МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН
ФАКУЛТЕТ „МЕДИЦИНА”

DEPARTMENT OF CARDIOLOGY, PULMONOLOGY AND ENDOCRINOLOGY

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Title: Anticoagulant and Antiplatelet Therapy in Ischemic Heart Disease – New Opportunities

Abstract

Dissertation submitted for the award of the educational and scientific degree of Doctor (PhD)

Doctoral program Cardiology

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The dissertation consists of 142 pages.

It contains a total of 23 figures and 11 tables.

The bibliography includes 130 references, of which 112 are in Latin script and 18 are in Cyrillic.

The dissertation was reviewed and approved for public defense by the Extended Departmental Council of the Department of Cardiology, Pulmonology and Endocrinology, Medical University – Pleven.

The clinical material related to the dissertation was collected at the Clinic of Cardiology, and the main investigations were carried out at the Clinic of Cardiology and the Clinic of Immunology, Medical University – Pleven.

The public defense of the dissertation will take place on _____ at _____ in Hall _____.

The dissertation materials are available for review at the Library of the Medical University – Pleven.

Abbreviations

2D Echo – Two-dimensional echocardiography

ACE2 – Angiotensin-converting enzyme 2

BP – Blood pressure

APC – Acute plaque change

ARDS – Acute respiratory distress syndrome

BMI – Body mass index

CABG – Coronary artery bypass grafting

CAD – Coronary artery disease

CK – Creatine kinase

CK-MB – MB fraction of creatine kinase

COVID-19 – Coronavirus disease 2019 (caused by SARS-CoV-2)

ECG – Electrocardiogram

ELISA – Enzyme-linked immunosorbent assay

EF – Ejection fraction

HDL – High-density lipoprotein

hs-cTnT – High-sensitivity cardiac troponin T

IL-1 – Interleukin-1

IL-1Ra – Interleukin-1 receptor antagonist

IL-6 – Interleukin-6

IU – International units

CT – Computed tomography

LDL – Low-density lipoprotein

MI – Myocardial infarction

UA – Unstable angina

NO – Nitric oxide

NSTEMI – Non-ST-elevation myocardial infarction

nm – Nanometer

ACS – Acute coronary syndrome

AMI – Acute myocardial infarction

PCI – Percutaneous coronary intervention

PCR – Polymerase chain reaction

pg/mL – Picograms per milliliter

SBP – Systolic blood pressure

SCA – Selective coronary angiography

CVD – Cardiovascular disease

STEMI – ST-elevation myocardial infarction

DM – Diabetes mellitus

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Introduction

Cardiovascular diseases (CVDs) account for approximately 60–65% of overall mortality worldwide. Among the leading causes of cardiovascular death is acute coronary syndrome (ACS), which is characterized by a sudden reduction in coronary blood flow, most commonly resulting from narrowing or occlusion of a coronary artery. The underlying pathological process is atherosclerosis.

Although atherosclerosis is present in virtually all patients with coronary artery disease, not every patient develops ACS. Over the years, several hypotheses have been proposed to explain the destabilization of atherosclerotic plaques. Currently, the chronic inflammation hypothesis is considered the most widely accepted mechanism.

Macrophages play a pivotal role in the development of atherosclerotic plaques. Following the phagocytosis of oxidized low-density lipoprotein (oxLDL), macrophages transform into foam cells. These foam cells secrete pro-inflammatory cytokines, including interleukin-1 (IL-1) and interleukin-6 (IL-6), which contribute to plaque progression and instability.

The major risk factors for plaque destabilization have also been well established. They are classified as modifiable (arterial hypertension, diabetes mellitus, smoking, and metabolic syndrome) and non-modifiable (sex, age, race, and family history). These risk factors define the patient's cardiovascular risk profile and help estimate the likelihood of developing ACS.

In late November 2019, the first case of COVID-19 was reported in Wuhan, China. The first confirmed case in Bulgaria was registered on March 8, 2020. Although COVID-19 primarily affects the respiratory system, it has been demonstrated to induce inflammatory responses in multiple organs, including the cardiovascular system.

Early studies investigating the effects of COVID-19 revealed a marked release of pro-inflammatory cytokines, particularly IL-1 and IL-6, resulting in excessive recruitment of inflammatory cells such as neutrophils and monocytes.

This inflammatory response has two major consequences for the cardiovascular system: accelerated formation of atherosclerotic plaques and direct myocardial injury caused by acute viral infection.

Similar to other viral infections, the inflammatory response induced by COVID-19 promotes rupture of the fibrous cap of atherosclerotic plaques, thereby triggering acute coronary syndrome. Accelerated plaque formation is likewise driven by inflammation, primarily through direct endothelial injury and dysfunction.

COVID-19 has also been shown to affect skeletal muscle mass and density. Experimental studies suggest that SARS-CoV-2 enters muscle tissue via ACE2 receptors, contributing to muscle loss, reduced bone mass, and joint inflammation.

The inflammatory cytokines IL-1 and IL-6, released during the inflammatory response, may further promote tissue fibrosis through increased activation of muscle fibroblasts.

The progressive loss of skeletal muscle mass and strength is defined as sarcopenia. Numerous studies have demonstrated an association between sarcopenia and adverse outcomes in patients with ACS, leading to recommendations for the incorporation of structured physical rehabilitation during hospitalization. However, the COVID-19 pandemic and the implementation of isolation measures substantially limited the application of such rehabilitation programs, thereby complicating the management and prolonging the recovery of patients with ACS.

Furthermore, several reports published during the pandemic documented prolonged delays between the onset of ACS symptoms and patients seeking medical attention. These delays significantly increased the risk of complications and contributed to longer hospital stays.

Aim and Objectives

Aim:

To investigate the impact of COVID-19 as a potential risk factor for the development of acute coronary syndrome (ACS) by evaluating patients' clinical characteristics and inflammatory biomarkers (IL-1 and IL-6) in patients with established ACS and a history of COVID-19 infection.

Objectives

1. To establish two study groups: patients with ACS and a previous COVID-19 infection, and patients with ACS without a history of COVID-19 infection, who were hospitalized at the Clinic of Cardiology, University Hospital "Dr. Georgi Stranski", Pleven, between April 2020 and April 2021.
2. To compare the two study groups in sex, age, and family history.
3. To compare the cardiovascular risk profiles of the study and control groups.
4. To determine the time interval between COVID-19 infection and the occurrence of the ischemic event.
5. To compare the number of affected coronary vessels and the primary admission diagnosis between the two groups.
6. To compare cardiovascular risk factors in patients with ACS and previous COVID-19 infection with those in patients with ACS without prior COVID-19 infection in a cohort of 158 participants included in Research Project No. 1/2021.
7. To compare the results obtained from this cohort with those of the larger study population consisting of 515 patients.
8. To compare the time interval between the onset of anginal symptoms and the patient's first medical consultation in the two groups.
9. To evaluate the impact of COVID-19 on skeletal muscle strength and determine the prevalence of sarcopenia in both groups.

10. To investigate inflammatory biomarkers, particularly IL-1 and IL-6, as potential predictors of ACS in patients with previous COVID-19 infection.
11. To assess the prevalence of previous ischemic cardiovascular events in both study groups.

IV. Clinical Material and Methods

1. Study Population

A retrospective observational study was conducted among patients hospitalized at the Clinic of Cardiology, University Hospital "Dr. Georgi Stranski", Pleven, between April 2020 and April 2021, with clinically, laboratory, and angiographically confirmed acute coronary syndrome (ACS). ACS included the following clinical entities: unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI).

A total of 515 consecutive patients were enrolled in the study and were divided into two groups according to the presence or absence of previous COVID-19 infection.

Inclusion Criteria

1. Age ≥18 years.
2. Angiographic confirmation of ACS.
3. Complete medical history, including the major cardiovascular risk factors.
4. Polymerase chain reaction (PCR)-confirmed COVID-19 status.

Exclusion Criteria

1. Age <18 years.
2. Absence of PCR testing for COVID-19.
3. Absence of selective coronary angiography (SCA) confirming ACS.

2. **Inflammatory Biomarker Substudy**

A second retrospective study included 158 patients with angiographically confirmed ACS in whom inflammatory biomarkers were evaluated.

Patients were divided into two groups:

- patients with active COVID-19 infection or a previous COVID-19 infection occurring within six months before the ACS event, and
- patients with no history of COVID-19 infection.

Inclusion Criteria

1. Age ≥18 years.
2. Angiographic confirmation of ACS.
3. Complete medical history, including the major cardiovascular risk factors.
4. PCR-confirmed COVID-19 status.
5. Ability to provide written informed consent for participation in the study.

Exclusion Criteria

1. Age <18 years.
2. Absence of PCR testing for COVID-19.
3. Inability to provide written informed consent.
4. Refusal to provide written informed consent.
5. Absence of selective coronary angiography confirming ACS.

3. **Assessment of Sarcopenia**

In addition to the clinical evaluation, all participants completed the SARC-F questionnaire, a validated screening tool for the assessment of sarcopenia.

4. **Laboratory Investigations**

Inflammatory biomarkers interleukin-1 (IL-1) and interleukin-6 (IL-6) were measured using the enzyme-linked immunosorbent assay (ELISA) method.

5. **Instrumental Investigations**

All patients underwent:

- Two-dimensional echocardiography (2D echocardiography); and
- Selective coronary angiography (SCA).

Results

Analysis of the study population demonstrated a predominance of male patients in both groups. In **Group I**, 64 patients (59.8%) were male and 43 (40.2%) were female, whereas in **Group II**, 276 patients (67.7%) were male and 132 (32.3%) were female (**$\chi^2 = 2.319$, df = 1, p = 0.128**). No statistically significant difference was observed in the mean age between the two groups, with both having a mean age of **67 years** (**t = 0.004, df = 513, p = 0.997**).

Analysis of family history showed that **99 patients (92.5%)** in Group I had no family history of cardiovascular disease, compared with **344 patients (84.3%)** in Group II, representing a statistically significant difference (**$\chi^2 = 4.751$, df = 1, p = 0.029**).

Table 1. Demographic characteristics of patients with acute coronary syndrome

Демографски характеристики	**Пациенти с анамнеза за Covid-19=107**	**Пациенти без анамнеза за Covid-19=408**	**Общо**
Пол			
Жени	43 (41.2%)	132 (32.3%)	175 (34.0%)
Мъже	64 (59.8%)	276 (67.7%)	340 (66.0%)
Възраст, *г.* (Mean ± SD)	67.0 ± 10.542	67.0 ± 10.283	67.0 ± 10.327
Фамилна анамнеза (брой, %)			
Да	8 (7.5%)	64 (15.7%)	72 (14.0%)
Не	99 (92.5%)	344 (84.3%)	443 (86.0%)

Age subgroup analysis demonstrated a similar distribution of ACS across the different age categories.

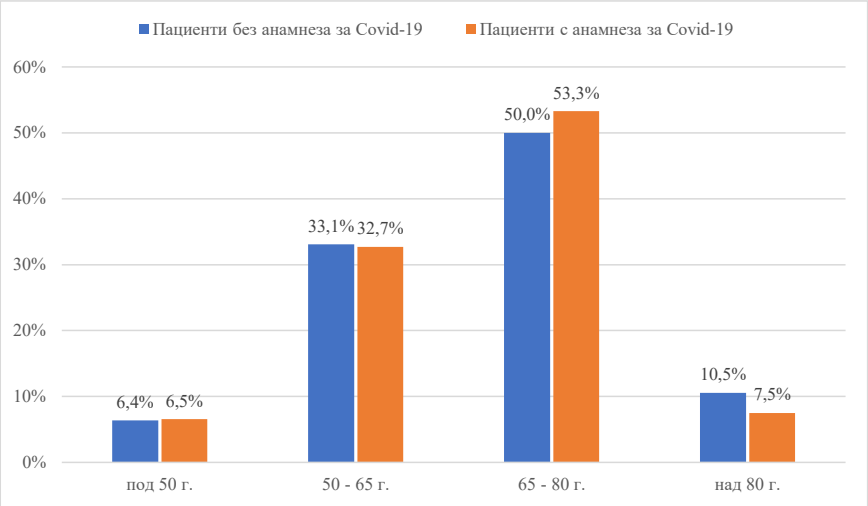

Figure 1. Distribution of patients with ACS according to age at diagnosis.

Within **Group I**, subgroup analysis demonstrated that the largest proportion of patients (**32.7%**) presented with **active or newly diagnosed COVID-19 infection** at the time of ACS. A second peak in ACS incidence (**29.9%**) was observed within six months after recovery from COVID-19. Overall, only **14.0%** of patients had experienced COVID-19 more than six months before hospitalization, including **6.5%** with an interval of 6–9 months and **7.5%** with an interval of 9–12 months.

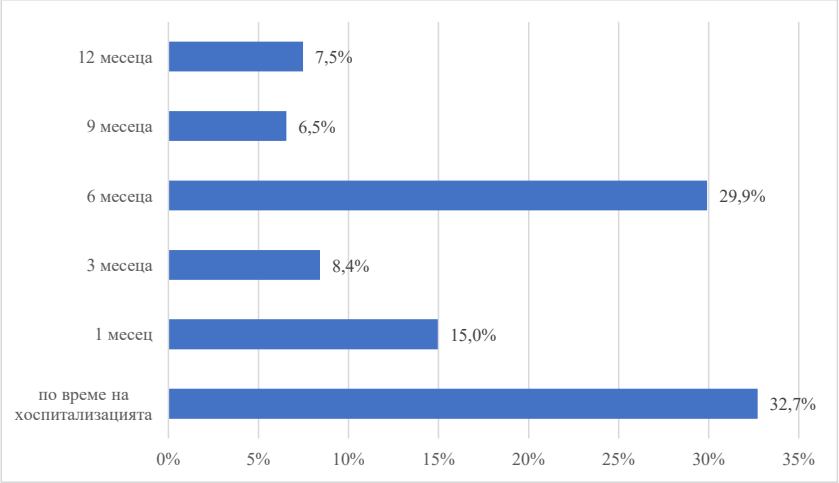

Figure 2. Time interval between COVID-19 diagnosis and the occurrence of acute coronary syndrome.

Analysis of the clinical presentation revealed that **unstable angina (UA)** was the predominant diagnosis in both groups, accounting for **76.6%** of cases in Group I and **68.9%** in Group II. **ST-elevation myocardial infarction (STEMI)** was diagnosed in **22.4%** and **27.9%**, respectively, while **NSTEMI** represented only **0.9%** and **3.2%** of cases.

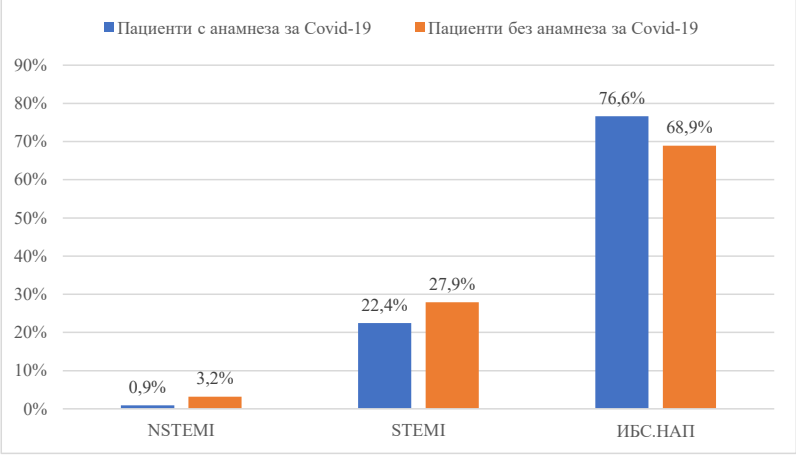

Figure 3. Distribution of admission diagnoses among patients with acute coronary syndrome.

Significant differences were observed in several cardiovascular risk factors between the two groups.

Diabetes mellitus was present in **35 patients (32.7%)** in Group I and **181 patients (44.3%)** in Group II (**χ^2 = 98.685, df = 1, p < 0.001**). The absence of diabetes mellitus was recorded in **72 patients (67.3%)** and **227 patients (55.7%)**, respectively (**χ^2 = 80.351, df = 1, p < 0.001**).

Smoking was significantly less common among patients with previous COVID-19 infection. Smokers accounted for **18.7%** of Group I and **34.1%** of Group II (**χ^2 = 9.392, df = 1, p = 0.002; Phi = −0.135**).

Alcohol consumption did not differ significantly between the groups, being reported by **5.7%** of patients in Group I and **9.1%** in Group II (**χ^2 = 1.327, df = 1, p = 0.249**).

Table 2. Modifiable cardiovascular risk factors in patients with acute coronary syndrome

Хронични рискови фактори	Пациенти с анамнеза за Covid-19 n=107	Пациенти без анамнеза за Covid-19 n=408	Общо
Захарен диабет (брой, %)			
да	35 (32.7%)	181 (44.3%)	216 (41.9%)
не	72 (67.3%)	227 (55.7%)	299 (58.1%)
Тютюнопушене (брой, %)			
да	20 (18.7%)	139 (34.1%)	159 (30.9%)
не	87 (81.3%)	269 (65.9%)	356 (69.1%)
Алкохол (брой, %)			
да	6 (5.7%)	37 (9.1%)	43 (8.3%)
не	101 (94.3%)	371 (90.9%)	472 (91.7%)

Among diabetic patients, **type 2 diabetes mellitus** predominated, accounting for **97.1%** of diabetic patients in Group I and **87.3%** in Group II. Newly diagnosed diabetes mellitus was more frequent in Group II (**11.0%**) than in Group I (**2.9%**).

Коментирал [EM1]: **Chi-square test (пациенти с анамнеза за Covid-19/пациенти без анамнеза за Covid-19):** χ^2 = 4.727, df=1, p = 0.030, Phi = -0.096 – има разлика

Chi-square test (със захарен диабет): χ^2 = 98.685, df=1, p = 0.000 – има разлика

Chi-square test (без захарен диабет): χ^2 = 80.351, df=1, p = 0.000 – има разлика

Коментирал [EM2]: **Chi-square test (пациенти с анамнеза за Covid-19/пациенти без анамнеза за Covid-19):** χ^2 = 9.392, df=1, p = 0.002, Phi = -0.135 – има разлика

Коментирал [EM3]: **Chi-square test (пациенти с анамнеза за Covid-19/пациенти без анамнеза за Covid-19):** χ^2 = 1.327, df=1, p = 0.249 – няма разлика

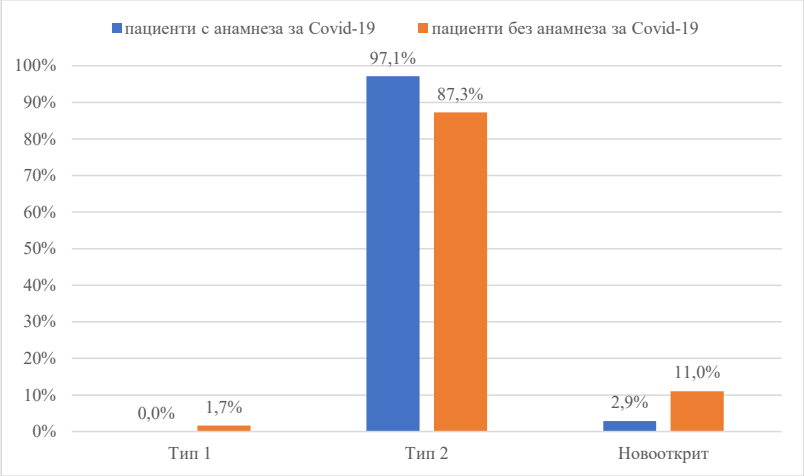

Figure 4. Distribution of diabetes mellitus type among patients with ACS.

No statistically significant differences were observed in the severity of arterial hypertension between the two groups ($\chi^2 = 5.325$, **df = 3, p = 0.150**).

Similarly, no significant differences were found regarding the number of diseased coronary vessels ($\chi^2 = 8.877$, **df = 4, p = 0.064**). Single-vessel disease was observed in **66.4%** of patients in Group I and **52.5%** in Group II, while two-vessel disease was present in **18.7%** and **27.5%**, respectively. Three-vessel disease occurred in **8.4%** versus **15.2%**, four-vessel disease in **5.6%** versus **4.4%**, and five-vessel disease in **0.9%** versus **0.2%**, respectively.

Table 3. Severity of arterial hypertension and extent of coronary artery disease in patients with ACS

Хронични рискови фактори	Пациенти с анамнеза за Covid-19 n=107	Пациенти без анамнеза за Covid-19 n=408	Общо
ХСБ (брой, %)			
1ва степен	6 (5.6%)	12 (2.9%)	18 (3.5%)
2ра степен	29 (27.1%)	89 (21.8%)	118 (22.9%)
3та степен	58 (54.2%)	267 (65.4%)	325 (63.1%)
Без ХСБ	14 (13.1%)	40 (9.8%)	54 (10.5%)
Съдове (брой, %)			
Един	71 (66.4%)	214 (52.5%)	285 (55.3%)
Два	20 (18.7%)	112 (27.5%)	132 (25.6%)
Три	9 (8.4%)	62 (5.5%)	71 (13.8%)

Коментирал [EM4]: **Chi-square test (пациенти с анамнеза за Covid-19/пациенти без анамнеза за Covid-19):** $\chi^2 = 5.325$, df=3, p = 0.150 – няма разлика

Четири	6 (5.6%)	18 (4.4%)	24 (4.7%)
Пет	1 (0.9%)	2 (0.1%)	3 (0.6%)

Коментирал [EM5]: **Chi-square test (пациенти с анамнеза за Covid-19/пациенти без анамнеза за Covid-19):** $\chi^2 = 8.877$, df=4, p = 0.064 – няма разлика

A retrospective observational study was conducted at the Clinic of Cardiology, University Hospital "Dr. Georgi Stransski", Pleven, including **158 patients** with angiographically confirmed **acute coronary syndrome (ACS)**. Patients were divided into two groups: **Group I**, comprising patients with active COVID-19 infection or a history of COVID-19 within six months before the ACS event, and **Group II**, comprising patients with no history of COVID-19 infection. Demographic characteristics, cardiovascular risk factors, and inflammatory biomarkers (**IL-1** and **IL-6**) were evaluated.

1. Demographic Characteristics

No statistically significant difference was observed in the mean age between patients with previous COVID-19 infection (Group I: **67.7 ± 12.617 years**) and those without previous infection (Group II: **64.6 ± 8.910 years**) (**Mann–Whitney U = 2432.000, z = –1.938, p = 0.053**).

Sex distribution was also comparable between the two groups. In Group I, **66 patients (68.8%)** were male and **30 (31.2%)** were female, whereas in Group II **46 patients (74.1%)** were male and **16 (25.9%)** were female (**χ^2 = 0.541, df = 1, p = 0.462**).

Overall, the two study groups were considered comparable with respect to age and sex.

Table 4. Demographic characteristics of patients with ACS included in Project No. 1/2021.

Групи	**N**	**Възраст (години)**			
		Mean ± SD	**Me**	**IQR**	**Test of Normality***
Здрави	62	64.6 ± 8.910	64.0	14.0	0.213
Болни	96	67.7 ± 12.617	68.0	18.0	0.140
Общо	158	66.5 ± 11.379	67.0	16.0	0.231

** Shapiro-Wilk Test of Normality*

Коментирал [EHM6]: **Mann-Whitney U test**: U = 2432.000, z = -1.938. p = 0.053 – няма разлика

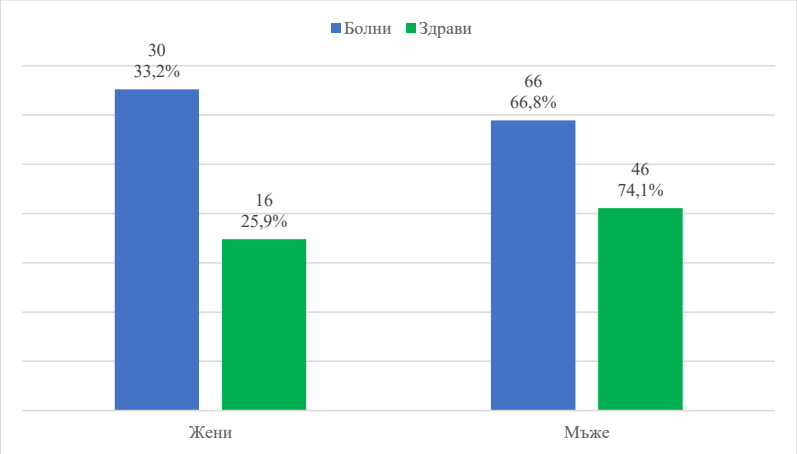

Figure 5. Sex distribution of patients with ACS included in Project No. 1/2021.

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2. Cardiovascular Risk Factors

The following cardiovascular risk factors were evaluated: family history of cardiovascular disease, type 2 diabetes mellitus, arterial hypertension, obesity, smoking, and alcohol consumption.

2.1 Family History

A positive family history was reported in **34 patients (35.4%)** in Group I and **20 patients (32.3%)** in Group II. No statistically significant difference was found between the groups (**$\chi^2 = 3.630$, df = 1, p = 0.057**).

2.2 Diabetes Mellitus

Diabetes mellitus was present in **40 patients (41.7%)** in Group I and **36 patients (58.1%)** in Group II, with no statistically significant difference between groups (**$\chi^2 = 0.211$, df = 1, p = 0.646**).

However, the proportion of patients **without diabetes mellitus** was significantly higher among those with previous COVID-19 infection (**58.3%**) than among patients without previous infection (**41.9%**) (Odds Ratio **0.516**, 95% CI **0.270–0.986**, *p* = **0.045**).

2.3 Arterial Hypertension

The prevalence and severity of arterial hypertension were also evaluated.

No statistically significant differences were observed for **Grade I** hypertension (**4.2% vs. 9.7%; $\chi^2 = 0.400$, p = 0.527**) or **Grade III** hypertension (**47.9% vs. 71.0%; $\chi^2 = 0.044$, p = 0.833**).

In contrast, **Grade II hypertension** was significantly more frequent among patients with previous COVID-19 infection (**31.3%**) than among patients without previous infection (**12.9%**) (**$\chi^2 = 12.737$, df = 1, p < 0.001**).

Notably, Grade III hypertension predominated in patients without previous COVID-19 infection, whereas among patients with previous COVID-19 infection the distribution between Grade II and Grade III hypertension was more balanced.

2.4 Obesity

Obesity, defined as **body mass index (BMI) ≥30 kg/m²**, was present in **24 patients (25.0%)** in Group I and **16 patients (25.8%)** in Group II, with no statistically significant difference (**$\chi^2 = 1.600$, df = 1, p = 0.206**).

2.5 Smoking

Smoking history was reported by **36 patients (37.5%)** in Group I and **28 patients (45.2%)** in Group II. The difference was not statistically significant (**$\chi^2 = 1.000$, df = 1, p = 0.317**).

2.6 Alcohol Consumption

Alcohol consumption differed significantly between the groups. It was reported by **16 patients (16.7%)** in Group I compared with **6 patients (9.7%)** in Group II (**$\chi^2 = 4.545$, df = 1, p = 0.033**).

Table 5. Cardiovascular risk factors among patients with ACS included in Project No. 1/2021.

Характеристика	Болни (n=96)	Здрави (n=62)	Общо (n=158)
Фамилна анамнеза			
да	34 (35.4%)	20 (32.3%)	54 (3.2%)
не	62 (64.6%)	42 (67.7%)	104 (65.8%)
Захарен диабет			
да	40 (41.7%)	36 (58.1%)	76 (48.1%)
не	56 (58.3%)	26 (41.9%)	82 (51.9%)
ХСБ			
Първа степен	4 (4.2%)	6 (9.7%)	10 (6.3%)

Коментирал [EHM7]: Chi-square test (да; болни /здрави): $\chi^2 = 3.630$, df=1, p = 0.057 – няма разлика

Коментирал [EHM8]: Chi-square test (да; болни /здрави): $\chi^2 = 0.211$, df=1, p = 0.646 – няма разлика

Втора степен	30 (31.3%)	8 (12.9%)	38 (24.1%)
Трета степен	46 (47.9%)	44 (71.0%)	90 (57.0%)
Липсва информация	16 (16.7%)	4 (6.4%)	20 (12.6%)
Обезитет			
да	24 (25.0%)	16 (25.8%)	40 (25.3%)
не	72 (75.0%)	46 (74.2%)	118 (74.7%)
Тютюнопушене			
да	36 (37.5%)	28 (45.2%)	64 (40.5%)
не	60 (62.5%)	34 (54.8%)	94 (59.5%)
Алкохол			
да	16 (16.7%)	6 (9.7%)	22 (13.9%)
не	80 (83.3%)	56 (90.3%)	136 (86.1%)

Коментирал [EHM9]:

Коментирал [EHM10]: Chi-square test (да; болни /здрави): $\chi^2 = 1.600$, df=1, p = 0.206 – няма разлика

Коментирал [EHM11]: Chi-square test (да; болни /здрави): $\chi^2 = 1.000$, df=1, p = 0.317 – няма разлика

Коментирал [EHM12]: Chi-square test (да; болни /здрави): $\chi^2 = 4.545$, df=1, p = 0.033 – има разлика

2.7 Left Ventricular Systolic Function

Left ventricular systolic function was assessed by **two-dimensional echocardiography** using the **Simpson biplane method** for the measurement of **left ventricular ejection fraction (LVEF)**.

The mean LVEF was **51.81 ± 9.416%** in patients with previous COVID-19 infection and **50.84 ± 8.290%** in patients without previous infection. No statistically significant difference was observed between the two groups (**Mann–Whitney U = 3062.000, z = 0.307, p = 0.759**).

Table 6. Left ventricular systolic function in patients with ACS included in Project No. 1/2021.

Показатели	Mean ± SD	Me	Min	Max	IQR
Simpson's LVEF, %	51.43 ± 8.977	52.0	30.0	68.0	14.0
болни	51.81 ± 9.416	51.5	30.0	68.0	14.0
здрави	50.84 ± 8.290	52.0	30.0	65.0	11.0

Коментирал [EHM13]: Independent-Samples Mann-Whitney U Test: U = 3062.000, z = 0.307, p = 0.759 – няма разлика

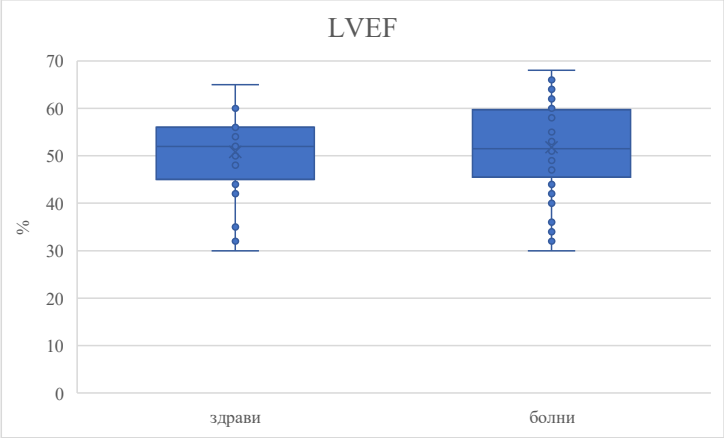

Figure 6. Comparison of left ventricular ejection fraction in patients with ACS included in Project No. 1/2021.

2.8. Laboratory Investigations

Laboratory parameters were compared between the two study groups by assessing the **lipid profile** and **biochemical markers of myocardial injury**.

2.9. Lipid Profile and Cardiac Biomarkers

The mean **total cholesterol (TC)** concentration in patients with a history of COVID-19 infection was **6.14 ± 7.138 mmol/L**, compared with **5.40 ± 1.038 mmol/L** in patients without previous COVID-19 infection (**Mann–Whitney U = 2644.000, z = –1.182, p = 0.237**).

The mean **LDL cholesterol** levels were **3.20 ± 1.037 mmol/L** in patients with previous COVID-19 infection and **3.25 ± 1.124 mmol/L** in those without previous infection (**U = 2820.000, z = –0.556, p = 0.579**).

Similarly, the mean **triglyceride (TG)** concentrations were **1.74 ± 1.213 mmol/L** in the COVID-19 group and **1.96 ± 1.276 mmol/L** in the non-COVID-19 group (**U = 2698.000, z = –0.990, p = 0.322**).

No statistically significant differences in lipid profile parameters were observed between the two groups.

Table 7. Lipid profile analysis in patients with ACS included in Project No. 1/2021.

Липиден профил	Mean ± SD	Me	Min	Max	IQR
LDL, *mmol/L*	3.21 ± 1.068	3.20	0.69	6.14	1.71
болни	3.20 ± 1.037	3.15	1.15	6.14	1.52
здрави	3.25 ± 1.124	3.21	0.69	5.72	1.74
TG, *mmol/L*	1.83 ± 1.239	1.74	0.48	7.58	0.98
болни	1.74 ± 1.213	1.46	0.48	7.58	0.79
здрави	1.96 ± 1.276	1.47	0.63	6.32	1.41
TC, *mmol/L*	5.85 ± 5.602	5.27	1.07	54.15	1.58
болни	6.14 ± 7.138	5.00	1.07	54.15	1.58
здрави	5.40 ± 1.038	5.56	2.91	7.25	1.76

Коментирал [EHM14]: **Independent-Samples Mann-Whitney U Test:** U = 2820.000, z = -0.556, p = 0.579 – няма разлика

Коментирал [EHM15]: **Independent-Samples Mann-Whitney U Test:** U = 2698.000, z = -0.990, p = 0.322 – няма разлика

Коментирал [EHM16]: **Independent-Samples Mann-Whitney U Test:** U = 2644.000, z = -1.182, p = 0.237 – няма разлика

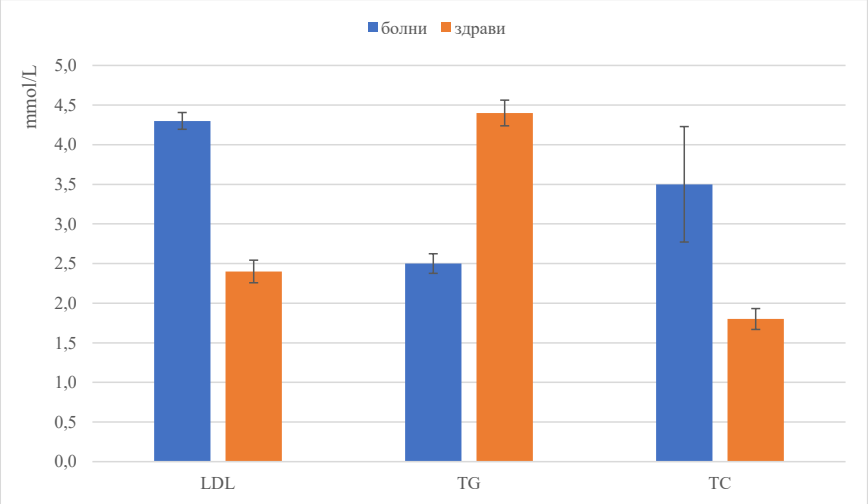

Figure 7. Subgroup analysis of the lipid profile in patients with ACS included in Project No. 1/2021.

A comparative analysis was also performed for the biochemical markers of myocardial injury, including **creatine kinase (CK)**, **CK-MB**, and **high-sensitivity cardiac troponin T (hs-cTnT)**.

The mean **CK** level was **252.4 ± 402.379 µmol/L** in patients with previous COVID-19 infection and **263.1 ± 462.734 µmol/L** in patients without previous COVID-19 infection (**U = 3004.000, z = 0.100, p = 0.921**).

The mean **CK-MB** concentration was **34.36 ± 37.817 U/L** in the COVID-19 group compared with **43.70 ± 75.910 U/L** in the non-COVID-19 group (**U = 3104.000, z = 0.456, p = 0.648**).

The mean **high-sensitivity cardiac troponin T (hs-cTnT)** level was **0.20 ± 0.519 ng/mL** in patients with previous COVID-19 infection and **0.11 ± 0.248 ng/mL** in those without previous infection (**U = 3282.000, z = 1.090, p = 0.276**).

None of the myocardial injury biomarkers demonstrated statistically significant differences between the two study groups.

2.9. Serum Potassium and Additional Laboratory Parameters

Serum potassium levels obtained from the electrolyte panel performed upon hospital admission were also compared between the two study groups. The mean serum potassium concentration was **4.32 ± 0.646 mmol/L** in patients with a history of COVID-19 infection and **4.37 ± 0.773 mmol/L** in patients without previous COVID-19 infection. No statistically significant difference was observed between the groups (**Mann–Whitney U = 2926.000, z = –0.178, p = 0.858**).

Table 8. Biochemical laboratory parameters in patients with ACS included in Project No. 1/2021.

Биохимични показатели	Mean ± SD	Me	Min	Max	IQR
КФК, *µmol/L*	256.6 ± 425.667	118.0	34.0	2345.0	125.0
болни	252.4 ± 402.379	121.0	34.0	2345.0	148.0
здрави	263.1 ± 462.734	118.0	62.0	2001.0	110.0
K, *mmol/L*	4.34 ± 0.697	4.22	3.08	7.60	0.79
болни	4.32 ± 0.646	4.25	3.08	5.50	0.85
здрави	4.37 ± 0.773	4.22	3.20	7.60	0.70

Коментирал [EHM17]: Independent-Samples Mann-Whitney U Test: U = 3004.000, z = 0.100, p = 0.921 – няма разлика

Коментирал [EHM18]: Independent-Samples Mann-Whitney U Test: U = 2926.000, z = -0.178, p = 0.858 – няма разлика

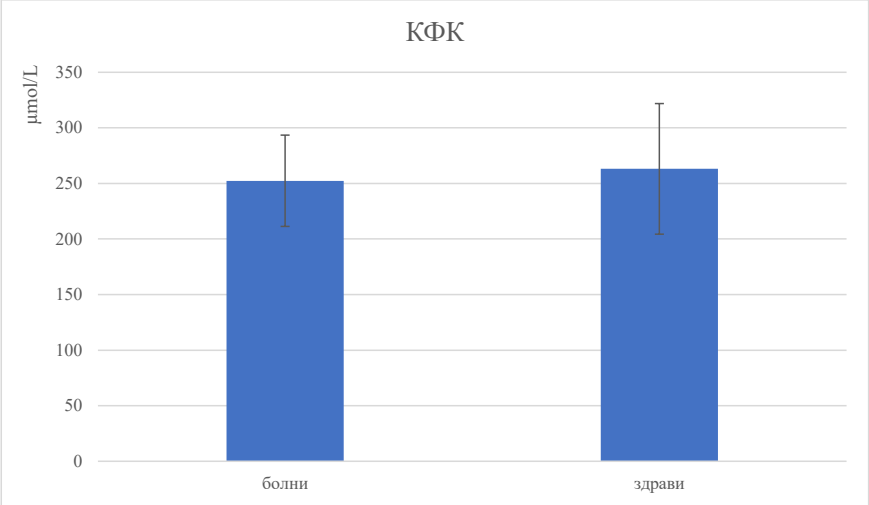

Figure 8. Subgroup analysis of serum creatine kinase (CK) levels in patients with ACS included in Project No. 1/2021.

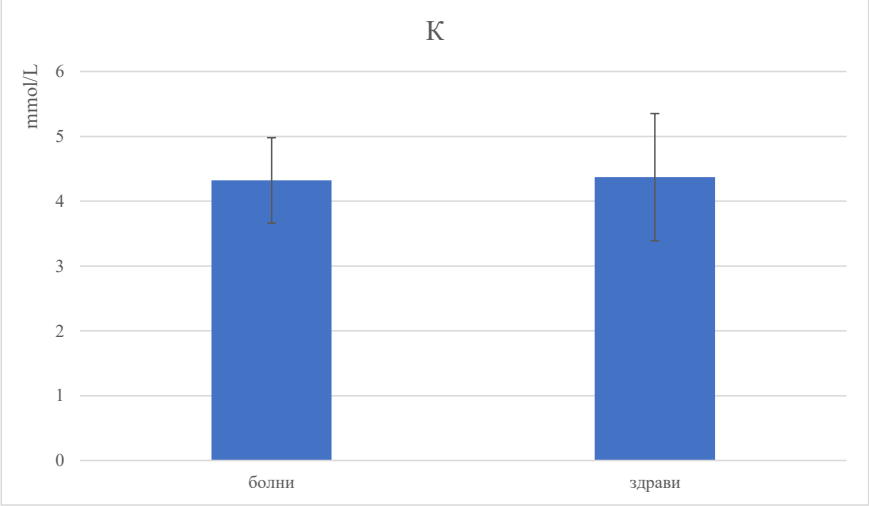

Figure 9. Subgroup analysis of serum potassium levels in patients with ACS included in Project No. 1/2021.

Table 9. Cardiac enzyme analysis in patients with ACS included in Project No. 1/2021.

Ензими	Mean ± SD	Me	Min	Max	IQR
CK-MB, *U/L*	38.03 ± 55.903	23.0	10.0	420.0	19.0
болни	34.36 ± 37.817	22.0	11.0	223.0	19.0
здрави	43.70 ± 75.910	23.0	10.0	420.0	28.0
cTnI, *ng/ml*	0.16 ± 0.434	0.024	0.006	2.980	0.066
болни	0.20 ± 0.519	0.035	0.006	2.980	0.064
здрави	0.11 ± 0.248	0.024	0.006	1.230	0.083

Коментирал [EHM19]: Independent-Samples Mann-Whitney U Test: U = 3104.000, z = 0.456, p = 0.648 – няма разлика

Коментирал [EHM20]: Independent-Samples Mann-Whitney U Test: U = 3282.000, z = 1.090, p = 0.276 – няма разлика

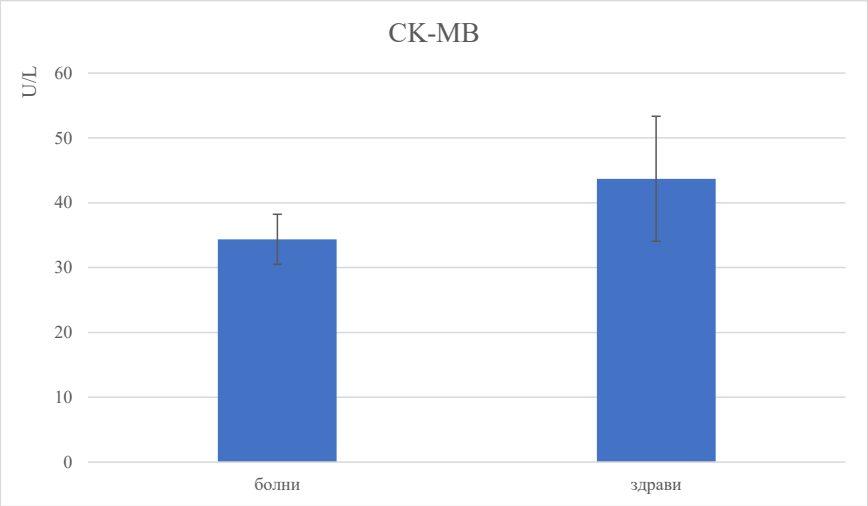

Figure 10. Subgroup analysis of serum CK-MB levels in patients with ACS included in Project No. 1/2021.

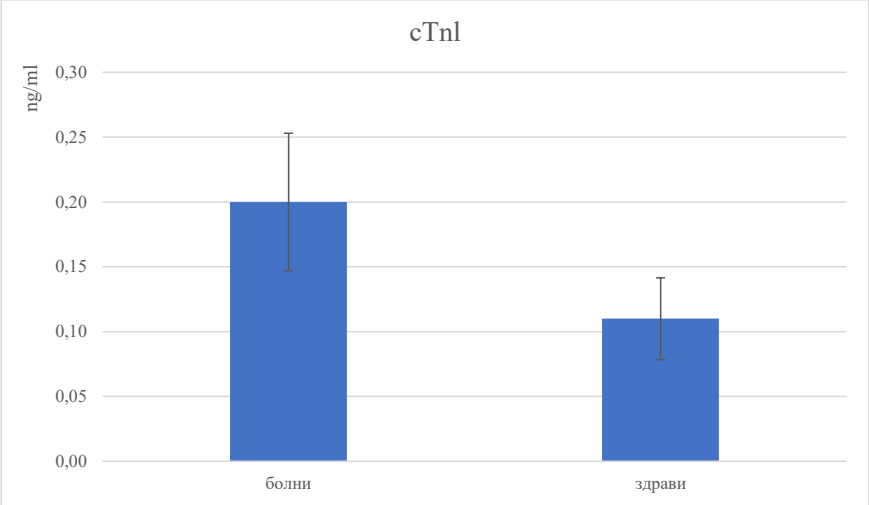

Figure 11. Subgroup analysis of high-sensitivity cardiac troponin T (hs-cTnT) levels in patients with ACS included in Project No. 1/2021.

2.10. Analysis of IL-1 and IL-6 as Predictors of Sarcopenia

Serum concentrations of **interleukin-1 (IL-1)** and **interleukin-6 (IL-6)** were measured using the **enzyme-linked immunosorbent assay (ELISA)** method to evaluate their potential role as biomarkers of inflammation and predictors of sarcopenia.

The mean serum **IL-1** concentration was **2.2 ± 1.293 pg/mL** in patients with previous COVID-19 infection compared with **0.6 ± 0.442 pg/mL** in patients without previous COVID-19 infection. This difference was highly statistically significant (**Mann–Whitney U = 5628.000, z = 9.464, p < 0.001**).

In contrast, the mean **IL-6** concentration was **10.2 ± 9.792 pg/mL** in patients with previous COVID-19 infection and **24.0 ± 10.895 pg/mL** in patients without previous COVID-19 infection. This difference was also highly statistically significant (**U = 652.000, z = –8.277, p < 0.001**).

Both inflammatory biomarkers demonstrated statistically significant differences between the two study groups.

Table 10. Inflammatory and sarcopenia-related biomarkers in patients with ACS included in Project No. 1/2021.

Интерлевкини	Mean ± SD	Me	Min	Max	IQR
IL-1, *pg/mL*	1.6 ± 1.312	1.4	0.2	7.4	1.6
болни	2.2 ± 1.293	1.7	0.9	7.4	1.2
здрави	0.6 ± 0.442	0.4	0.2	1.7	0.3
IL-6, *pg/mL*	15.6 ± 12.237	12.5	1.5	52.1	16.7
болни	24.0 ± 9.792	8.1	1.5	52.1	8.7
здрави	10,2 ± 10.895	21.9	12.2	52.1	16.0

Коментирал [EHM21]: **Independent-Samples Mann-Whitney U Test:** U = 5628.000, z = 9.464, p = 0.000 – има разлика

Коментирал [EHM22]: **Independent-Samples Mann-Whitney U Test:** U = 652.000, z = -8.277, p = 0.000 – има разлика

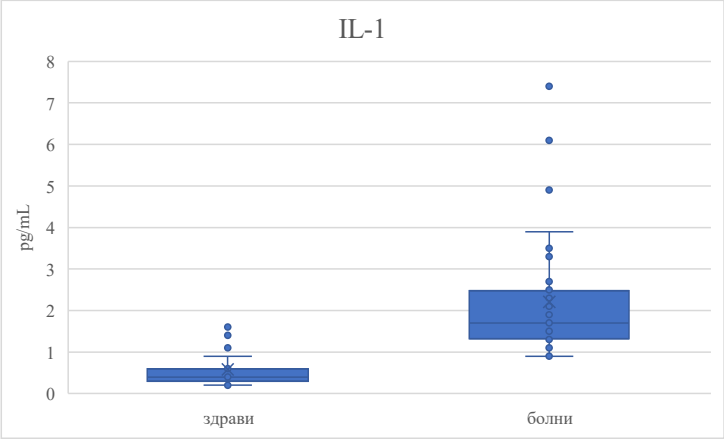

Figure 12. Subgroup analysis of serum IL-1 concentrations in patients with ACS included in Project No. 1/2021.

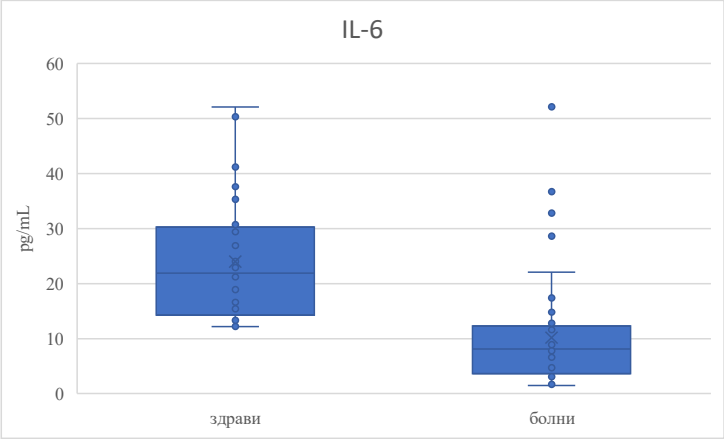

Figure 13. Subgroup analysis of serum IL-6 concentrations in patients with ACS included in Project No. 1/2021.

2.11. Time from Onset of Anginal Symptoms to Seeking Medical Care

Based on a detailed medical history obtained prior to hospital admission, the time interval between the onset of **anginal symptoms** and the patient's decision to seek medical attention was compared between the two study groups.

During the first **five days** after symptom onset, the proportion of patients seeking medical care was comparable between the two groups. However, between **Day 5 and Day 10**, patients with active or previous COVID-19 infection were significantly more likely to delay seeking medical attention (**$\chi^2 = 16.667$, df = 1, $p < 0.001$**).

Furthermore, among patients who sought medical care **more than 10 days** after the onset of symptoms, all had either active COVID-19 infection or a history of COVID-19.

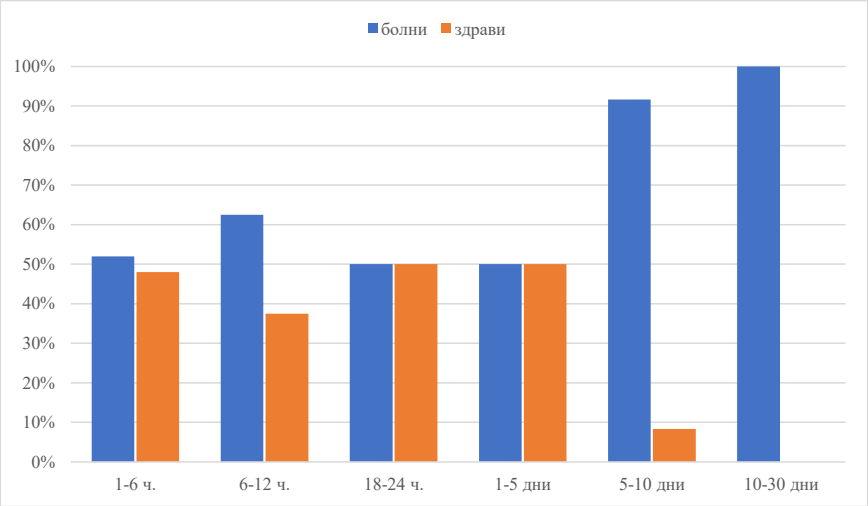

Figure 14. Subgroup analysis of the time interval between the onset of anginal symptoms and seeking medical care in patients with ACS included in Project No. 1/2021.

2.12. Previous Ischemic Events

The final cardiovascular risk factor evaluated in the two study groups was the presence of a **previous ischemic event**, documented both anamnestically and through the patients' medical records.

No statistically significant difference was observed in the prevalence of previous ischemic events between the two groups. A previous ischemic event was documented in **22.9%** of patients with previous COVID-19 infection (Group I) and **32.3%** of patients without previous COVID-19 infection (Group II) (**χ^2 = 0.095, df = 1, p = 0.758**).

In contrast, the **absence of a previous ischemic event** was significantly more frequent among patients with previous COVID-19 infection (**77.1%**) than among those without previous infection (**67.7%**) (**χ^2 = 8.828, df = 1, p = 0.003**).

Overall, no statistically significant difference was found in the distribution of patients according to the presence or absence of a previous ischemic event (**χ^2 = 1.684, df = 1, p = 0.194**).

Figure 15. Subgroup analysis of patients with ACS included in Project No. 1/2021 according to the presence of a previous ischemic event.

Table 11. Analysis of the presence or absence of a previous ischemic event in patients with ACS included in Project No. 1/2021.

Предишен тромбоцитен инцидент	**Здрави**	**Болни**	χ^2	**df**	**p-value**
Има предишен тромбоцитен инцидент	20 (32.3%)	22 (22.9%)	0.095	1	0.758
Няма предишен тромбоцитен инцидент	42 (67,7%)	74 (77.1%)	8.828	1	0.003
Общо	62 (100%)	96 (100%)	1.684	1	0.194

3. SARC-F Questionnaire Assessment

Following informed consent, all participants completed the **SARC-F questionnaire** during their hospitalization in the Cardiology Clinic. Patients were provided with sufficient time to understand the questionnaire, and each question was explained individually before completion.

Among patients with **active or previous COVID-19 infection**, only **2 patients (2.1%)** achieved a score of **0–1 points**, indicating a very low likelihood of sarcopenia. In contrast, **12 patients (19.7%)** in the group without previous COVID-19 infection scored **0–1 points**.

A **SARC-F score of 2–4 points** was observed in **16 patients (16.8%)** in Group I compared with **34 patients (49.6%)** in Group II.

Scores ranging from **4 to 6 points** were recorded in **49 patients (51.4%)** in the COVID-19 group and **10 patients (17.3%)** in the non-COVID-19 group.

A **SARC-F score greater than 6 points**, indicating a high probability of clinically significant sarcopenia, was found in **29 patients (31.6%)** with previous or active COVID-19 infection compared with **7 patients (13.4%)** in the control group.

Discussion and Conclusion

1. The analysis of patients with acute coronary syndrome (ACS) hospitalized during the COVID-19 pandemic at the Department of Cardiology, University Hospital "Dr. Georgi Stranski", Pleven, demonstrated a similar mean age in patients with previous/active COVID-19 infection (Group I) and patients with ACS without a history of COVID-19 infection (Group II). The mean age in both groups (67 years) is consistent with the average age reported in most published studies from Europe and North America.

Sex distribution was also comparable between the two groups, with a predominance of male patients (59.8% in Group I and 67.7% in Group II). These findings are consistent with the gender distribution reported in studies from Europe and Japan.

The age distribution of ACS was also of interest. The majority of ACS cases occurred between 65 and 80 years of age, while almost all patients (86% in Group I and 83.1% in Group II) were between 50 and 80 years old.

Within Group I, the highest incidence of ACS occurred during active COVID-19 infection (32.7%). Following recovery from COVID-19, the incidence of ACS gradually declined during the first six months, after which a second peak was observed at approximately six months (29.9%).

Analysis of admission diagnoses demonstrated a predominance of ischemic heart disease presenting as unstable angina pectoris (IHD-UAP) in both groups (76.6% in Group I and 68.9% in Group II).

The proportion of STEMI was similar in both groups. This finding differs from most published studies and suggests that many patients presented with atypical anginal symptoms not associated with significant electrocardiographic changes.

Evaluation of cardiovascular risk factors revealed a statistically significant difference in the prevalence of diabetes mellitus, affecting 32.7% of patients in Group I compared with 44.3% in Group II ($\chi^2 = 98.685$, df = 1, $p = 0.000$). These findings suggest a lower contribution of diabetes mellitus among patients with previous COVID-19 infection and support several of the studies discussed in the Discussion section.

Smoking prevalence was also lower in Group I (18.7%) compared with Group II (34.1%) ($\chi^2 = 9.392$, df = 1, $p = 0.002$, Phi = −0.135), which is in agreement with

findings reported from countries with socioeconomic conditions comparable to those in Bulgaria.

No statistically significant differences were observed regarding arterial hypertension or alcohol consumption, findings that are supported by numerous published studies.

The extent of coronary artery disease assessed by invasive coronary angiography also showed no statistically significant differences between the groups. Nevertheless, single-vessel coronary artery disease predominated (66.4% in Group I versus 52.5% in Group II). Patients without previous COVID-19 demonstrated a more gradual decrease in the prevalence of double-, triple-, and multivessel coronary artery disease.

Overall, the analysis demonstrated similar demographic characteristics but a somewhat lower-risk cardiovascular profile among patients with previous COVID-19 infection.

2. We further compared an independent cohort of 158 patients with ACS and previous COVID-19 infection (Group I) with patients presenting with ACS without previous COVID-19 infection (Group II), and compared these findings with those obtained in the larger study population.

The demographic characteristics remained consistent with those described above, including male predominance and similar mean age (67.7 years in Group I and 64.6 years in Group II).

No statistically significant difference was observed regarding family history of cardiovascular disease, although the p-value approached significance ($p = 0.057$).

Similarly, the prevalence of diabetes mellitus did not differ significantly between the two groups.

Regarding hypertension, patients in Group I more frequently presented with grade II hypertension, whereas grade III hypertension predominated in Group II.

Alcohol consumption was significantly more common among patients with previous COVID-19 infection (16.7% versus 9.7%; $p = 0.033$).

Body mass index (BMI) did not differ significantly between the two groups.

Overall, both groups exhibited comparable cardiovascular risk profiles, although patients with previous COVID-19 tended to have less severe hypertension but a higher prevalence of regular alcohol consumption.

Assessment of left ventricular systolic function using transthoracic echocardiography revealed no significant differences between the groups, findings that are consistent with previously published studies.

Likewise, lipid profile analysis demonstrated no statistically significant differences.

Markers of myocardial injury and serum electrolyte levels also did not differ significantly between the two groups.

Because of these findings, we investigated sarcopenia as a possible mechanism contributing to ACS development.

Serum IL-1 and IL-6 concentrations differed significantly between the groups. Mean IL-1 concentration was 2.2 ± 1.293 pg/mL in Group I and 0.6 ± 0.442 pg/mL in Group II ($U = 5628.000$, $z = 9.464$, $p = 0.000$).

Conversely, IL-6 concentrations were 10.2 ± 9.792 pg/mL in Group I and 24.0 ± 10.895 pg/mL in Group II ($U = 652.000$, $z = -8.277$, $p = 0.000$).

These findings indicate persistent inflammatory activity and an increased inflammatory risk in patients with previous COVID-19 infection, consistent with several large international studies.

Time from symptom onset to seeking medical attention was also evaluated. No significant difference was observed during the first five days after symptom onset; however, after the fifth day, delayed presentation was significantly more common among patients with previous COVID-19 infection. Similar findings have been reported in several studies and have been attributed to patients' fear of seeking hospital care during the pandemic.

Finally, previous ischemic events were analyzed. A significant difference was observed only among patients without a previous ischemic event, with a higher proportion in Group I. This finding suggests that ACS following COVID-19 frequently represents a first cardiovascular event, an observation for which currently limited evidence is available in the international literature.

CONCLUSIONS

1. The study was based on two patient cohorts:
    - a cohort of 515 patients admitted to the Department of Cardiology, University Hospital "Dr. Georgi Stransski", Pleven, who underwent interventional treatment;
    - a second cohort of 158 patients divided into two subgroups according to the same criteria as the larger cohort.
2. No significant differences were observed between the groups regarding mean age (67 years) or sex distribution, with a predominance of male patients.
3. A positive family history of cardiovascular disease was less frequent among patients with active or previous COVID-19 infection.
4. Type 2 diabetes mellitus and smoking were more prevalent in the control group, with diabetes mellitus present in 44.3% of patients.
5. No statistically significant differences were found between the groups regarding arterial hypertension or alcohol consumption.
6. Two distinct peaks were identified in the interval between COVID-19 infection and the occurrence of an acute ischemic event: the first during active COVID-19 infection (32.7%) and the second approximately six months after recovery (29.9%).
7. The predominant admission diagnosis in both groups was ischemic heart disease presenting as unstable angina pectoris (IHD-UAP), accounting for 76.6% and 68.9% of cases, respectively.
8. In the later patient cohort included in the project, the proportion of smokers was higher than that observed in the initial cohort of 515 patients.
9. Muscle strength and the prevalence of sarcopenia, assessed using the SARC-F questionnaire, were significantly more impaired in patients with active or previous COVID-19 infection than in the control group.
10. Serum IL-1 concentrations were significantly higher in patients with active or previous COVID-19 infection than in the control group.

11. Serum IL-6 concentrations were significantly higher in the control group than in patients with active or previous COVID-19 infection.
12. A history of previous ischemic events was more common in the control group than among patients with active or previous COVID-19 infection.

SCIENTIFIC CONTRIBUTIONS

Scientific and Theoretical Contributions

1. This study is the first in Bulgaria to characterize the cardiovascular risk profile of patients with active or previous COVID-19 infection. **(Original contribution)**
2. A distinct pattern of IL-1 and IL-6 concentrations was identified, suggesting their potential role as predictive biomarkers for the development of acute coronary syndrome in patients with active or previous COVID-19 infection. **(Original contribution)**

Scientific and Practical Contributions

1. Two peaks in the occurrence of acute coronary syndrome were identified: during active COVID-19 infection and approximately six months after recovery, followed by a gradual decline in ACS incidence. **(Original contribution)**
2. Patients with active or previous COVID-19 infection demonstrated a lower overall burden of traditional cardiovascular risk factors compared with the control group. **(Original contribution)**
3. The interval between the onset of anginal symptoms and seeking medical attention was prolonged among patients with COVID-19 infection, confirming observations reported in previous studies. **(Confirmatory contribution)**
4. Ischemic heart disease presenting as unstable angina pectoris (IHD-UAP) was identified as the predominant clinical presentation in patients with active or previous COVID-19 infection. **(Original contribution)**
5. A higher prevalence of sarcopenia was demonstrated among patients with active or previous COVID-19 infection, confirming findings reported in the international literature. **(Confirmatory contribution)**