International Heart and Vascular Disease Journal. Volume 13, № 45, March 2025
ISSN: 2311-1623 (Print)
ISSN: 2311-1631 (Online)
http://www.heart-vdj.com

Prediction of recurrent cardiovascular events within the first year after a type 2 acute myocardial infarction in men under 45 years of age

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In recent years, cardiovascular diseases (CVD) have remained among the top three causes of mortality and disability worldwide. In this regard, the search for and study of risk factors, identification of pathogenetic mechanisms underlying cardiovascular complications, and improvement of primary and secondary prevention methods continue. Currently, there is emerging evidence on important components of the hemostatic system, including extracellular formations of various sizes — microvesicles of different or-

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igins. Research into their role in the pathogenesis of acute arterial thromboses is ongoing. Microvesicles (EVs) are extracellular structures that trigger a cascade of mechanisms affecting changes in the vascular endothelium.

The aim of the study is to investigate the role of microvesicles of different origins in the risk of recurrent cardiovascular events within 1 year after a type 2 acute myocardial infarction (AMI) in men under 45 years of age.

Methods. The study included young men under 45 years of age with type 2 AMI, confirmed by coronary angiography and elevated markers of myocardial injury. The control group consisted of young men under 45 years of age without chronic diseases. The main group consisted of 60 patients, the control group included 30 healthy volunteers. The study investigated microvesicles of platelet (CD41+), endothelial (CD31+ — platelet endothelial adhesion molecule), and leukocyte origin (CD45+) in peripheral blood. To determine the quantity of microvesicles, venous blood samples were examined using 0.32% sodium citrate anticoagulant and processed within 2 hours after blood collection. The samples were processed through sequential centrifugation. All measurements were performed on a CytoFlex flow cytometer (Beckman Coulter, USA) equipped with three lasers: Red (638 nm), Blue (488 nm), and Violet (405 nm). Extracellular vesicles were separated by diameter, and measurements were taken for unstained samples to set positive gates. Subsequently, the material was stained, mixed again, and measured after diluting the sample 100 times using phosphate-buffered saline (PBS x10, Bio-Rad). In each analysis, 1 million events were recorded at a flow rate of 60 µl/min. For statistical processing, the parameter of the number of events (extracellular events) per 1 µl was used.

Results. While studying microvesicles, it was found that the levels of CD31+ EVs (endothelial) and CD45+ EVs (leukocyte-derived) were comparable in healthy volunteers and patients with type 2 AMI under 45 years of age. A decrease in the expression of CD31+ EVs by the endothelial glycocalyx was observed, which may indicate its consumption. A difference was observed in the number of CD45+ EVs in patients with recurrent cardiovascular events (CVE) within one year, while during a CVE episode, the number of CD31+ EVs decreased.

Conclusion. During the acute phase of type 2 AMI, the level of CD45+ EVs was statistically significantly higher in patients who subsequently experienced recurrent CVEs within one year.

Keywords: type 2 acute myocardial infarction, microvesicles, cardiovascular complications, risk factors, hemostasis, recurrent cardiovascular events

Conflict of interests: none declared.

Recieved: 20.12.2024
Review date: 21.01.2025
Accepted: 02.01.2025

For citation: Gorbacheva N.S., Veselovskaya N.G., Nikolaeva M.G. et al. Prediction of recurrent cardiovascular events within the first year after a type 2 acute myocardial infarction in men under 45 years of age. International Journal of Heart and Vascular Diseases. 2025. 13(45):23–29. DOI: 10.24412/2311-1623-2025-45-28-36

Introduction

In recent years, cardiovascular diseases (CVD) have remained among the top three causes of mortality and disability worldwide. This has led to ongoing research into risk factors (RF), the identification of pathogenetic mechanisms underlying cardiovascular complications (CVC), and the improvement of primary and secondary prevention methods [1–3].

Currently, traditional RFs for CVD (hypertension, obesity, smoking, alcohol consumption, dyslipidemia, diabetes mellitus) and non-traditional RFs (gender, age) have been extensively studied [4–6]. The persistent trend in CVD morbidity and mortality highlights the need to explore potential causes and new RFs [7].

In recent decades, factors related to vascular-platelet and coagulation hemostasis, the fibrinolytic system (coagulation factors II and XIII), and microvesicles (EVs) of various origins have been actively studied in the context of arterial thrombosis risk in young patients without traditional atherosclerosis RFs [8, 9]. Previously, EVs were studied in oncology, obstetrics and gynecology, and wound surface surgery, with emerging evidence of their role in cardiology. As is known, microvesicles (EVs), which are extracellular formations, trigger a cascade of mechanisms causing changes in the vascular endothelium, thereby contributing to thromboembolic complications [10–12].

Moreover, imbalances in the hemostatic system are influenced by gene polymorphisms and comorbid somatic diseases, which can lead to acute vascular events [13, 14].

During acute myocardial infarction (AMI), blood cells and endothelial cells release EVs, the quantity of which can be measured in peripheral blood [15].

It can be hypothesized that changes in the concentration of EVs of various origins after AMI may underlie recurrent cardiovascular events (CVE). EVs have also been shown to actively participate in angiogenesis and myocardial remodeling [16, 17].

To date, the functions of exogenous EVs derived from cultured pluripotent cells in vitro have been sufficiently studied, but there is limited data on the functions of endogenous EVs in type 2 AMI. Theoretical insights into the potential role of EVs of various origins in the development of type 2 AMI and early complications after vascular events have provided the basis for their investigation in a cohort of young men with type 2 AMI and early recurrent CVEs.

The aim of the study is to investigate the role of microvesicles of different origins in the risk of recurrent cardiovascular events within 1 year after a type 2 AMI in men under 45 years of age

Methods

The study was conducted at the Altai Regional Cardiological Dispensary in Barnaul. Laboratory diagnostic tests and flow cytometry were performed in the hemostasis laboratory of the Regional Clinical Hospital of Emergency Medical Care No. 2.

To determine the quantity of microvesicles, venous blood samples were examined using 0.32% sodium citrate anticoagulant and processed within 2 hours after blood collection. The samples were processed through sequential centrifugation. All measurements were performed on a CytoFlex flow cytometer (Beckman Coulter, USA) equipped with three lasers: Red (638 nm), Blue (488 nm), and Violet (405 nm). Extracellular vesicles were separated by diameter, and measurements were taken for unstained samples to set positive gates. Subsequently, the material was stained, mixed again, and measured after diluting the sample 100 times using phosphate-buffered saline (PBS x10, Bio-Rad). In each analysis, 1 million events were recorded at a flow rate of 60 µl/min, ensuring that no objects larger than 1000 nm were present in the analyzed area. For statistical processing, the parameter of the number of events (extracellular events) per 1 µl was used.

The study included 90 men under 45 years of age (41.5±1.5 years), of whom 30 were healthy volunteers and 60 were diagnosed with type 2 AMI with ST-segment elevation without comorbidities. The diagnosis of type 2 AMI was established based on coronary angiography data, which showed no atherosclerotic lesions in the coronary arteries, and an elevated concentration of troponin I in peripheral blood. All patients admitted with AMI were assessed for traditional RFs of AMI, coagulation hemostasis, and the fibrinolytic system. Blood serum analysis was also performed to determine microvesicles (EVs) of various origins using flow cytometry.

Prospective follow-up was conducted for 1 year from the time of patient inclusion in the study. Adverse CVEs included recurrent AMI, death, and acute cerebrovascular accident (stroke).

In our study, microvesicles of platelet (CD41+), endothelial (CD31+ — platelet endothelial adhesion molecule), and leukocyte origin (CD45+) in peripheral blood were investigated.

Limitations of the study included a relatively small cohort of patients (60 individuals) with type 2 AMI without atherosclerotic lesions, and limited data in the literature on microvesicles and their role in arterial thromboses.

Inclusion criteria: Men with type 2 AMI under 45 years of age, signed informed consent, elevated markers of myocardial injury, and absence of stenotic lesions according to coronary angiography.

Exclusion criteria: Female sex and men over 45 years of age; presence of severe comorbidities (chronic obstructive pulmonary disease, liver or kidney failure, oncological diseases); previously diagnosed type 1 or type 2 diabetes mellitus; history of stroke; post-infarction cardiosclerosis; cardiac arrhythmias and conduction disorders (atrial fibrillation, atrioventricular or sinoatrial block).

Statistical analysis

Statistical processing and graphical representation of data were performed using computer programs MedCalc 20.0.27 and Statistica 12.0 (StatSoft).

To assess the influence of the studied factors and indicators, the method of multiple binary logistic regression was used. Based on stepwise analysis, several factors (predictors) statistically significantly influencing the outcome ($p < 0.05$) were included in the regression model, with one of the study indicators being EVs. To compare two independent groups regarding quantitative characteristics, the Nagelkerke

DOI: 10.24412/2311-1623-2025-45-28-36

coefficient was used. The obtained data are presented as the arithmetic mean (M) and standard deviation (SD), with differences considered statistically significant at $p < 0.05$.

Results

While analyzing traditional RFs, the mean values of the body mass index (BMI) were obtained: 26.76±3.36 kg/m² in the control group and 31.29±14.00 kg/m² in the main group. In the subgroup with CVE, the BMI was 30.4±4.6 kg/m², and in the subgroup without CVE, it was 30.8±6.2 kg/m², showing no significant difference between the comparison groups.

The total cholesterol (TC) level in the main group was 6.71±1.23 mmol/L, while in the control group, it was 4.78±1.34 mmol/L. When comparing TC results in the subgroup with CVE (6.89±2.1 mmol/L) and without CVE (6.34±1.6 mmol/L), no significant differences were found between these groups.

During the study, platelet-derived EVs (CD41+) in blood plasma were determined in both groups. Comparative analysis of blood plasma in patients from the main and control groups revealed a concentration of 953.65±164 per μL in the main group and 1005.3±98.7 per μL in the control group. In the subgroup of type 2 AMI with CVE, the concentration of CD41+ EVs was 892±95.4 per μL, and without CVE, it was 934.8±76.5 per μL. Comparative analysis between the groups showed no significant differences (p=0.965) (Fig. 1).

While analyzing leukocyte-derived microvesicles (CD45+), their concentration in the control group was 2267.9±1104.43 per μL, and in the main group, it was 2486.91±1003.56 per μL, also showing no significant difference between the comparison groups (p=0.568) (Fig. 2).

In the analysis of endothelial-derived EVs (CD31+), their concentration in the control group was 123.061±72.8 per μL, and in the main group, it was 87.43±62.55 per μL. In the type 2 AMI subgroup with CVE, the concentration of CD31+ EVs was 118.14±87.12 per μL, and without CVE, it was 102.56±73.35 per μL.

A decrease in their concentration was observed in the type 2 AMI patient group, which was likely due to the binding of CD31+ to blood cells and the endothelial glycocalyx (a consumption mechanism) (Fig. 3).

While evaluating CD31+ EVs as predictors of recurrent CVEs after 1 year using ROC analysis, the sensitivity was 48.1%, and the specificity was 72.7%, indicating a good quality of the predictive model for recurrent CVEs based on CD45+ EV levels (Fig. 4).

According to the regression analysis model, a statistically significant influence of microvesicles (CD41+ EVs, CD31+ EVs, and CD45+ EVs) on the dependent variable — the likelihood of CVE development — was identified (χ^2=128.7; p<0.001). It was also shown that these predictors determine the outcome (dependent

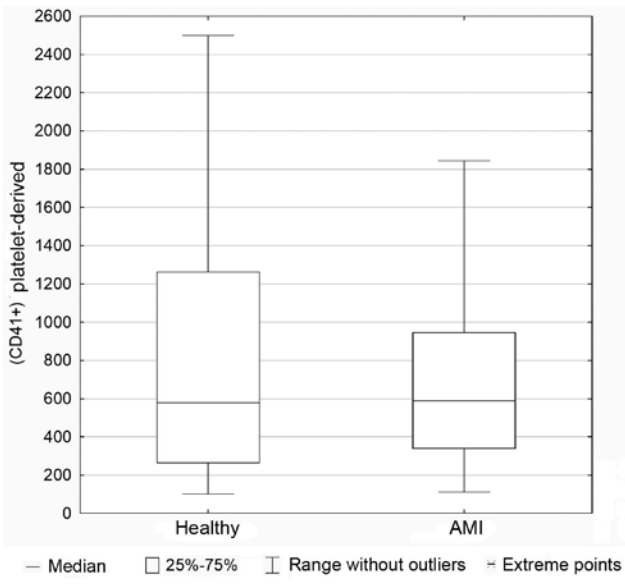

Fig. 1. CD41+ EVs in the comparison group of healthy men and patients with type 2 AMI

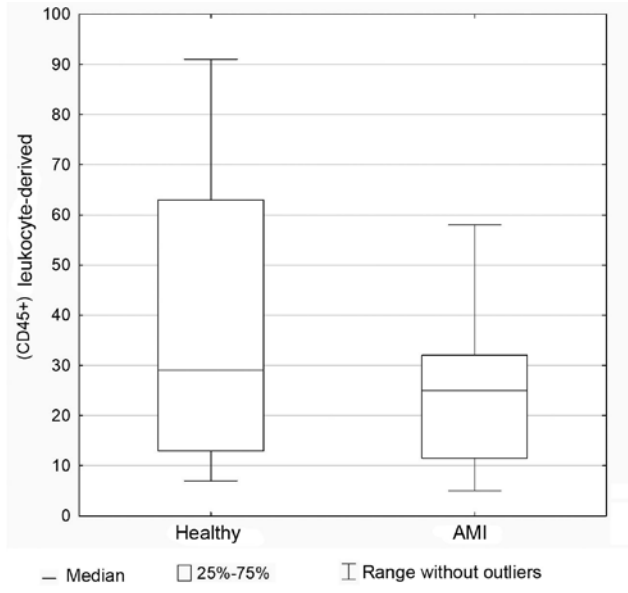

Fig. 2. CD45+ EVs in the comparison group of healthy men and patients with type 2 AMI

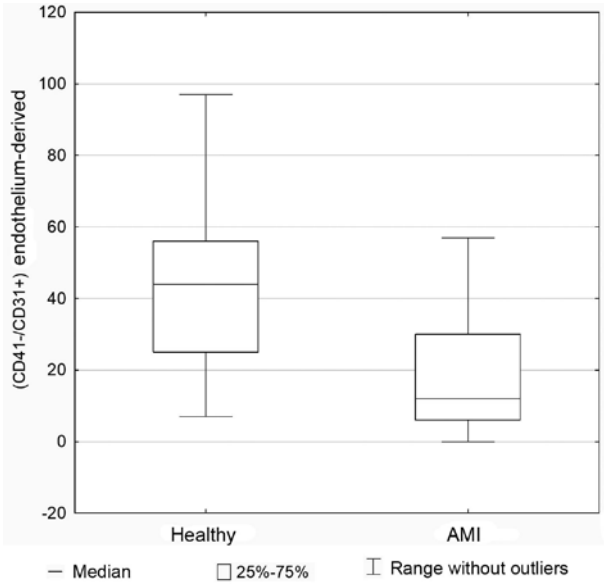

Fig. 3. CD31+ EVs in the comparison group of healthy men and patients with type 2 AMI

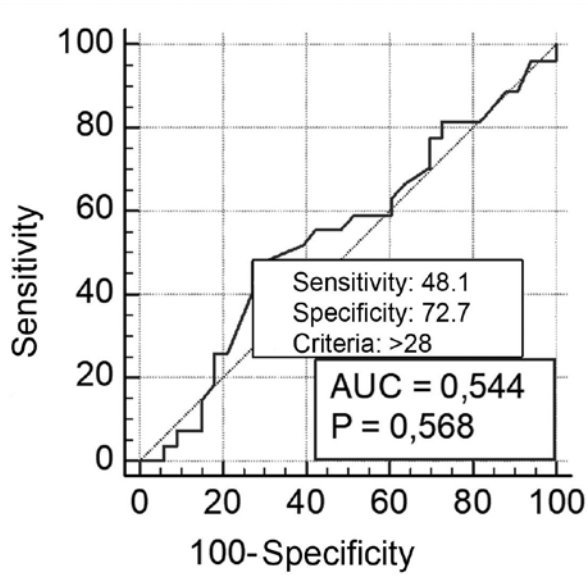

Fig. 4. ROC-curve of EVs CD 45+

variable) by 40.9%. Thus, the obtained data indicate the need for further research into microvesicles in the risk of CVE development, especially in young individuals.

Discussion

In recent decades, CVDs have maintained their leading position in morbidity, mortality, and disability. According to the published Global Burden of Disease (GBD) report on December 11, 2023, the mortality rate from coronary heart disease (CHD) is 108.8 deaths per 100,000 people. Statistics show that 1 million people die annually from CVDs in Russia[1]. AMI is one of the forms of cardiovascular catastrophes. According to statistics, the number of patients with AMI is increasing annually, with a trend toward younger patients. Among young people aged 20–24 years in Russia, mortality from AMI increased by 82% from 2018 to 2023, and among those aged 30–35 years, it increased by 63% [18]. The prevalence of AMI in men is almost 5 times higher than in women[2].

Based on statistical data, young men with type 2 AMI, who show an increased incidence of CVCs after acute coronary events, are of particular interest. This patient group remains understudied.

According to epidemiological studies, traditional RFs have been well studied [4–6], but non-traditional risk factors, such as indicators of coagulation hemostasis (factor II [18], factor XIII [19]), and EVs [11], which were previously studied in oncological processes [20], gynecological pathology [21], and obstetrics [22], have a profound and significant impact.

In our study, among the numerous types of microvesicles, CD45+ EVs, CD31+ EVs, and CD41+ EVs, which have tropism for the vascular wall, were selected.

CD45+ EVs initiate signaling events through their C-terminal PTPase domain, leading to endothelial-mesenchymal transition — a dynamic process in which endothelial cells acquire mesenchymal properties that promote vascular remodeling and tissue proliferation. Comparative analysis revealed that elevated levels of CD45+ EVs in patients with recurrent CVEs may indicate a primary excessive inflammatory response.

CD31+ EVs activation is associated with signaling events that trigger the anti-atherogenic production of NO by the endothelium, causing vasodilation mediated by CD31+ EVs. Additionally, CD31+ EVs are important for switching cellular phenotypes necessary for accelerating wound healing after the inflammation phase.

A decrease in the concentration of CD31+ EVs was observed in the type 2 AMI patient group, likely due to

[1] Morbidity of the population by major classes of diseases in 2000-2023. Federal State Statistics Service (Rosstat). — (Data from the Russian Ministry of Health, calculated by Rosstat) 29.11.2024 Russian

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the binding of CD31+ to blood cells and the endothelial glycocalyx (a consumption mechanism).

CD41+ EVs are the most numerous and coagulation-active, accounting for about 70–90% of the total EVs in blood plasma. They affect the vascular endothelium, inducing changes by transferring arachidonic acid to endothelial cells and enhancing platelet adhesion due to the presence of collagen-binding receptor proteins in their composition [23].

Thus, for a comprehensive assessment of RFs for recurrent CVEs in patients after a first-time AMI, along with traditional RFs, it is necessary to evaluate hemostatic system indicators to predict complications and select secondary prevention methods.

Conclusion

Thus, the study of microvesicles revealed that the levels of CD31+ EVs (endothelial) and CD45+ EVs (leukocyte-derived) were comparable in healthy volunteers and patients with type 2 AMI under 45 years of age. A decrease in the expression of CD31+ EVs by the endothelial glycocalyx was observed, which may indicate a consumption mechanism. During the acute phase of type 2 AMI, the level of CD45+ EVs was statistically significantly higher in patients who subsequently experienced recurrent CVEs within one year.

Further research in this direction will contribute to understanding the mechanisms of arterial thromboses and early CVEs in young patients, as well as identifying high-risk groups in combination with genetic testing for thrombogenic mutations. This will enable the development of individualized preventive programs, potentially including adjustments to antiplatelet/anticoagulant therapy.

Conflict of interests: none declared.

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