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The Significance and Prognostic Role of Platelet Microvesicles in the Development of Thrombotic Complications in Patients with Cardiac Rhythm Disorders

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Abstract Cardiac rhythm disorders, particularly atrial fibrillation (AF), are associated with a heightened risk of thromboembolic events, making effective management crucial for patient outcomes. This study investigates the role of platelet microvesicles (PMVs) in the development of thrombotic complications in patients with cardiac rhythm disorders, specifically those with ischemic myocardial damage. PMVs, which are small, phospholipid-enriched extracellular vesicles, have been identified as significant contributors to the hypercoagulable state observed in these patients. The study involved the quantification and characterization of PMVs in different patient groups and assessed their correlation with platelet aggregation and the incidence of thrombotic events. The results demonstrate that elevated levels of PMVs are strongly associated with increased thrombotic risk, particularly due to the presence of procoagulant factors such as phosphatidylserine (PS) and tissue factor (TF) on their surfaces. These findings suggest that PMV levels could serve as a prognostic biomarker for thrombotic complications, offering a potential tool for identifying high-risk patients who may benefit from intensified antithrombotic therapy. The study concludes that while PMVs hold promise as both biomarkers and therapeutic targets, further research is necessary to establish standardized thresholds for PMV levels and to develop targeted therapies that mitigate their procoagulant effects without disrupting physiological processes.

Keywords Platelet Microvesicles, Cardiac Rhythm Disorders, Thrombotic Complications, Atrial Fibrillation, Ischemic Myocardial Damage

1. Introduction

Cardiac rhythm disorders, particularly atrial fibrillation, are among the most common cardiovascular conditions and are associated with a significantly increased risk of thromboembolic events. The pathophysiology of thrombosis in these patients is complex and multifactorial, involving endothelial dysfunction, blood stasis, and hypercoagulability. Recently, attention has been focused on the role of platelet microvesicles (PMVs) as critical contributors to the thrombotic risk in patients with cardiac rhythm disorders [1,3,6,8].

Platelet microvesicles are small, phospholipid-enriched extracellular vesicles, typically ranging from 100 to 1000 nm in diameter. They are released from platelets upon activation, apoptosis, or during physiological cell aging. The biogenesis of PMVs involves the outward budding and fission of the plasma membrane, a process often triggered by increased intracellular calcium levels and cytoskeletal rearrangements [2,4,9].

The surface of PMVs is enriched with procoagulant phospholipids, particularly phosphatidylserine (PS), which is normally confined to the inner leaflet of the plasma membrane but becomes externalized during vesicle formation. PMVs also carry a variety of bioactive molecules, including coagulation factors, adhesion molecules, and receptors, that facilitate their involvement in hemostasis and thrombosis [2,5,7].

The procoagulant potential of PMVs is largely attributed to their surface expression of PS and tissue factor (TF). PS provides a negatively charged surface that supports the assembly of coagulation factor complexes, particularly the tenase and prothrombinase complexes, leading to the rapid generation of thrombin. Thrombin, in turn, converts fibrinogen to fibrin, culminating in clot formation [6,10].

Additionally, some PMVs express TF, a potent initiator of the extrinsic coagulation pathway. TF-bearing PMVs can activate the coagulation cascade directly, leading to the

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amplification of thrombin generation and subsequent fibrin formation. This dual mechanism of action makes PMVs highly effective at promoting thrombosis, particularly in a hypercoagulable state such as that seen in cardiac rhythm disorders [3,6,11].

Several studies have demonstrated elevated levels of PMVs in patients with cardiac rhythm disorders, especially those with atrial fibrillation. These elevated PMV levels have been correlated with markers of hypercoagulability, including increased thrombin generation and fibrin formation, as well as with clinical outcomes such as stroke and systemic embolism [5,12,13].

Research has also shown that the concentration of PMVs is significantly higher in patients with cardiac rhythm disorders who develop thrombotic complications compared to those who do not. This suggests that PMV levels could serve as a biomarker for thrombotic risk in these patients. Furthermore, the presence of TF-positive PMVs in the peripheral blood of these patients has been associated with an increased risk of thromboembolism, providing further evidence of the prothrombotic role of PMVs in this population [14-17].

The prognostic significance of PMVs in cardiac rhythm disorders is supported by studies showing that higher levels of PMVs are associated with an increased risk of stroke and myocardial infarction. The positive predictive value of elevated PMV levels for the development of thrombotic complications has been reported to be high, indicating that patients with increased PMV concentrations are at substantial risk for adverse thrombotic events [4,8,18,21].

The use of PMV levels as a prognostic tool could help identify high-risk patients who may benefit from more aggressive antithrombotic therapy. However, further research is needed to establish standardized thresholds for PMV levels and to determine the optimal therapeutic strategies for patients with elevated PMV levels [19,20].

The mechanisms by which PMVs contribute to thrombosis in cardiac rhythm disorders are multifaceted. PMVs can enhance platelet aggregation, facilitate the assembly of coagulation factor complexes, and promote the formation of a stable fibrin clot. Additionally, PMVs can interact with endothelial cells and leukocytes, further amplifying the prothrombotic state [19,22-24].

In atrial fibrillation, for example, the irregular and often rapid heart rate can lead to blood stasis in the atria, which is a key factor in thrombus formation. The presence of PMVs in this context can exacerbate the prothrombotic environment by providing a surface for coagulation factor activation and by promoting platelet aggregation, thereby increasing the risk of thrombus formation and subsequent embolization [7,16,25].

Given the role of PMVs in thrombosis, targeting PMVs or their procoagulant activity could be a potential therapeutic strategy for preventing thrombotic complications in patients with cardiac rhythm disorders. Antithrombotic therapies, such as anticoagulants and antiplatelet agents, may reduce the release of PMVs or inhibit their procoagulant effects, thereby lowering the risk of thrombosis [23,26].

There is also interest in the development of therapies that specifically target the components of PMVs, such as PS or TF, to disrupt their procoagulant activity. However, the challenge lies in selectively targeting pathological PMVs without affecting their physiological roles in hemostasis and immune regulation [9,14].

Platelet microvesicles are emerging as important players in the pathophysiology of thrombotic complications in patients with cardiac rhythm disorders. Their ability to promote coagulation through the expression of procoagulant phospholipids and tissue factor, along with their elevated levels in these patients, underscores their significance as both biomarkers and potential therapeutic targets. Future research should focus on elucidating the precise mechanisms by which PMVs contribute to thrombosis in cardiac rhythm disorders and on developing targeted therapies to mitigate this risk [2,7,20].

In this context, our study aims to evaluate the role of platelet microvesicles (PMVs) in the development of thrombotic complications in patients with cardiac rhythm disorders, particularly those associated with ischemic heart disease. Specifically, the objectives of the study include quantifying and characterizing PMVs in different patient groups, assessing the correlation between PMV levels and platelet aggregation, and determining the prognostic significance of PMV levels in predicting thrombotic events. By achieving these objectives, we seek to better understand the contribution of PMVs to the hypercoagulable state observed in these patients, and to explore their potential as biomarkers for identifying individuals at high risk of thrombotic complications.

2. Materials and Methods

Study Design

This study was conducted to investigate the role of platelet microvesicles (PMVs) in the development of thrombotic complications in patients with cardiac rhythm disorders, particularly those associated with ischemic heart disease (IHD). The study comprised both a quantitative and qualitative assessment of microvesicle content across various patient groups, as well as an evaluation of the prognostic significance of PMV levels in predicting thrombotic complications.

Patient Selection

A total of 250 patients were enrolled in the study, which included three groups based on PMV levels:

- Group I: Patients with low PMV levels (<25 million/mL; n=120).
- Group II: Patients with intermediate PMV levels (25-35 million/mL; n=79).
- Group III: Patients with high PMV levels (>35 million/mL; n=51).

Patients were selected based on the presence of cardiac rhythm disorders, with additional subgroups for ischemic

myocardial damage, acute coronary syndrome (ACS) with coronary angiography (CAG), and healthy controls. All patients provided informed consent before participation.

Sample Collection and Preparation

Peripheral blood samples were collected from patients at various time points: preoperatively, immediately post-CAG, and on the 3rd and 7th days following surgical intervention. Blood samples were processed to isolate plasma, from which PMVs were subsequently extracted.

Quantification and Characterization of Microvesicles

Microvesicles were quantified and characterized using flow cytometry, a technique that allows for the identification and analysis of particles based on size, granularity, and fluorescence. Microvesicles were labeled with fluorochrome -conjugated antibodies against specific markers, including:

- **Phosphatidylserine (PS)**: Detected using annexin V binding assays.
- **Platelet markers (CD41 and CD61)**: Used to identify platelet-derived microvesicles.
- **Leukocyte marker (CD45)**: Used to identify leukocyte-derived microvesicles.
- **Erythrocyte marker (CD235)**: Used to identify erythrocyte-derived microvesicles.
- **Tissue factor (TF)**: Used to assess the procoagulant potential of the microvesicles.

Fluorescence intensity and the percentage of microvesicles positive for each marker were recorded.

Platelet Aggregation Assays

Platelet aggregation was assessed using a Multiplate analyzer, measuring the response to various agonists, including ADP, collagen, and adrenaline. The extent of platelet aggregation was correlated with PMV levels to evaluate their potential role in hypercoagulability.

Prognostic Analysis

The prognostic significance of PMV levels was evaluated by assessing their predictive value for thrombotic events. ROC curve analysis was performed to determine the sensitivity, specificity, and predictive values (positive and negative) of PMV levels in predicting thrombotic complications.

Statistical Analysis

Data were analyzed using SPSS software. Correlations between PMV levels and clinical outcomes, including thrombotic events and mortality, were assessed using Pearson's correlation coefficient. ROC curve analysis was employed to determine the diagnostic accuracy of PMV levels. Statistical significance was defined as $p < 0.05$.

3. Results and Discussions

In the initial phase of the study, a quantitative and qualitative assessment of microvesicle content was conducted across the groups. The hemostatic parameters of the patients under investigation are presented in Table 1.

The study revealed a significant increase in the concentration of microvesicles in the peripheral blood of patients with ischemic heart disease prior to surgical intervention (43.8×10^6 per mL of plasma vs. 26.9×10^6 in the group of healthy individuals, p=0.001).

Monitoring the dynamics of microvesicle content demonstrated a decrease in their concentration in the peripheral blood following coronary angiography, with levels reaching 39.1×10^6 particles per mL by the 7th day.

In the group of patients who experienced a fatal outcome, the preoperative microvesicle concentration in peripheral blood did not differ from that of the group with a favorable outcome at the time of the study (43.8×10^6 vs. 43.1×10^6, p=0.35). However, in the fatal outcome group, a significantly higher concentration of microvesicles was observed on the 3rd day post-operation compared to the group with a favorable outcome (42.6×10^6 vs. 22.9×10^6, p=0.003).

Microvesicles are shed from the plasma membrane of virtually all cell types during activation, apoptosis, or as a part of normal cellular processes and aging. They range from 100 to 1000 nm in diameter and are the only type of extracellular vesicles that can be directly observed in the optical range using microscopy or flow cytometry. Like the plasma membrane, the microvesicular membrane contains phosphatidylserine (PS). However, unlike cells, microvesicles lack ATP-dependent mechanisms to maintain membrane asymmetry, resulting in the constant presence of phosphatidylserine on the outer membrane layer. Microvesicles are the primary non-cellular source of procoagulant surfaces in blood plasma, even rivaling platelets in this function.

The study of peripheral blood microvesicles in patients with atrial fibrillation associated with ischemic heart disease showed the presence of phosphatidylserine on the membrane surfaces, as determined by the binding of annexin V labeled with a fluorochrome.

Table 1. Platelet Aggregation Intensity in the Control Group and Thrombosis Group, Median (25%-75% IQR)

Groups	Mcv, x106 ml	ADP, mm	Collagen, mm	Adrenaline, mm	MA TEG, mm	CI TEG, %
Control	26,9 (24,3-31,8)	46,5 (36,3-54,1)	43,2 (38,5-54,7)	41,5 (37,2-51,3)	48,9 (37,5-53,6)	0,1 (0,0-1,2)
IHD	43,8 (40,2-45,8)**	71,4 (69,1-74,7)**	69,8 (65,3-75,1)*	71,1 (68,1-75,4)*	73,2 (70,2-76,4)**	2,4 (1,9-2,7)**
CAG	34,5 (30,5-36,9)	50,4 (42,7-56,3)	41,8 (37,2-56,1)	43,2 (38,7-54,1)	47,6 (34,8-56,4)	1,2 (0,8-1,4)

Note: * *p ≤ 0.001*, ** - *p ≤ 0.01 - compared to the control group*

The expression of phosphatidylserine (PS) on the microvesicles of the patients studied varied significantly in both percentage and fluorescence intensity, as determined by flow cytometry (Figure 1).

The procoagulant activity of plasma microvesicles cannot be limited to their ability to carry negatively charged sites that initiate blood coagulation (PS-positive). Circulating microvesicles can also carry active coagulation factors such as IXa, XIa, or XIIa, as demonstrated by several studies. Platelet- and erythrocyte-derived microvesicles directly activate blood coagulation via the contact pathway, whereas monocyte-derived microvesicles carry tissue factor and activate the extrinsic pathway.

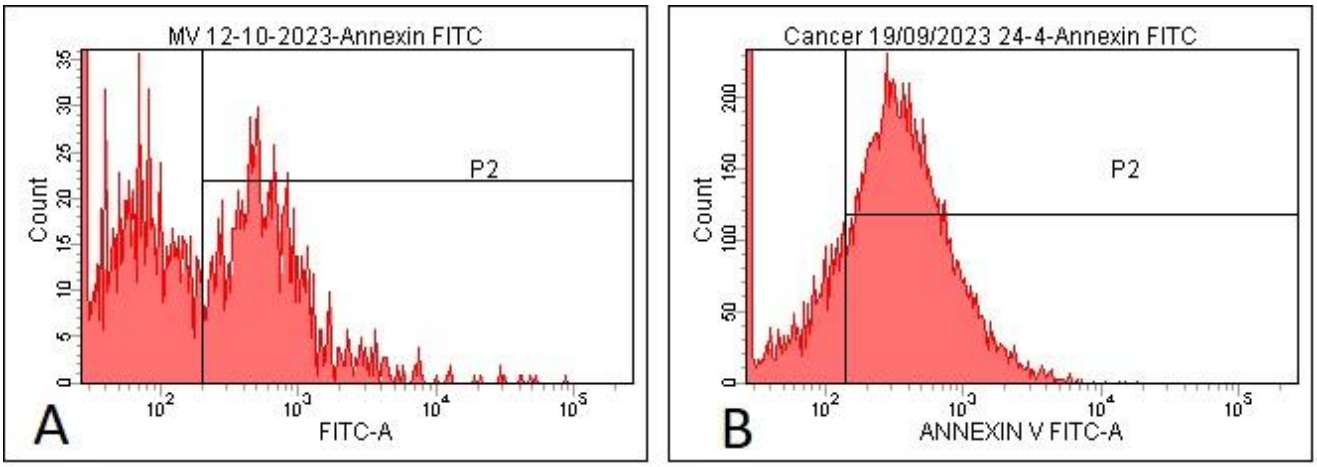

Figure 1. Expression of Phosphatidylserine (PS) on Microvesicles in the Control Group and Patients with Ischemic Heart Disease and Cardiac

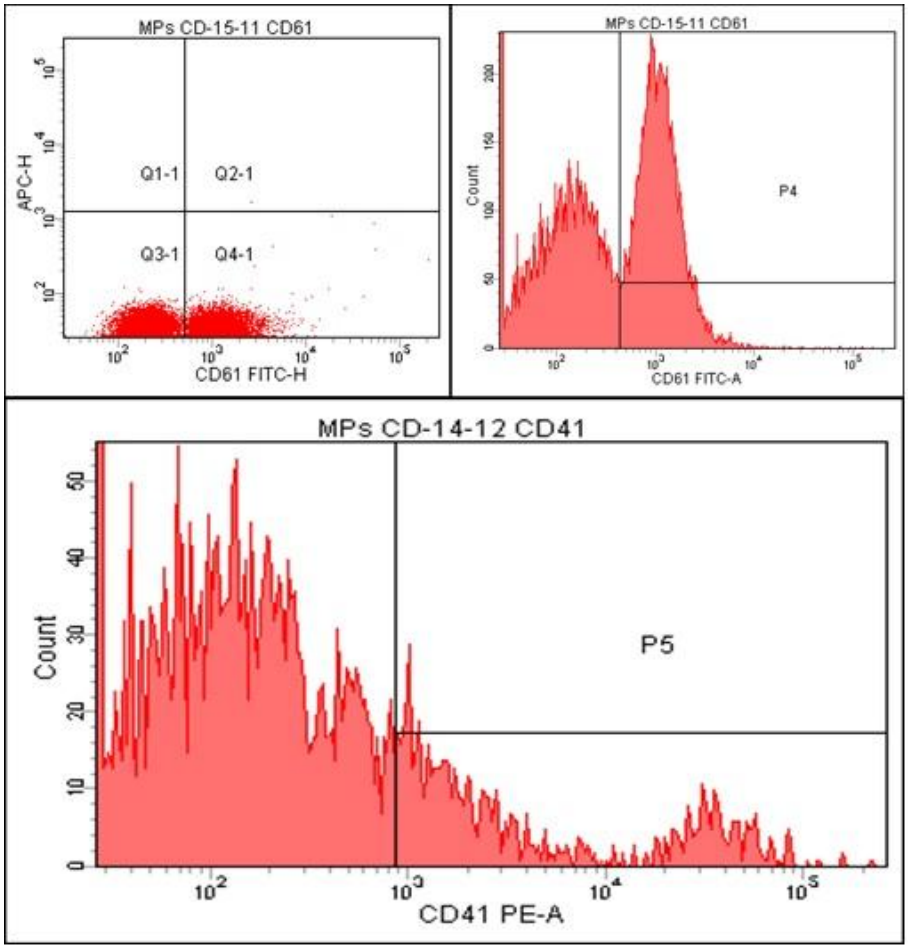

Figure 2. Histograms of CD41 and CD61 Markers on Microvesicles in Patients with Ischemic Heart Disease and Cardiac Rhythm Disorders

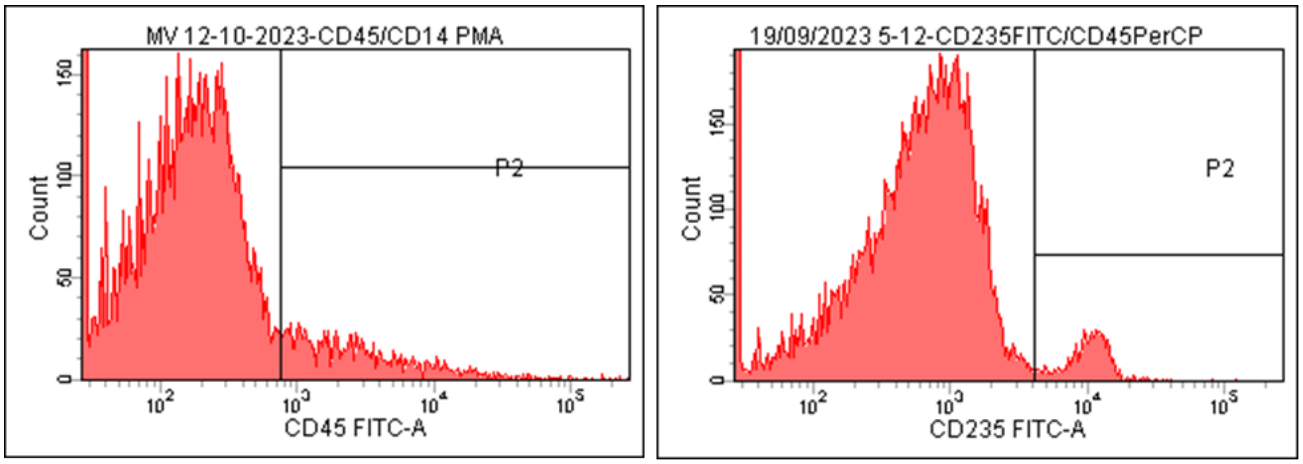

Figure 3. Histograms of CD45 and CD235 Markers on Microvesicles in Patients with Cardiac Rhythm Disorders Associated with Ischemic Myocardial Damage

The prothrombotic effect may be mediated by the expression of tissue factor (TF) directly by tumor cells and vascular endothelial cells during inflammation. However, it has been observed in several studies that extracellular vesicles positive for TF circulate in peripheral blood. Among extracellular vesicles, microvesicles are distinguished by their procoagulant activity.

Our studies on the phenotype of microvesicles in patients with cardiac rhythm disorders associated with ischemic myocardial damage revealed a predominance of platelet-derived microvesicles in peripheral blood, as determined by flow cytometry through the expression of platelet markers CD41 and CD61 on the particles (Figure 2).

In the samples analyzed from patients with cardiac rhythm disorders associated with ischemic myocardial damage, microvesicles of leukocytic and erythrocytic origin were detected, identified by the cellular markers CD45 and CD235, respectively (Figure 3).

The analysis of tissue factor (TF) expression on microvesicles in the peripheral blood of the patient group revealed the presence of TF-positive microparticles (Figure 4).

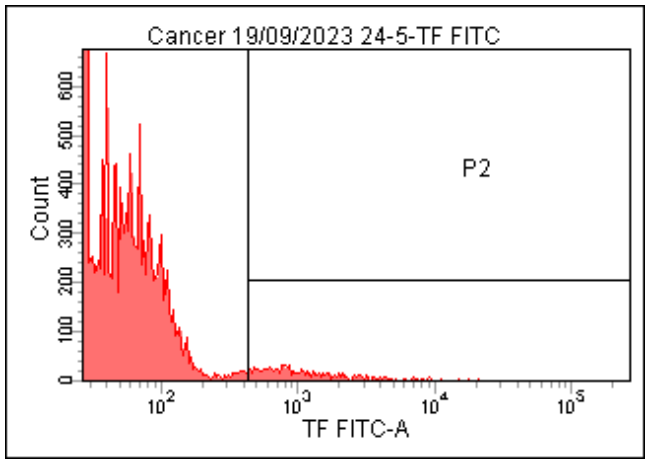

Figure 4. Histograms of Tissue Factor (TF) Expression on Microvesicles in Patients with Cardiac Rhythm Disorders Associated with Ischemic Myocardial Damage

The presence of tissue factor (TF)-positive microvesicles in the peripheral blood of patients with cardiac rhythm disorders associated with ischemic myocardial damage, which possess a direct procoagulant role, may indicate a potential risk for the development of thrombotic conditions.

The study was concluded with an evaluation of the prognostic role of platelet microvesicle (mcv) determination in predicting thrombotic complications. Based on laboratory assessments of mcv, all study participants were redistributed into research groups (Table 2). The first group included patients with low mcv levels (<25 million/mL); the second group comprised patients with intermediate mcv levels (25-35 million/mL); and the third group consisted of patients with high mcv levels (>35 million/mL).

Table 2. Distribution of Patients by Platelet Microvesicle Content on the 3rd Day Post-Surgery

Characteristics	I Group, n=120	II Group, n=79	III Group, n=51	Total, n=250
Age ± SD, years	52,8±7,4	58,2±9,1	53,9±7,2	57,4±8,3
Male (%)	24 (20,0)	28 (35,4)	45 (88,2)	97 (38,8)
Diagnosis in entering, n (%)				
Healthy	50 (41,7)	0 (0,0)	0 (0,0)	50 (20,0)
ACS with CAG	5 (4,2)	19 (24,1)	5 (9,8)	30 (12,0)
Gallstone Disease	61 (50,8)	34 (43,0)	5 (9,8)	100 (40,0)
IHD with rhythm disturbance	4 (3,3)	26 (32,9)	41 (80,4)	70 (28,0)
Mortality, n (%)	1 (0,8)	2 (2,5)	12 (23,5)	13 (5,2)

It was established that the level of mcv content correlates with platelet hyperaggregation: low platelet aggregation levels corresponded to low mcv levels ($r=0.984$, $r^2=0.962$, $p=0.001$). Patients who experienced thrombotic episodes were predominantly found in the group with high levels of platelet microvesicles.

In the next phase, the prognostic significance of determining blood platelet microvesicle levels as a factor

for thrombosis development was assessed (Table 3) and evaluated as a predictor of complications (Figure 5).

Table 3. Evaluation of the Prognostic Value of Platelet Microvesicle Levels in Diagnosing Thrombotic Complications

Indicator	Values
Diagnostic Sensitivity, %	86,9 (78,3-95,2)
Diagnostic Specificity, %	31,2 (25,3-47,9)
Positive Predictive Value, %	97,8 (91,5-98,7)
Negative Predictive Value, %	26,3 (21,1-34,5)

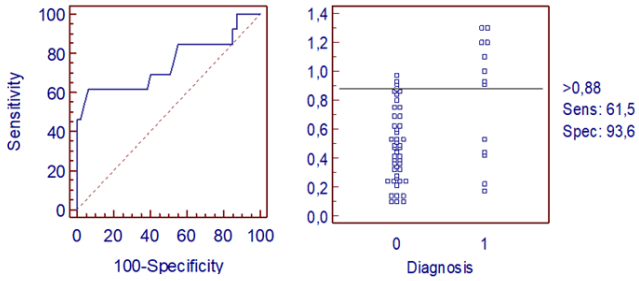

Figure 5. Distribution of Deceased and Surviving Patients and ROC Curve of Microvesicle Content

It was determined that the median sensitivity of the diagnostic test was 86.9%, while the specificity was 31.2%. The positive predictive value was 97.8% (indicating a high likelihood of thrombosis development in patients with elevated mcv levels), whereas the negative predictive value was 26.3% (indicating a lower likelihood of thrombosis development in patients with low mcv levels).

The study was concluded with an analysis of the relationship between mortality and mcv levels. Mortality due to thrombosis within 28 days after hospitalization was 23.5% in the group with high mcv levels and 0.8% in the group with low mcv levels. The area under the ROC curve (AUC) for mcv content was 0.831±0.083, with a 95% confidence interval (CI) of 0.719 - 0.941. Sensitivity was 61.5% (54.7-82.3), and specificity was 93.6% (83.5-98.1). The optimal cutoff point was 0.88. The positive predictive value was 74.5%, and the negative predictive value was 87.5%.

In conclusion, patients with cardiac rhythm disorders associated with ischemic myocardial damage exhibit elevated levels of circulating procoagulant microvesicles, which correlate with an increased risk of thrombosis. The procoagulant activity of microvesicles is likely due to the presence of phosphatidylserine and tissue factor on their surfaces. These microvesicles serve as carriers of procoagulant activity throughout the systemic circulation. The content of circulating procoagulant microvesicles correlates with the risk of thrombosis, including thrombosis associated with cancer.

4. Conclusions

The study underscores the significant role of platelet microvesicles (PMVs) in the development of thrombotic complications among patients with cardiac rhythm disorders, particularly those associated with ischemic myocardial damage. Elevated levels of PMVs, characterized by their procoagulant activity due to the presence of phosphatidylserine (PS) and tissue factor (TF), have been shown to correlate with increased risks of stroke and myocardial infarction.

The findings suggest that monitoring PMV levels could serve as a valuable prognostic tool in identifying high-risk patients who may benefit from more aggressive antithrombotic therapy. However, the study also highlights the need for further research to establish standardized thresholds for PMV levels and to explore targeted therapeutic strategies that can mitigate the procoagulant effects of PMVs without compromising their physiological roles in hemostasis and immune regulation.

In conclusion, this research contributes to the growing body of evidence supporting the use of PMVs as biomarkers for thrombotic risk in patients with cardiac rhythm disorders. Future studies should focus on refining the diagnostic and therapeutic applications of PMV measurement to improve patient outcomes and reduce the incidence of thrombotic complications.

REFERENCES

[1] Y. C. Lau, M. Proietti, E. Guiducci, A. D. Blann, and G. Y. H. Lip, “Atrial Fibrillation and Thromboembolism in Patients With Chronic Kidney Disease,” Journal of the American College of Cardiology, vol. 68, no. 13, pp. 1452–1464, Sep. 2016, doi: 10.1016/j.jacc.2016.06.057.

[2] J. O. Burton et al., “Elevated levels of procoagulant plasma microvesicles in dialysis patients,” PloS one, vol. 8, no. 8, p. e72663, 2013.

[3] M. M Polovina, G. YH Lip, and T. S Potpara, “Endothelial (dys) function in lone atrial fibrillation,” Current pharmaceutical design, vol. 21, no. 5, pp. 622–645, 2015.

[4] A. E. Berezin and A. A. Berezin, “Extracellular vesicles and thrombogenicity in atrial fibrillation,” International Journal of Molecular Sciences, vol. 23, no. 3, p. 1774, 2022.

[5] Å . Thulin, J. Lindbäck, C. B. Granger, L. Wallentin, L. Lind, and A. Siegbahn, “Extracellular vesicles in atrial fibrillation and stroke,” Thrombosis Research, vol. 193, pp. 180–189, 2020.

[6] B. J. Hirsh, R. S. Copeland-Halperin, and J. L. Halperin, “Fibrotic Atrial Cardiomyopathy, Atrial Fibrillation, and Thromboembolism,” Journal of the American College of Cardiology, vol. 65, no. 20, pp. 2239–2251, May 2015, doi: 10.1016/j.jacc.2015.03.557.

[7] G. R. S. Athayde et al., “Impact of left atrial compliance improvement on functional status after percutaneous mitral valvuloplasty,” Cathet Cardio Intervent, vol. 93, no. 1, pp. 156–163, Jan. 2019, doi: 10.1002/ccd.27831.

[8] K. H. Kim et al., “Left atrial remodelling in patients with successful percutaneous mitral valvuloplasty: determinants and impact on long-term clinical outcome,” Heart, vol. 96, no. 13, pp. 1050–1055, 2010.

[9] K. H. Kim et al., "Left atrial remodelling in patients with successful percutaneous mitral valvuloplasty: determinants and impact on long-term clinical outcome," Heart, vol. 96, no. 13, pp. 1050–1055, 2010.

[10] T. Watson, E. Shantsila, and G. Y. Lip, "Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited," The Lancet, vol. 373, no. 9658, pp. 155–166, 2009.

[11] X. Loyer, A.-C. Vion, A. Tedgui, and C. M. Boulanger, "Microvesicles as Cell–Cell Messengers in Cardiovascular Diseases," Circulation Research, vol. 114, no. 2, pp. 345–353, Jan. 2014, doi: 10.1161/CIRCRESAHA.113.300858.

[12] L. Wisgrill et al., "Peripheral blood microvesicles secretion is influenced by storage time, temperature, and anticoagulants," Cytometry Pt A, vol. 89, no. 7, pp. 663–672, Jul. 2016, doi: 10.1002/cyto.a.22892.

[13] J. Jiang et al., "Platelet Membrane-Fused Circulating Extracellular Vesicles Protect the Heart from Ischemia / Reperfusion Injury," Adv Healthcare Materials, vol. 12, no. 21, p. 2300052, Aug. 2023, doi: 10.1002/adhm.202300052.

[14] I. Melki, N. Tessandier, A. Zufferey, and E. Boilard, "Platelet microvesicles in health and disease," Platelets, vol. 28, no. 3, pp. 214–221, Apr. 2017, doi:10.1080/09537104.2016.1265924.

[15] Y. Pan et al., "Platelet-derived microvesicles (PMVs) in cancer progression and clinical applications," Clin Transl Oncol, vol. 25, no. 4, pp. 873–881, Nov. 2022, doi: 10.1007/s12094-022-03014-3.

[16] M. Aatonen, M. Grönholm, and P. Siljander, "Platelet-Derived Microvesicles: Multitalented Participants in Intercellular Communication," Semin Thromb Hemost, vol. 38, no. 01, pp. 102–113, Feb. 2012, doi: 10.1055/s-0031-1300956.

[17] A. E. Berezin and A. A. Berezin, "Platelet-derived vesicles: diagnostic and predictive value in cardiovascular diseases," J Unexplored Med Data, vol. 4, no. 4, 2019, Accessed: Aug. 12, 2024. [Online]. Available: https://pdfs.semanticscholar.org/9c17/b9d52f423e5f8a4931e260cf55d8994b527a.pdf.

[18] I. Cruz-Gonzalez et al., "Predicting success and long-term outcomes of percutaneous mitral valvuloplasty: a multifactorial score," The American journal of medicine, vol. 122, no. 6, pp. 581-e11, 2009.

[19] I. Cruz-Gonzalez et al., "Predicting success and long-term outcomes of percutaneous mitral valvuloplasty: a multifactorial score," The American journal of medicine, vol. 122, no. 6, pp. 581-e11, 2009.

[20] I. Savelieva, A. Bajpai, and A. John Camm, "Stroke in atrial fibrillation: Update on pathophysiology, new antithrombotic therapies, and evolution of procedures and devices," Annals of Medicine, vol. 39, no. 5, pp. 371–391, Jan. 2007, doi: 10.1080/07853890701320662.

[21] S. Rafaqat, S. Gluscevic, D. Patoulias, S. Sharif, and A. Klisic, "The Association between Coagulation and Atrial Fibrillation," Biomedicines, vol. 12, no. 2, p. 274, 2024.

[22] H. Wang et al., "The microvesicle/CD36 complex triggers a prothrombotic phenotype in patients with non-valvular atrial fibrillation," J Cellular Molecular Medi, vol. 24, no. 13, pp. 7331–7340, Jul. 2020, doi: 10.1111/jcmm.15311.

[23] A. A. Khan and G. Y. Lip, "The prothrombotic state in atrial fibrillation: pathophysiological and management implications," Cardiovascular research, vol. 115, no. 1, pp. 31–45, 2019.

[24] B. Coll-Vinent et al., "Transient endothelial dysfunction is present shortly after cardioversion in patients with lone atrial fibrillation," Thrombosis research, vol. 117, no. 3, pp. 235–240, 2006.

[25] I. F. Palacios, P. L. Sanchez, L. C. Harrell, A. E. Weyman, and P. C. Block, "Which Patients Benefit From Percutaneous Mitral Balloon Valvuloplasty?: Prevalvuloplasty and Postvalvuloplasty Variables That Predict Long-Term Outcome," Circulation, vol. 105, no. 12, pp. 1465–1471, Mar. 2002, doi: 10.1161/01.CIR.0000012143.27196.F4.

[26] I. F. Palacios, P. L. Sanchez, L. C. Harrell, A. E. Weyman, and P. C. Block, "Which Patients Benefit From Percutaneous Mitral Balloon Valvuloplasty?: Prevalvuloplasty and Postvalvuloplasty Variables That Predict Long-Term Outcome," Circulation, vol. 105, no. 12, pp. 1465–1471, Mar. 2002, doi: 10.1161/01.CIR.0000012143.27196.F4.