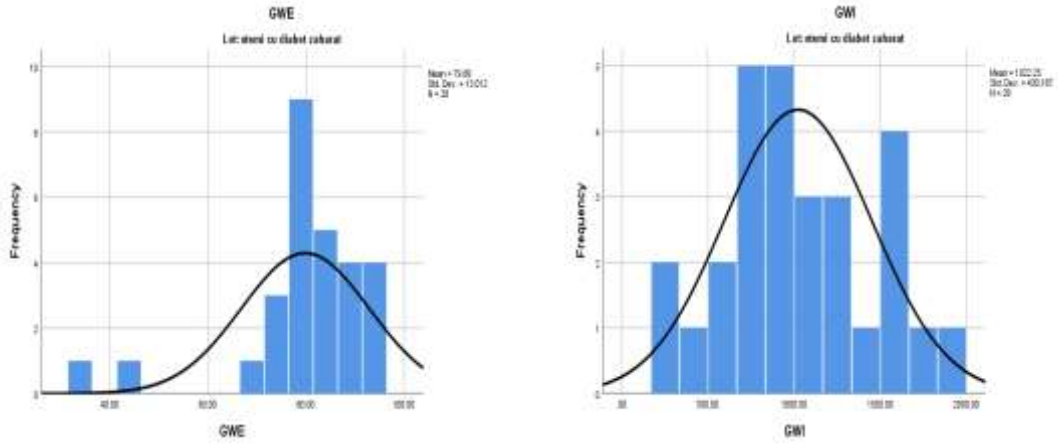


Fig. 4.4. Distribution of patients based on GWE and GWI in the STEMI with diabetes mellitus group

In the group of patients with chronic coronary syndrome (SCC), the baseline mean value of GCW was 1793.87 ± 396.0, the mean value of GWW was 136.5 ± 85.6, the mean value of GWE was 90.1 ± 6.9, and the mean value of GWI was 1630.1 ± 403.5.

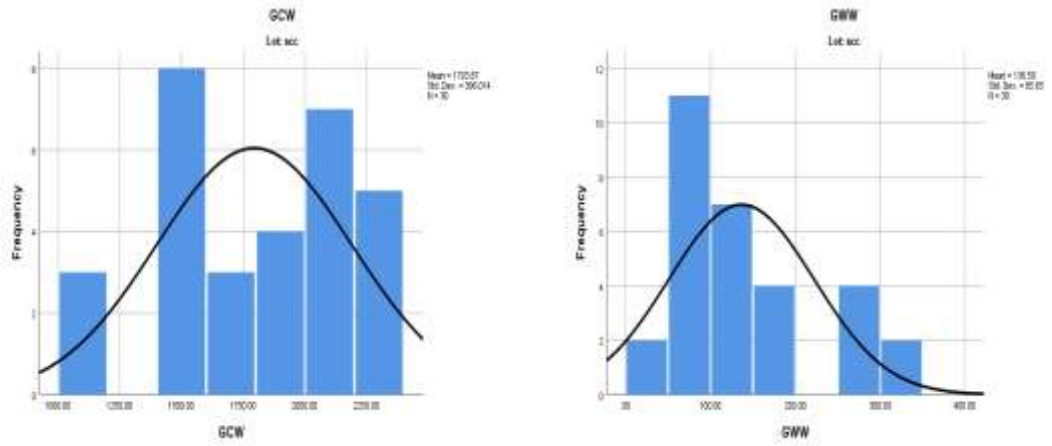


Fig. 4.5. Distribution of patients based on GCW and GWW in the CCS group

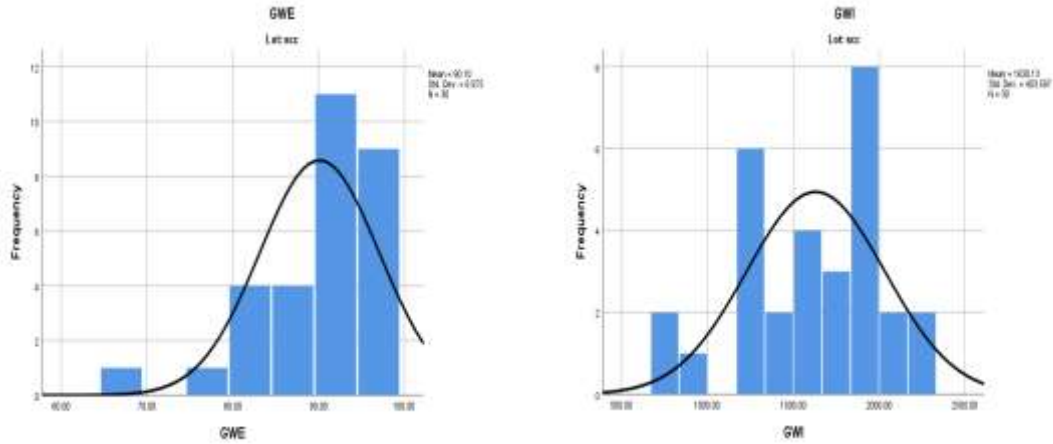


Fig. 4.6. Distribution of patients based on GWE and GWI in the CCS group

Using T-test and Levene's test, we found that all mechanical work parameters were significantly higher in the STEMI group compared to the SCC group, with the following results: for GCW, t=-5.69, p=0.0001; for GWW, t=2.15, p=0.03; for GWE, t=-3.50, p=0.001; and for GWI, t=-5.08, p=0.0001 (Fig. 4.7).

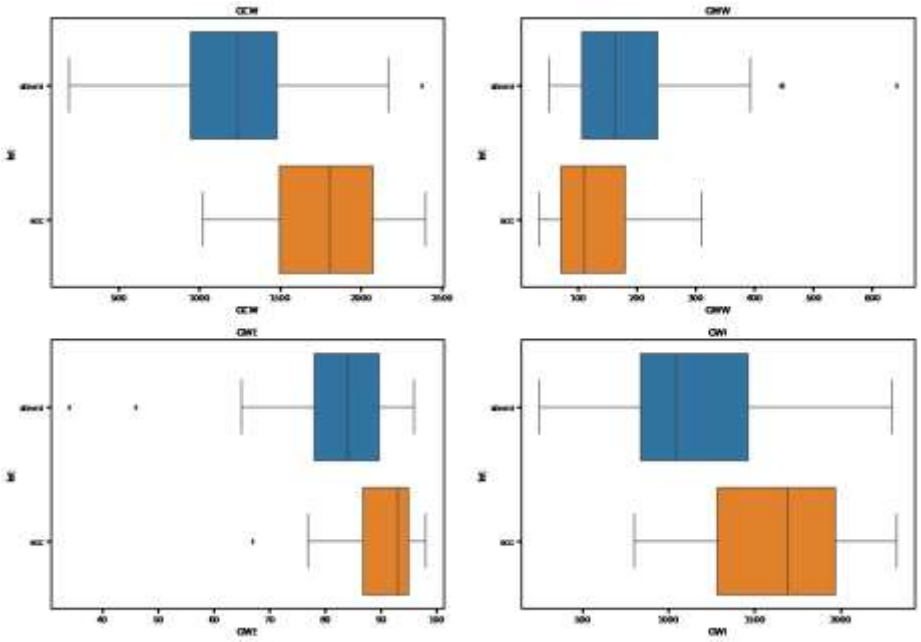


Fig. 4.7. – Comparison of statistically significant myocardial work parameters between patients with STEMI and CCS at baseline

Using Pearson correlation tests, we evaluated the main myocardial work parameters and their correlation with circulating microRNAs and MMPs in the general cohort at baseline. miR133 showed significant negative correlations with GCW (R=-0.29, p=0.01), GWE (R=-0.40, p=0.001), and GWI (R=-0.31, p=0.008), with no other significant associations for the remaining microRNAs. Regarding circulating MMPs, both MMP1 and MMP9 demonstrated significant correlations with myocardial work parameters in the general cohort. These results are illustrated in the heat map below (Fig. 4.8).

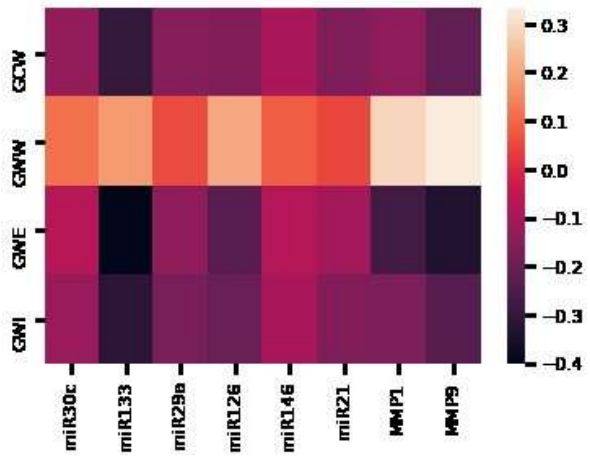


Figura 4.8. The heat map of Pearson correlations between myocardial work parameters and circulating microRNAs and MMPs in the general cohort at baseline

Subsequently, we analyzed the correlation between microRNAs and circulating MMPs in each group using Pearson correlation tests and obtained the following data. In the cohort of patients with STEMI associated with diabetes mellitus, MMP9 was positively correlated with GWW (R=0.46, p=0.01), and no significant correlations were found for microRNAs or MMP1 (Fig. 4.9). Moreover, in the cohort of patients with STEMI without diabetes mellitus, GWE was negatively correlated with miR133 (R=-0.61, p=0.004), miR126 (R=-0.60, p=0.04), and MMP9 (R=-0.44, p=0.01), while GWW had a positive correlation with miR133 (R=0.49, p=0.02) (Fig. 4.10).

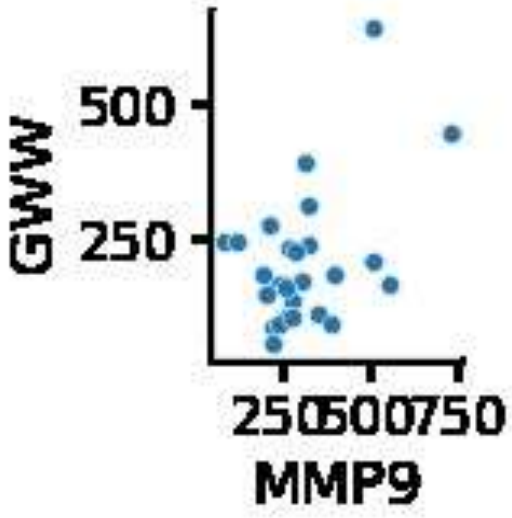


Fig 4.9. Statistically significant correlation of MMP9 with GWW in patients with STEMI and diabetes mellitus at baseline

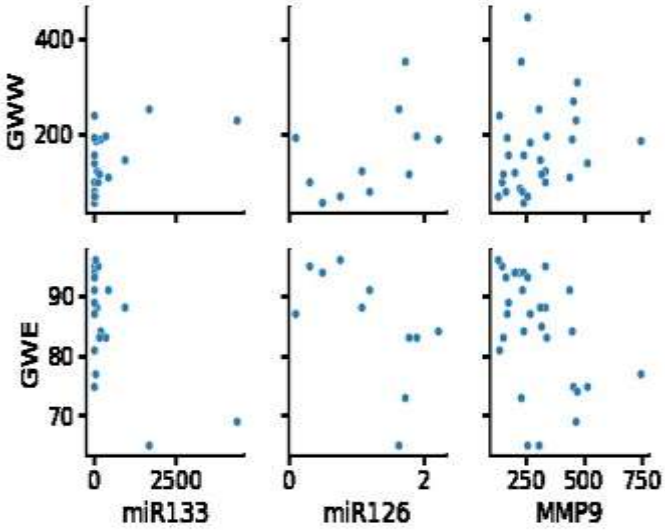


Fig 4.10. Statistically significant correlations of miR133, miR126, and MMP9 with GWW and GWE in patients with STEMI without diabetes mellitus at baseline

Evaluation of myocardial work parameters at 1 year was conducted by correlating circulating microRNAs and MMPs using Pearson correlation tests, and the following results were obtained. In the general cohort, miR133 was negatively correlated with GCW (R=-0.33, p=0.01),

GWE (R=-0.38, p=0.004), and GWI (R=-0.32, p=0.01). No significant correlations were found with the other microRNAs or circulating MMPs (Fig. 4.11).

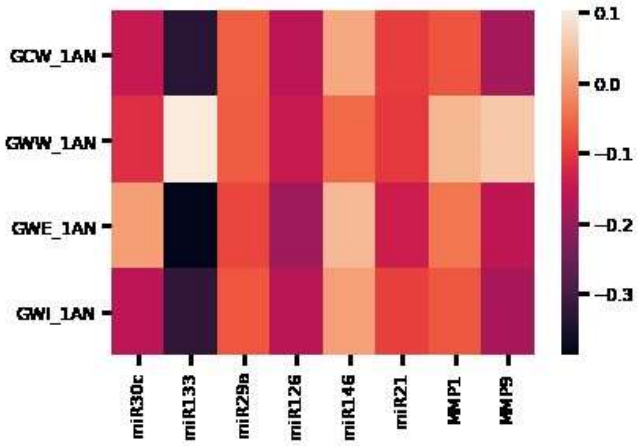


Figura 4.11. Heat map of Pearson correlations of myocardial work parameters with circulating microRNAs and MMPs in the general cohort at 1 year

Using logistic regression models, respectively the Omnibus test for model coefficients, Nagelkerke R2, the Hosmer and Lemeshow test for evaluating the predictive power of each microRNA, respectively MMP analyzed for myocardial work parameters, no predictive values emerged for the improvement of myocardial work parameters at a year.

4.4.Discussions

Myocardial work (MW) analysis has recently been proposed as a new method for assessing left ventricular systolic function. It is considered superior to ejection fraction and global longitudinal strain, as it is not dependent on left ventricular filling.[47] Previous studies have evaluated the clinical applications of this method in various cardiovascular pathologies.[48]

This research demonstrates the involvement of miR133, miR126, miR146, and miR21, which are known to potentially affect coronary artery disease through their impact on unstable atherosclerotic plaques, with myocardial work parameters in both diabetic and non-diabetic patients. In our study, miR133 had higher values associated with increased GWW and reduced GWE in patients with STEMI without diabetes at baseline. The evaluation at one year showed significant correlations with GCW, GWE, and GWI in the entire cohort and in patients with STEMI and diabetes. On the other hand, miR126 and miR146, involved in inflammatory processes

as demonstrated in previous chapters and in atherosclerotic plaque progression and myocardial necrosis [49], were associated with GWE in STEMI patients, showing a negative correlation, and with GCW in SCC patients, showing a positive correlation. This indicates their possible role in post-myocardial infarction myocardial remodeling.

There is insufficient data in the literature to demonstrate the possible effects of matrix metalloproteinases on left ventricular systolic function as determined by myocardial work parameters.[50] However, our study shows that both MMP1 and MMP9 have statistically significant correlations with these parameters. MMP9 had a negative correlation with GWE at baseline in STEMI patients without diabetes and with GCW and GWI at one year in the same cohort, and a positive correlation with GWW in STEMI patients with diabetes at baseline. MMP1 had higher values associated with greater GWW in SCC patients. No statistically significant predictive models were obtained for circulating microRNAs or MMPs, indicating the need for further, larger studies to evaluate the potential prognostic and diagnostic roles of these biomarkers.

4.5.Conclusions

In conclusion, myocardial work parameters have promising prognostic and diagnostic potential in patients with coronary atherosclerotic disease. Circulating microRNAs and MMPs play a complementary role alongside echocardiographic parameters in defining and stratifying left ventricular systolic function and the post-myocardial infarction remodeling process. These biomarkers, together with myocardial work parameters, could have a prognostic role, facilitating cardiovascular risk stratification, improving prevention strategies, and thereby reducing cardiovascular mortality.

5. Finite Element Model for Analyzing the Influence of the Infarcted Myocardium Percentage on the Ejection Fraction

5.1. Finite Element Method in the Biomechanics of the Cardiovascular System

The Finite Element Method (FEM) is the most widely used numerical method in industry and research for simulating the behavior of various physical systems under a considerable variety of interactions. Due to its ability to encompass complex geometric features, material properties, boundary conditions, and local interactions between components, FEM has also been applied in biomechanics, particularly in the cardiovascular system. Among other things, it can model the

heart's pumping function to improve the diagnosis and treatment of cardiac pathologies. Traditional computer-aided design (CAD) methods used for designing geometries of inert mechanical structures have limited applicability in heart biomechanics, as they often apply to generic, simplified geometries given the heart's complex anatomy. To accurately reproduce anatomical details, medical imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) are used. For heart biomechanics models that include blood flow, the primary parameters required are the density of blood, averaging 1060 g/cm³, and its dynamic viscosity, approximately 4 mPa·s.

5.2. Presentation of the Proposed Model

In the case of myocardial infarction affecting the left ventricle, a model that predicts the reduction of the ejection fraction based on the volume of infarcted tissue is of interest. In this work, a preliminary model is proposed to address this issue, based on fluid-solid interaction, where the solid is represented by the geometry of the left ventricle and the fluid by the volume of blood contained within it. The geometry of the left ventricle was downloaded in STEP format from the GRABCAD website, which offers users a variety of CAD models for different structures. The CAD model has the advantage of a realistic geometry, as it was created based on medical imaging. This is shown in Figure 5.1.

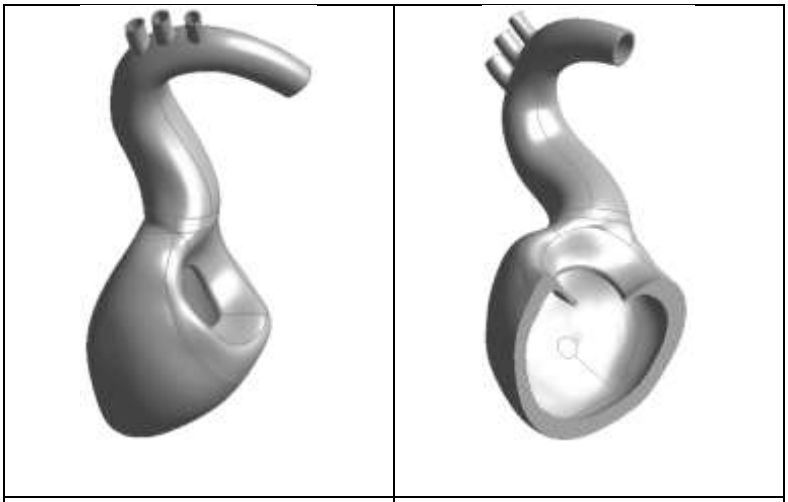
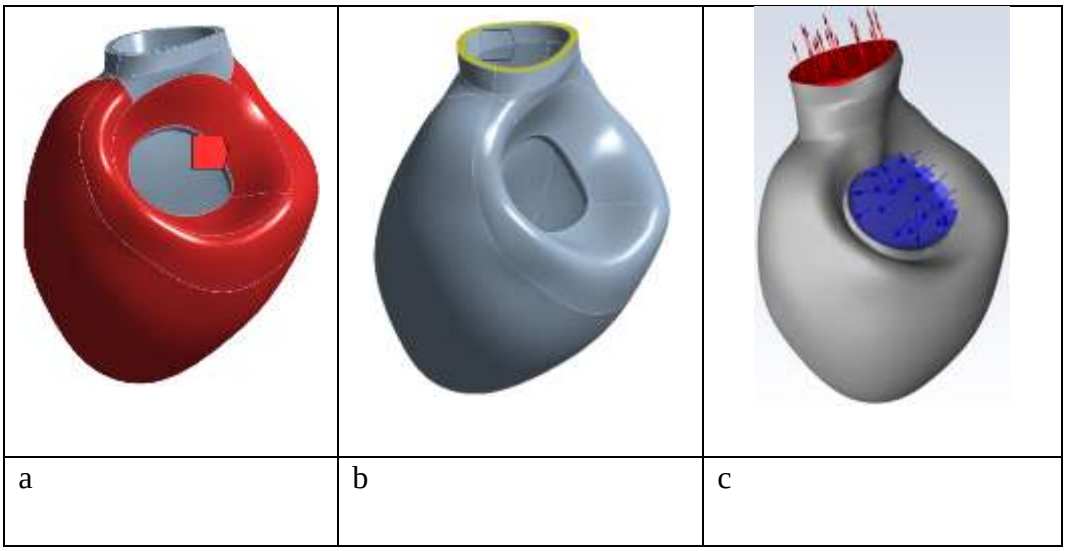


Fig. 5.1. CAD Model of the Left Ventricle – View (a) and Section (b)

The material of the myocardium was defined as a composite reinforced with bidirectional fabric, designed to replicate the muscle fibers, making it an orthotropic composite. This approach is based on the material characteristics presented by Bagnoli and co-authors [50], but it simplifies the modeling process by avoiding the explicit modeling of muscle fibers. The analysis focuses on simulating ventricular contraction starting from the initial, relaxed state. In the proposed model, this requirement can be met by applying a uniform external pressure on the surfaces indicated in red in Figure 5.2 - a. To prevent rigid body motion, which is inherent due to the lack of static equilibrium, it is necessary to constrain all degrees of freedom without affecting the results of interest. In this regard, all degrees of freedom (translations and rotations) were constrained on the surface marked in yellow in Figure 5.2 - b. In the fluid model, a pressure of 100 mmHg was applied at the entrance to the ventricle (the surface marked in blue in Figure 5.2 - b). The exit pressure was defined, although it serves as a monitored result rather than a boundary condition (the red surface in Figure 5.2 - c).



a	b	c

Fig. 5.2. Boundary Conditions for the Solid Model: External Pressure (a), Constraints (b), and Boundary Conditions for the Fluid Model - Inlet Pressure in the Ventricle (c)

5.3. Directions for Further Analysis

Based on the proposed model, future research will focus on the following simulation-based directions: Analyzing the influence of the infarcted myocardium percentage, as well as the

location of the infarcted area, on the ejection fraction; Analyzing the influence of flow parameters, particularly the dynamic viscosity of blood, on the ejection fraction.

5.4. Conclusions

The model proposed in this chapter is preliminary and is part of a broader study aimed at developing a complex model that includes realistic multiphysical interactions based on clinical data. The goal of the model is to create a computer simulation foundation for cardiac hemodynamics. Such a model can provide a deeper understanding of the mechanisms governing the heart's pumping function, with applications in the diagnosis and treatment of acute myocardial infarction.

6. Final Conclusions and Personal Contributions

Regarding the assessment of functional cardiac changes in patients with acute myocardial infarction, whether or not they have diabetes, and the relationship between these changes and circulating microRNAs and MMPs, which was the main objective of this thesis, the conclusions are presented on the following pages.

1. Analyzing **left ventricular systolic function**, we demonstrated that biplane and three dimensional ejection fraction, cardiac output, and myocardial deformation parameters (radial strain, longitudinal strain, and longitudinal strain rate, as well as untwisting rate) were significantly reduced in patients with STEMI compared to those with CCS. This finding also applied to right ventricular function parameters, where both diameter and longitudinal strain of the RV were lower in patients with acute myocardial infarction. Furthermore, the evaluation of ventricular function parameters at one year showed that ejection fraction, global longitudinal strain, and longitudinal strain rate were significantly increased at one year compared to the enrollment time in patients with STEMI and diabetes, demonstrating the benefit of current myocardial revascularization therapy in ventricular remodeling and reverse remodeling.
2. Regarding **circulating microRNAs and MMPs**, our results show that expression levels of *miR133, miR146, miR29a, and miR21* are significantly higher in STEMI patients compared to CCS patients. We also found that miRNA126 and miR133 were associated with ejection fraction and myocardial deformation parameters in STEMI patients compared to those with chronic atherosclerotic disease. Additionally, miR21, miR30c, and miR29a were

associated with left ventricular deformation parameters in both STEMI and SCC patients, but with inverse correlation. In our study, both MMP1 and MMP9 had negative correlations with ejection fraction and left ventricular deformation parameters in STEMI patients, with no significant correlations with right ventricular parameters. However, MMP9 also had higher values in patients with lower longitudinal strain, basal rotation, and left ventricular torsion in the CCS group.

3. Our study also demonstrated that *miR133 has statistically significant predictive value* for increased radial strain in both STEMI and diabetic patients, although it did not have predictive power for other ventricular function parameters. However, miR133, miR29a, miR30c, miR146, and miR21 had significant predictive value for acute myocardial infarction diagnosis.
4. Regarding **atrial function**, our study showed that miR126 levels and serum levels of MMP1 and MMP9 are significantly associated with atrial function parameters, including maximum and minimum atrial volumes and preatrial contraction volume, active and total ejection fraction, as well as deformation parameters such as positive strain, negative strain, and global atrial strain in STEMI patients. Concerning the right atrium, we found significant correlations of miR30c, miR133, miR126, and miR146 with atrial deformation parameters in the general cohort and in CCS patients. The one-year evaluation showed that MMP1 and MMP9 levels correlate with active and total left atrial ejection fraction in STEMI patients without diabetes, while in STEMI patients with diabetes, miR133 and miR29a were associated with left atrial deformation parameters.
5. Regarding **vascular function** and the role of circulating microRNAs, we demonstrated that higher values of miRNA126 correlated negatively with arterial stiffness parameters CAVI and IGB, while miR29a showed a positive correlation with CAVI in STEMI patients with diabetes and a negative correlation with CAVI and IGB in CCS patients.
6. In Chapter 8 of this research, for the first time in the literature, we evaluated **myocardial work parameters** and the influence of circulating microRNAs and MMPs on them. We demonstrated that *miR133, miR126, miR146, and miR21*, known to have potential effects on coronary artery disease through unstable atherosclerotic plaques, are associated with myocardial work parameters in both diabetic and non-diabetic patients, with higher values in those with constructive myocardial work and lower myocardial efficiency.

7. In the final chapter of the personal contributions section, I simulated **a model using the finite element method** to provide predictions regarding the decrease in left ventricular ejection fraction following an acute myocardial infarction.

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