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CLINICAL SIGNIFICANCE OF PENTRAXIN 3 IN PATIENTS WITH ISCHEMIC HEART DISEASE WHO ARE NOT ELIGIBLE FOR A REVASCULARIZATION PROCEDURE

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LIST OF ABBREVIATIONS

HsCRP	High sensitivity C-reactive protein
BMI	Body Mass Index
LVEDD	Left ventricular end-diastolic diameter
LVESD	Left ventricular end-systolic diameter
FS	Fractional shortening
SCA	Selective coronary angiography
ESC	European Society of Cardiology
ASCVD	Atherosclerotic Cardiovascular Disease
PTX3	Pentraxin 3
PCI	Percutaneous coronary intervention
MACE	Major adverse cardiovascular events
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
ELISA	Enzime-linked immunosorbent assay
QCA	Quantitative coronary angiography

1. INTRODUCTION

Ischemic heart disease (IHD) is the leading cause of death and disability worldwide. In 2020, 244,1 million people were affected by IHD globally, with men and people living in economically underdeveloped countries being affected to a greater extent (Tsao et al., 2022). Coronary atherosclerosis is the primary substrate for the development of acute and chronic coronary syndromes. Atherosclerosis is a multifactorial process with a long latent period and inflammation plays a central role in its pathogenesis. The role of inflammation in the development of atherosclerosis was noted as early as the XIX century by the pathologists Rokitansky and Virchow, however, the pathogenetic mechanisms then were not fully understood (Mayerl et al., 2006). Today, the inflammation is recognized as a primary, initiating and sustaining factor in the progression and complications of atherosclerosis.

Recognizing atherosclerosis as an inflammatory process has sparked interest in inflammatory biomarkers with a focus on assessing their predictive value in patients with ischemic heart disease. Various inflammatory biomarkers have been studied in the context of IHD, but their full clinical integration into daily practice remains challenging. Pentraxins are a group of proteins that share a phylogenetically conserved common structure and a specific amino acid sequence that is unique to each protein. They are divided into short and long pentraxins. The first group includes C-reactive protein (CRP) and serum amyloid P, while the second group includes pentraxin 3 (PTX3). The difference between the specific structures of each protein in the superfamily lies in the N-terminal region, which explains their different biological functions (Wang et al., 2020; Casula et al., 2017). High-sensitivity C-reactive protein (hsCRP) has been the most extensively studied to date (Arnett et al., 2019; Mach et al., 2025). Pentraxin 3, discovered in the 1990s, is the prototype of the long pentraxins. It is a specific biomarker reflecting local vascular inflammation and is produced by cells involved in the progression of atherosclerosis: endothelial cells, macrophages and smooth muscle cells (Jenny et al., 2014). Pentraxin 3 is part of the innate immune system (Breviario et al., 1992). Its pathogenic role is associated with the enhancement of endothelial dysfunction, inhibition of neoangiogenesis and increase in oxidative stress (Xingyan et al., 2024). The biomarker is involved in numerous interactions and is elevated in inflammatory processes, acute kidney injury, chronic kidney disease, in patients on dialysis, bronchial asthma, liver cirrhosis, stroke, autoimmune diseases (rheumatoid arthritis, progressive systemic sclerosis), small-vessel vasculitides (Churg-Strauss syndrome, Wegener’s granulomatosis), inflammatory bowel disease, pregnancy, preeclampsia, carcinomas (breast, lung, hepatocellular, prostate, glioma,

melanoma, liposarcoma) (Cozzi et al., 2012; Deban et al., 2011; Fazzini et al., 2001; Iwata et al., 2009; Kato et al., 2008; Luchetti et al., 2000; Mauri et al., 2010; Nenova et al., 2023; Zhang et al., 2022). The presence of these diseases result in low specificity for assessing cardiovascular risk and associated vascular inflammation. In healthy volunteers, median PTX3 levels are ~ 2 ng/mL (Ristagno et al., 2019). There are currently no established reference values for the biomarker.

2. AIM AND TASKS OF THE STUDY

2.1. Aim

To determine the risk of rehospitalizations in patients with ischemic heart disease who are not eligible for a revascularization procedure, by measuring pentraxin 3 values.

2.2. Tasks

1. To determine the baseline values of pentraxin 3 in the study group;
2. To correlate the baseline values of pentraxin 3 with the results of the Seattle Angina Questionnaire scales;
3. To record the number of rehospitalizations involving repeat selective coronary angiography with or without percutaneous coronary intervention due to worsening of angina symptoms (unstable angina pectoris, fatal and non-fatal myocardial infarction) in patients during the follow-up period;
4. To make a correlation between baseline pentraxin 3 levels and the events described in task 3;
5. To identify a high-risk group based on the monitored markers.

3. PARTICIPANTS AND METHODS

3.1. Participants

The study included 80 patients. The study was conducted at the Clinic of Interventional Cardiology, St. Marina University Hospital, Varna, Bulgaria from December 2022 to January 2025. Patients were followed-up for rehospitalizations due to worsening of angina symptoms (unstable angina pectoris, fatal and non-fatal myocardial infarction) with subsequent selective coronary angiography (SCA) ± percutaneous coronary intervention (PCI) up to September 2025.

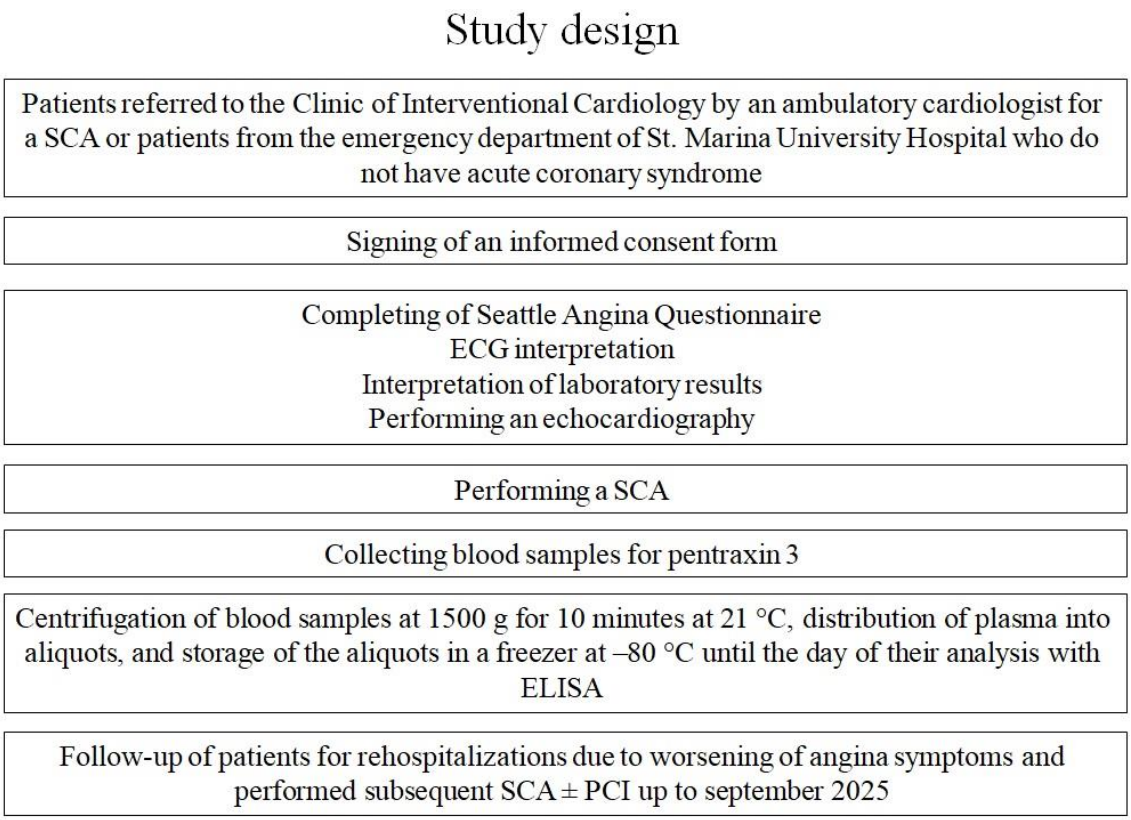
3.1.1. Inclusion criteria

- Age ≤ 70 years;
- Coronary atherosclerosis confirmed by selective coronary angiography, without need for a revascularization procedure, with established < 70% luminal stenosis in at least one coronary artery as determined by quantitative coronary angiography or patients with diffuse coronary atherosclerosis who are not eligible for a revascularization procedure;
- Signed informed consent;

3.1.2. Exclusion criteria

- Chronic kidney disease or acute kidney injury (glomerular filtration rate eGFR < 60 mL/min/1,73m^2);
- Evidence for an inflammatory disease (pneumonia, urinary tract infection, etc.);
- Autoimmune disease;
- Cancer;
- Pregnancy;
- Polycystic ovary syndrome;
- Confirmed liver cirrhosis;
- Severe (hemodynamically significant) valvular heart disease;
- Patients without angiographic findings of coronary atherosclerosis;
- Percutaneous coronary intervention within the last year;

3.2. STUDY DESIGN



3.3. Methods

3.3.1. Structured interview

A medical history was taken from each participant and the information obtained was recorded in the participant's clinical record. We collected information regarding age, sex, risk factors for cardiovascular disease, comorbidities, family history and current medical therapy with the respective doses. A license to use the Seattle Angina Questionnaire (SAQ) for the purpose of the study was obtained from CV Outcomes, Inc., USA. Information regarding angina symptoms was recorded from each patient after completing the questionnaire on their own or with the assistance of the main investigator. An officially translated version of the questionnaire in Bulgarian was used (Spertus et al., 1995).

3.3.2. Clinical examination

The clinical examination included measurement of blood pressure, heart rate, height, weight and the calculation of Body Mass Index (BMI) using the standard formula for each participant: BMI = body weight (kg)/height (m^2).

3.3.3. Laboratory tests

Blood samples for the analysis of biochemical parameters - troponin I (TnI), LDL-C, HDL-C, total cholesterol, triglycerides, and C-reactive protein - were processed at the central clinical laboratory of St. Marina University Hospital, Varna. Blood samples for plasma pentraxin 3 were collected in the morning following selective coronary angiography and after a 12-hour fast. They were distributed into two vacutainers, each containing 3 mL of venous blood, using a closed plasma separation system with EDTA anticoagulant from Beckton, Dickinson and Company. Sample analysis was performed using a ready-to-use Human PTX3 ELISA kit from Thermo Fisher Scientific, USA, catalogue number Invitrogen EH386RB, with a sensitivity (detection limit) of 0,08 ng/mL and a linear range of 0,08–20 ng/mL. All recommendations for sample processing were followed in accordance with the manufacturer's protocol.

3.3.4. Instrumental tests

To rule out the presence of hemodynamically significant valvular heart disease and to determine the dimensions of the left ventricle and left ventricular systolic function, each patient underwent a transthoracic echocardiography prior to performing a selective coronary angiography, measuring the left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) from standard parasternal long-axis view (PLAX). Using the Teicholz method, the left ventricular fractional shortening (FS) was calculated as an accessible and reproducible parameter of left ventricular systolic function.

Every patient underwent a selective coronary angiography after establishing indications and obtaining informed consent for the procedure. Patients with a luminal stenosis of < 70% as determined by quantitative coronary angiography (QCA) in at least one coronary artery or patients with diffuse coronary atherosclerosis who were not eligible for a revascularization procedure were enrolled in the study.

3.3.5. Statistical methods and analysis

3.3.5.1. Statistical estimation method

a. Point estimates: To calculate the arithmetic mean of continuous variables, the following formula was used: $X = [\Sigma X]/n$. In cases of non-normal data distribution, as with the investigated biomarker pentraxin 3, the median of the distribution was used, representing the central value of a dataset. When the median was used as a measure of central tendency, the interquartile range (IQR) was applied as a measure of dispersion, indicating the values corresponding to the 25th and 75th percentiles of the distribution. Standard deviation (σ, SD) was used as a measure of variability of the variables.

b. Interval estimates:

Confidence interval (CI): In this study, 95% confidence intervals were used.

Significance level (p): In our study, the level of significance was set at $\alpha = 0,05$, corresponding to a 5% probability of a Type I error. The results of the statistical tests were considered statistically significant at $p < 0,05$.

3.3.5.2. Graphical methods:

To illustrate the study results and the identified associations, the following graphical methods were used: bar charts, scatter plots, boxplots, ROC curves and error bars. ROC curves and the area under the curve (AUC) were used to evaluate the sensitivity and specificity of pentraxin 3 as an indicator of the severity and frequency of anginal symptoms, assessed through the „Physical Limitation“ and „Angina Frequency“ scales of the SAQ, as well as the rehospitalizations due to worsening of angina symptoms (unstable angina pectoris, fatal and non-fatal myocardial infarction) during the follow-up period. AUC values of 0,5 indicate random classification, 0,6–0,7 indicate poor discriminatory ability, 0,7–0,8 moderate discriminatory ability, 0,8–0,9 good discriminatory ability, and 0,9–1,0 excellent discriminatory ability. Time to rehospitalization was presented using a Kaplan–Meier curve.

3.3.5.3. One-way analysis of variance (ANOVA):

One-way ANOVA was used to compare mean values when analyzing more than two independent subgroups. At Shapiro–Wilk $p > 0,05$, the data were considered normally distributed, and at Levene's test $p > 0,05$, it was assumed that there were no significant differences in variances. In the presence of a statistically significant difference between groups after performing ANOVA, a post hoc Tukey HSD test was conducted.

3.3.5.4. Independent Student's t-test:

The independent Student's t-test was used to compare two independent subgroups when normal distribution was assumed: Shapiro–Wilk $p > 0,05$.

3.3.5.5. Non-parametric analysis:

Pearson's chi-square (χ^2) test was used for the analysis of ordinal categorical variables, while Fisher's exact test was applied when the expected frequency was < 5. For comparison of more than two independent subgroups with non-normally distributed data (Shapiro–Wilk $p < 0,05$), the non-parametric Kruskal–Wallis test was used. Plasma pentraxin 3 values show a non-normal distribution, therefore, non-parametric tests were applied. The Mann–Whitney U test and Pearson's chi-square (χ^2) test were used. Changes in the frequency of medication use between hospital admission and discharge were assessed using McNemar's test for paired binary variables.

3.3.5.6. Correlation analysis:

Spearman's method was used to assess the correlation between two quantitative variables with a non-normal distribution. The Spearman correlation coefficient (ρ) ranges from −1 to 1, with the sign indicating the direction of the association: $\rho > 0$ indicates a positive association, $\rho < 0$ indicates a negative association, and $\rho = 0$ indicates no association. A ρ value of 0,2–0,39 demonstrates a weak correlation, 0,4–0,69 a moderate correlation, and ≥ 0,7 a strong correlation.

3.3.5.7. Univariate logistic regression analysis:

An univariate logistic regression analysis was performed to determine the probability of a rehospitalization due to worsening of angina symptoms during follow-up in the group with initially elevated PTX3 levels.

3.3.5.8. Multivariate logistic regression analysis:

A multivariate logistic regression analysis was performed to determine the independent contribution of the investigated biomarker in predicting subsequent rehospitalizations and to assess whether it retains its prognostic value. The biomarker was sequentially included in models together with traditional risk factors: smoking, diabetes mellitus type 2 and dyslipidemia. The odds ratio (OR) was used as it is considered an equivalent of relative risk. Variables that remained significant in the model were interpreted as independent predictors of future rehospitalizations. Graphically, the results of adding each individual traditional risk factor to the model including pentraxin 3 were presented using a Forest plot.

Data analysis was performed using a standard statistical software package for personal computers — SPSS® for Windows, version 19,0. Forest plot was created using RStudio (Posit Software, Boston, MA, USA).

4. RESULTS AND DISCUSSION

4.1. Demographic characteristics of the study participants

The study included 80 patients between 18 and 70 years of age, of whom n = 42 were men and n = 38 were women, representing 52,5% and 47,5% of the total cohort, respectively. Table 1 presents the main demographic and clinical characteristics of the participants included in the study.

Variable	Mean ± 1SD
N	80
Age	58,91 ± 7,21
BMI (kg/m^2)	30,43 ± 5,68
LVEDD (mm)	49,16 ± 5,48
FS (%)	30,76 ± 5,70
Creatinine (mmol/L)	75,0 ± 15,60
eGFR (mL/min/1,73m^2)	88,96 ± 18,86
Total cholesterol (mmol/L)	4,91 ± 1,38
LDL-C (mmol/L)	2,81 ± 1,23
HDL-C (mmol/L)	1,29 ± 0,38
Triglycerides (mmol/L)	1,85 ± 1,22
CRP (mg/L)	3,20 ± 4,10
PTX3 (ng/mL)	1,88 ± 2,96

Table 1. Demographic and clinical characteristics of the participants included in the study with mean values ± 1 SD.

Figure 1 shows the distribution of the cohort according to body mass index (BMI). Only 12,5% of all patients had a normal BMI (18,5–24,9) kg/m^2, while 87,5% were overweight (≥ 25,0) kg/m^2 or had varying degrees of obesity (≥ 30,0) kg/m^2. The mean BMI was 30,4 (95% CI 29,2–31,7) kg/m².

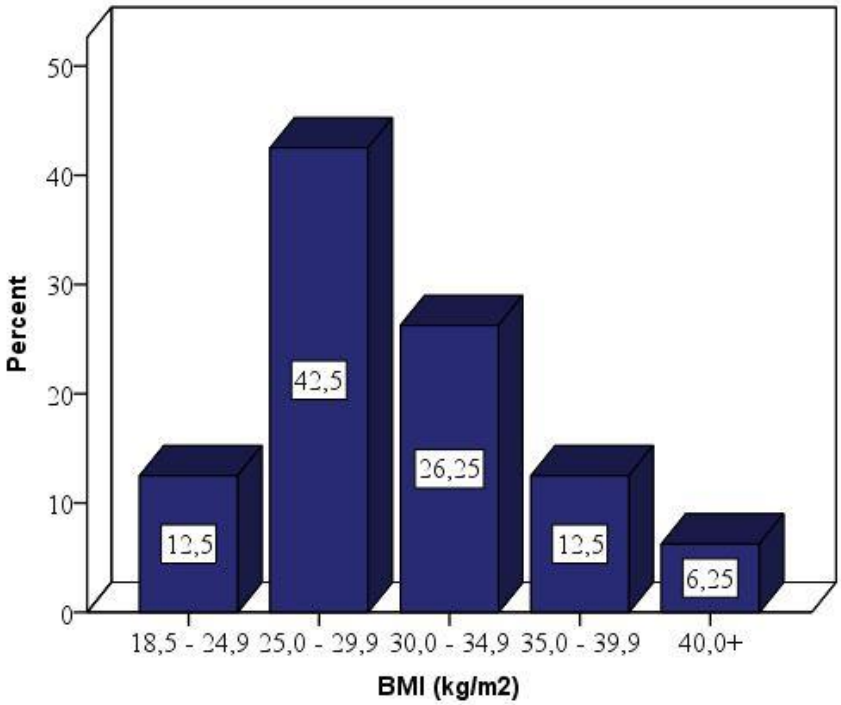


Figure 1. Bar chart showing the distribution of the cohort according to body mass index (BMI).

All patients underwent echocardiography on admission. From the echocardiographic examination in the standard parasternal long-axis view (PLAX), using one-dimensional ultrasound (M-mode), the left ventricular end-diastolic diameter (LVEDD), the left ventricular end-systolic diameter (LVESD) and fractional shortening (FS) were measured using the Teichholz method, as accessible and reproducible parameters for assessing left ventricular size and function. In our cohort, n = 73 patients had normal FS (25–45%), while n = 7 had reduced FS without signs of heart failure during the hospitalization and without hemodynamically significant acquired or congenital valvular heart disease.

Patients' antianginal therapy at home prior to hospitalization is presented in figure 2 and table 2. Highest proportion of patients were receiving one or two antianginal medications. The most commonly used being beta-blockers and dihydropyridine calcium channel blockers 60% and 42,5%, respectively. In a smaller proportion of cases, nitrates, ivabradine, and trimetazidine were used, while no patients were receiving ranolazine.

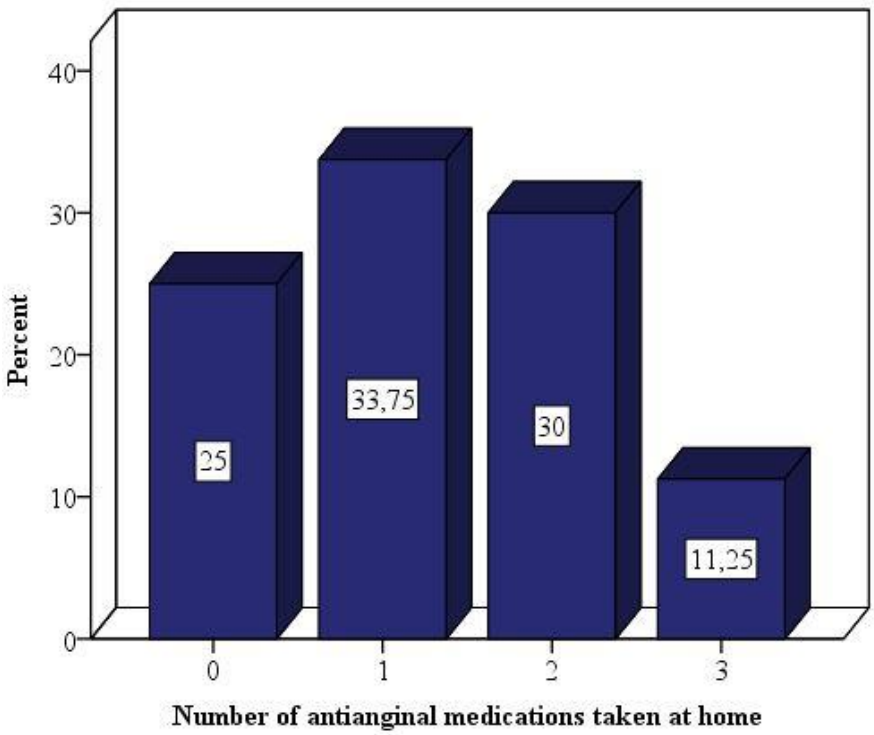


Figure 2. Bar chart showing the percentage distribution of the number of antianginal medications taken by patients at the time of hospital admission.

Medication	Frequency of use in the cohort
Beta-blocker	60%
Dihydropyridine calcium channel blockers	42,5%
Nitrates	13,8%
Ivabradine	3,8%
Trimetazidine	7,5%
Ranolazine	0%

Table 2. Frequency of use of antianginal medications in the study group.

All patients receiving combined antianginal therapy at home accounted for 41,3% (n = 33). From this group, 72,7% (n = 24) were on dual therapy, while 27,3% (n = 9) were on triple antianginal therapy. The most common combination among patients on dual antianginal therapy was a beta-blocker and a dihydropyridine calcium channel blocker (n = 18), whereas the most frequent combination among patients on triple antianginal therapy was a beta-blocker, a dihydropyridine calcium channel blocker and a nitrate (n = 3). Regarding medications affecting the renin–angiotensin–aldosterone system (angiotensin-converting

enzyme inhibitors and angiotensin receptor blockers), 67,5% (n = 54) of patients were receiving therapy with at least one drug from these two classes at the time of hospitalization.

4.2. Risk profile of the study cohort

For each patient, the risk profile for ischemic heart disease was assessed using the established traditional risk factors: smoking, arterial hypertension, type 2 diabetes mellitus, and dyslipidemia. The presence of a family history of ischemic heart disease was also taken into account when determining indications for performing a selective coronary angiography.

Family history of IHD was defined as the presence of one or more first-degree relatives with premature IHD (men < 55 years and women < 65 years) (Vrints et al., 2024).

Regarding smoking as a major modifiable risk factor, non-smokers were defined as individuals who had never smoked or had smoked ≤ 100 cigarettes in their lifetime. Smokers were defined as those who had smoked > 100 cigarettes in their lifetime and at least one cigarette in the past 30 days. Former smokers were defined as patients who had not smoked in the past 30 days. In our cohort, 42,5% (n = 34) were current smokers, while 57,5% (n = 46) were non-smokers or former smokers (Kramarow et al., 2023).

Arterial hypertension was defined in the study as blood pressure values ≥ 140/90 mmHg, a documented history of hypertension or ongoing antihypertensive pharmacotherapy at home. Arterial hypertension was present in 91,3% (n = 73) of patients, while in 8,8% (n = 7) no evidence of hypertension was found or no antihypertensive treatment was used. Among patients with confirmed hypertension, 11% (n = 8) were not receiving antihypertensive medication and 23,3% (n = 17) of all hypertensive patients had poorly controlled blood pressure according to the current criteria of the European Society of Cardiology (ESC) (McEvoy et al., 2024).

Type 2 diabetes mellitus was present in 26,3% (n = 21). Among them, 14,3% (n = 3) were receiving insulin therapy, while 85,7% (n = 18) were on oral hypoglycaemic treatment.

Dyslipidaemia is a major risk factor in patients with ischemic heart disease. Dyslipidaemia was defined as LDL-C > 3,0 mmol/L, triglycerides > 1,7 mmol/L and HDL-cholesterol < 1,0 mmol/L for men and < 1,2 mmol/L for women (Pappan et al., 2025). In order to standardize therapy with different types of statins, equivalent doses of routinely used statins in clinical practice were applied, based on the classification proposed by the American

College of Cardiology and the American Heart Association: rosuvastatin 5 mg = atorvastatin 10 mg = simvastatin 20 mg; rosuvastatin 10 mg = atorvastatin 20 mg = pitavastatin 4 mg; rosuvastatin 20 mg = atorvastatin 40 mg; rosuvastatin 40 mg = atorvastatin 80 mg (Grundy et al., 2019). Table 3 shows the distribution of patients according to the statin taken at home in each group based on the described equivalent doses, as well as the mean lipid profile values for each group on admission ± 1 SD. Patients receiving statins were divided into two groups: moderate-intensity and high-intensity statin therapy, according to the expected LDL-C reduction of approximately ~ 30% and ~ 50%, respectively.

Lipid profile (laboratory reference range)	**No statin**	**Rosuvastatin 5mg / Atorvastatin 10mg / Simvastatin 20mg**	**Rosuvastatin 10mg / Atorvastatin 20mg/ Pitavastatin 4mg**	**Rosuvastatin 20 mg / Atorvastatin 40mg**	**Rosuvastatin 40mg / Atorvastatin 80mg**
		Moderate-intensity statin therapy		**High-intensity statin therapy**	
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean
	n = 27	n = 11	n = 29	n = 12	n = 1
Total cholesterol mmol/L (2,7 – 5,18)	6,17 ± 1,25	4,31 ± 0,85	4,29 ± 1,00	4,01 ± 0,80	5,89
LDL–cholesterol mmol/L (0,00 – 2,60)	3,86 ± 1,25	2,27 ± 0,69	2,38 ± 0,90	1,92 ± 0,58	3,53
HDL-cholesterol mmol/L (1,0-1,60)	1,36 ± 0,36	1,38 ± 0,35	1,22 ± 0,38	1,25 ± 0,46	1,41
Triglycerides mmol/L (0,10-1,70)	2,36 ± 1,65	1,45 ± 0,63	1,50 ± 0,80	1,84 ± 1,05	2,07

Table 3. Distribution of patients according to statin type/dose at hospitalization and mean lipid fractions values ± 1 SD.

A statistically significant difference in lipid profile values for total cholesterol and LDL-cholesterol was observed between patients not receiving statin therapy and those receiving statins of different intensity. At Shapiro–Wilk $p > 0,05$, the data were considered normally distributed and Levene's test $p > 0,05$ showed no significant differences in variances, therefore, ANOVA was used: $p < 0,001$. To determine the direction of differences among the three groups (no statin therapy, moderate-intensity statin therapy and high-intensity statin therapy), a post hoc Tukey HSD analysis was performed. Patients not receiving statin therapy had significantly higher total cholesterol and LDL-C levels compared with patients receiving statins, regardless of statin type or dose $p < 0,001$ for all comparisons. No statistically significant difference in total cholesterol and LDL-cholesterol levels were found between the groups receiving statin therapy of different intensity $p > 0,05$.

Due to the non-normal distribution of HDL-C and triglyceride values (Shapiro–Wilk p < 0,05), the non-parametric Kruskal–Wallis test was used. No statistically significant difference was observed between patients not receiving statin therapy and those receiving statin therapy of different intensity, with p = 0,473 for HDL-C and p = 0,083 for triglycerides.

Patients with previous percutaneous coronary intervention performed more than one year before enrollment in the study were 12,5% (n = 10). All patients with prior coronary interventions had a baseline LDL-C < 2,6 mmol/L on admission in the department. They were receiving moderate- and high-intensity statin therapy in the corresponding equivalent doses: rosuvastatin 10 mg = atorvastatin 20 mg (n = 4) and rosuvastatin 20 mg = atorvastatin 40 mg (n = 6). However, only one patient (n = 1) achieved the target LDL-C level according to the current European Society of Cardiology guidelines (< 1,4 mmol/L) (Mach et al., 2025).

Patients were enrolled in the study following selective coronary angiography, which revealed < 70% luminal stenosis in at least one coronary artery using quantitative coronary angiography (QCA), or in case of diffuse coronary atherosclerosis not eligible for a revascularization procedure. The left anterior descending artery (LAD) was most frequently affected in 85%, followed by the right coronary artery (RCA) in 57,5% and the left circumflex artery (LCx) in 53,7% of cases. Table 4 presents the number of coronary arteries affected by intermediate-grade stenoses < 70% luminal narrowing assessed by QCA. The mean number of affected coronary arteries was 1,98 (95% CI 1,79–2,16).

Number of affected coronary arteries	**Percent (%) from all patients**	**N**
1	36,25%	29
2	30%	24
3	33,75%	27

Table 4. Number of affected coronary arteries as a percentage of the study cohort.

4.3. Pentraxin 3

Pentraxin 3 was measured at baseline in all 80 patients. Boxplot analysis identified 11 outlier values in the distribution of pentraxin 3 ≥ 2.491 ng/mL. The mean value of the biomarker in all patients was 1,88 ± 2,96 (95% CI 1,22–2,54) ng/mL, with a median of 0,9205 ng/mL.

Women had higher mean biomarker values: 2,35 ± 3,96 (95% CI 1,31–3,39) ng/mL, median 0,92 ng/mL (n = 38), whereas in men the mean values were 1,46 ± 1,54 (95% CI 0,98–1,94) ng/mL, median 0,94 ng/mL (n = 42), figure 3. No statistically significant increase in biomarker levels was observed with advancing age in either sex: in women, Spearman's correlation coefficient was ρ = 0,095, p = 0,570, while in men, Spearman's correlation coefficient was ρ = 0,058, p = 0,716. In our cohort, women were older than men, with a median age of 62 years (IQR 58–66) for women and 60 years (IQR 53–66) for men, however, this difference did not reach statistical significance Mann–Whitney U = 633,5, Z = -1,590, p = 0,112. Regarding left ventricular systolic function assessed by fractional shortening, the groups were similar: median FS in men was 30 (IQR 26–34)%, while in women it was 30,5 (IQR 26,5–34,5)%.

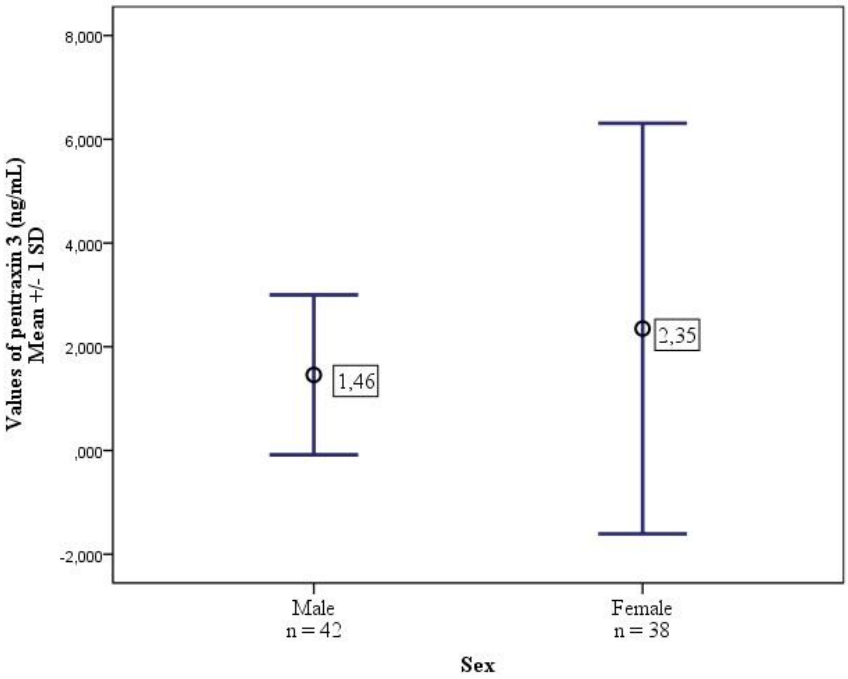


Figure 3. Error bar, illustrating the mean values of pentraxin 3 in men and women ± 1 SD.

Figures 4 and 5 graphically illustrate the distribution of pentraxin 3 values using a histogram with an overlaid Gaussian curve for men and women.

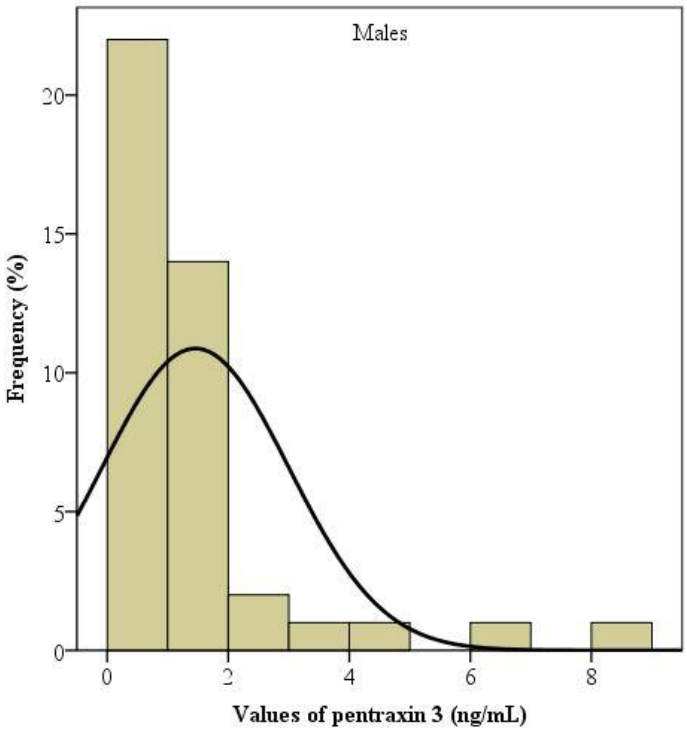


Figure 4. Histogram of pentraxin 3 values in men with a superimposed Gaussian curve.

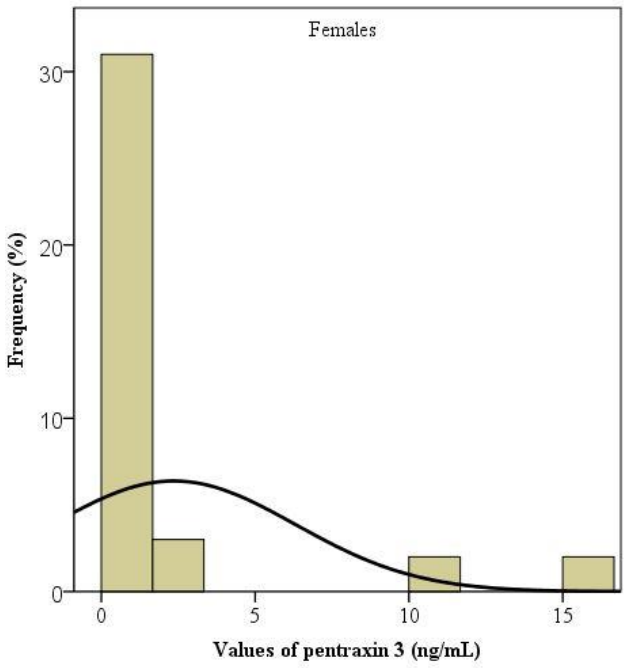


Figure 5. Histogram of pentraxin 3 values in women with a superimposed Gaussian curve.

The observed sex differences in biomarker levels are not attributable to difference in risk factors distribution. Table 5 presents the distribution of patients according to sex and risk profile. For comparison of categorical variables, Pearson's chi-square (χ^2) test was used for smoking, type 2 diabetes mellitus and dyslipidemia, while Fisher's exact test was applied for arterial hypertension. No statistically significant difference was found in the distribution of traditional risk factors between men and women in our cohort.

Risk factor	**Men**	**Women**	**p**
Smoking	58,8%	41,2%	0,330
N	20	14	
Arterial hypertension	54,8%	45,2%	0,248
N	40	33	
Type 2 diabetes mellitus	52,4%	47,6%	0,990
N	11	10	
Dyslipidemia	56%	44%	0,418
N	28	22	

Table 5. Traditional risk factors distribution in men and women enrolled in the study.

Table 6 presents the main demographic and clinical characteristics of the outliers and the rest of the cohort. Table 7 compares the distribution of traditional risk factors between these two groups.

Variable	Mean ± 1 SD	Mean ± 1 SD	p
N	69	11	
Age	59,12 ± 7,05	57,64 ± 8,42	0,710
BMI (kg/m^2)	30,74 ± 5,70	28,47 ± 5,38	0,246
LVEDD (mm)	49,39 ± 5,25	47,73 ± 6,90	0,154
FS (%)	30,86 ± 5,81	30,18 ± 5,17	0,718
Creatinine (mmol/L)	74,71 ± 16,06	76,82 ± 12,80	0,834
eGFR ($mL/min/1,73m^2$)	89,81 ± 19,48	83,63 ± 13,86	0,292
Total cholesterol (mmol/L)	4,84 ± 1,39	5,31 ± 1,32	0,211
LDL-C (mmol/L)	2,77 ± 1,28	3,04 ± 0,94	0,308
HDL-C (mmol/L)	1,31 ± 0,39	1,17 ± 0,28	0,201
Triglycerides (mmol/L)	1,76 ± 1,13	2,41 ± 1,61	0,068
CRP (mg/L)	3,34 ± 4,25	2,29 ± 3,02	0,394
PTX3 (ng/mL)	0,96 ± 0,35	7,65 ± 5,11	< 0,001

Table 6. Demographic and clinical characteristics of the outliers and the rest of the cohort, mean values ± 1 SD.

Risk factor	PTX3 < 2,491 ng/mL	PTX3 ≥ 2,491 ng/mL	p
N	69	11	
Smoking	44,9% (n = 31)	27,3% (n = 3)	0,338
Arterial hypertension	94,2% (n = 65)	72,7% (n = 8)	0,051
Type 2 diabetes mellitus	27,5% (n = 19)	18,2% (n = 2)	0,719
Dyslipidemia	58% (n = 40)	90,9% (n = 10)	0,046

Table 7. Presence of traditional risk factors in outliers and the rest of the cohort.

4.3.1. Discussion

In our cohort, patients have similar characteristics by sex, with no statistically significant difference in the distribution of traditional risk factors between men and women. We found that women had higher baseline levels of vascular inflammation assessed by PTX3. The difference in biomarker levels observed between sexes is consistent with the values reported in the literature by Yamasaki et al. in healthy Japanese population (Yamasaki et al., 2009). Dubin et al., in a cohort of 986 participants with a history of ischemic heart disease, reported elevated pentraxin 3 levels in patients who were older, caucasian, with reduced glomerular filtration rate, lower ejection fraction, concomitant heart failure or increased left ventricular mass (Dubin et al., 2012). In our cohort, the median age of women was only two years higher than that of men, nevertheless, the highest baseline pentraxin 3 values were observed in females. It is possible that persistently elevated baseline biomarker levels correlate with an increased long-term risk of adverse cardiovascular events in these individuals. This is particularly important given the lack of statistically significant difference between the routinely assessed clinical parameters of the main group (n = 69) and the outlier group (n = 11) in our study. A statistically significant difference between the two groups was found only with regard to dyslipidemia as a risk factor.

4.3.2. Pentraxin 3 and traditional risk factors

Regarding the patients' risk profile, no statistically significant difference in pentraxin 3 levels were found between smokers and non-smokers: Mann–Whitney U = 625, Z = -1,53, p = 0,126, as well as between patients with type 2 diabetes mellitus and those without: Mann–Whitney U = 616, Z = -0,038, p = 0,969. Arterial hypertension is a major risk factor for the development and progression of ischemic heart disease, however, no statistically significant difference in PTX3 levels was observed between patients with or without hypertension: Mann–Whitney U = 193,5, Z = -1,056, p = 0,291. According to the adopted criteria for dyslipidemia, no statistically significant difference in biomarker levels was observed between the group with and without dyslipidemia as a risk factor: Mann–Whitney U = 742,5, Z = -0,75, p = 0,941. No statistically significant correlation was found between plasma PTX3 levels and LDL-C as a continuous variable: Spearman's correlation coefficient $\rho = +0{,}058$, p = 0,607), nor between HDL-C and the inflammatory biomarker: Spearman's $\rho = -0{,}029$, p = 0,796. In the current ESC guidelines, non-HDL cholesterol is established as a key risk parameter included in the SCORE2 and SCORE2-OP risk calculators (Visseren et al., 2021).

Using this parameter, no statistically significant correlation with pentraxin 3 was observed: Spearman's ρ = 0,105, p = 0,355. Body mass index also showed no influence on PTX3 levels in our cohort: Spearman's ρ = -0,103, p = 0,364. Overall, these findings indicate no statistically significant differences in PTX3 levels between patient groups with or without traditional risk factors including smoking, type 2 diabetes mellitus, arterial hypertension or dyslipidemia. This pattern is illustrated in figure 6 using error bars.

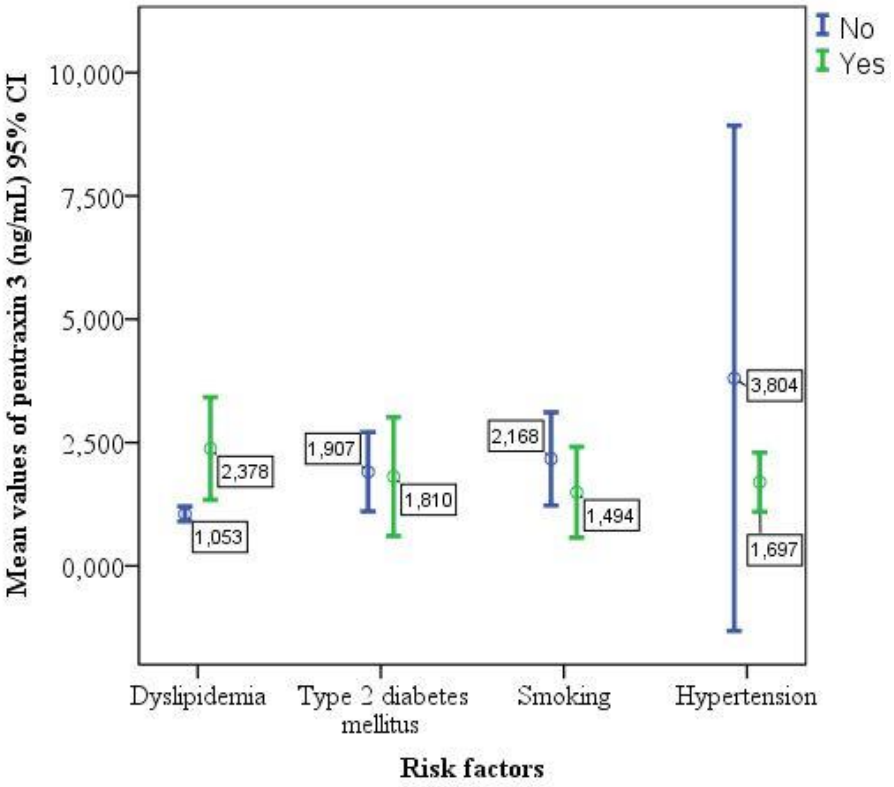


Figure 6. Error bars, illustrating the distribution of mean pentraxin 3 values in accordance with the presence of traditional risk factors.

4.3.3. Discussion

In our cohort, PTX3 levels did not show statistically significant difference between patient groups with or without traditional risk factors: smoking, type 2 diabetes mellitus, arterial hypertension and dyslipidemia. PTX3 appears to be independent of the presence of these risk characteristics, suggesting a potentially independent role in the pathogenesis of ischemic heart disease and in the progression of atherosclerosis. Our findings are supported by Inoue et al., who found that PTX3 is not influenced by lipid profile, diabetes mellitus, smoking, sex or obesity (Inoue et al., 2007). Whether elevated baseline levels of vascular

inflammation assessed with PTX3 can be considered an additional independent risk factor remains to be clarified. The results of published studies are heterogeneous. Lee et al. investigated biomarker levels in a Korean cohort without a history of statin use and found that, contrary to expectations, higher PTX3 levels may exert an atheroprotective and anti-inflammatory effect, showing an inverse correlation with traditional cardiovascular risk factors (Lee et al., 2019). The most likely explanation for the reported discrepancies between different studies lies in the difference between the respective cohorts, including race, age and comorbidities, as well as the difficulty in interpreting the individual contribution of each risk factor given the frequent coexistence of multiple risk factors in most patients.

4.3.4. Pentraxin 3 and C-reactive protein

We performed a correlation analysis between the two inflammatory biomarkers PTX3 and CRP. Both proteins are markers of inflammation and belong to the pentraxin family, however, their biological roles are different. Figure 7 illustrates a scatter plot of the two biomarkers in our cohort. Visual inspection suggests that higher CRP values are associated with lower PTX3 levels, and conversely, cases with elevated PTX3 values tend to show low CRP levels < 5 mg/L. The lack of a statistically significant correlation between the two inflammatory biomarkers was confirmed by Spearman's correlation coefficient $\rho = 0{,}045$, $p = 0{,}693$, indicating that the two variables are independent of each other.

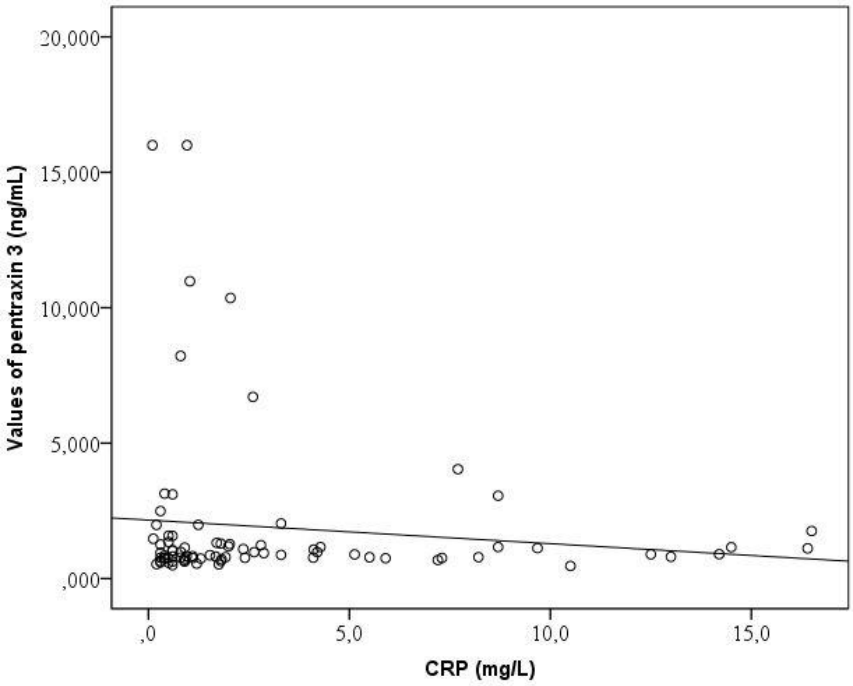


Figure 7. Scatter plot illustrating the lack of correlation between the values of PTX3 and CRP.

4.3.5. Discussion

These differences between the two proteins reflect their distinct biological roles. We observed marked variability in PTX3 levels at low CRP values, which may suggest that this marker is more sensitive and reflects a different aspect of the inflammatory process, namely baseline low-grade vascular inflammation. It is important to emphasize that all patients included in the study showed no clinical or laboratory evidence of an active inflammatory process. PTX3 is considered a more specific inflammatory marker reflecting vascular

inflammation because it is locally produced by cells directly involved in the atherosclerotic process, whereas CRP is produced by the liver. Our findings are supported by Miyazaki et al. in patients with myocardial infarction occurring at least 3 months prior to study inclusion. PTX3 did not correlate with CRP, in contrast to the positive correlation observed between CRP and SAA (Miyazaki et al., 2014). CRP is a non-specific systemic inflammatory biomarker, in clinical studies of patients with ischemic heart disease, the most commonly investigated form is high-sensitivity C-reactive protein (hsCRP). However, in a study reported by Soeki et al., in which both inflammatory markers were assessed, PTX3 was found to be more specific for atherosclerotic plaque instability (Soeki et al., 2011). According to Premnath et al., both proteins can predict the presence of coronary atherosclerosis, with PTX3 demonstrating superior predictive value (Premnath et al., 2022). Nevertheless, increasing hsCRP levels (< 1 mg/L, 1–3 mg/L, 3–10 mg/L, and > 10 mg/L) are considered to reflect an increased risk of all-cause mortality and major adverse cardiovascular events (MACE) (Carrero et al., 2019).

Both proteins, which belong to the pentraxin family, reflect different aspects of the inflammatory process. Although hsCRP has been studied more extensively, PTX3 is a specific marker of vascular inflammation, with potentially better predictive value and may have a role in supporting clinical assessment in patients with ischemic heart disease. It should be noted that hsCRP was not tested in our study.

4.3.6. Pentraxin 3 in patients with dyslipidemia on statin therapy

From the total cohort, 33,8% (n = 27) were not receiving statin therapy at the time of hospital admission, while 66,3% (n = 53) were already on statin treatment with varying intensity. Interestingly, no statistically significant difference in pentraxin 3 values was found between patients receiving statins and those not receiving statin therapy: Mann–Whitney U = 683, Z = -0,331, p = 0,741. Moreover, even after excluding patients not receiving statins and stratifying the remaining patients into moderate-intensity and high-intensity statin therapy groups (n = 53), no statistically significant difference in PTX3 levels was observed between these groups: Mann–Whitney U = 259, Z = -0,021, p = 0,984, figure 8.

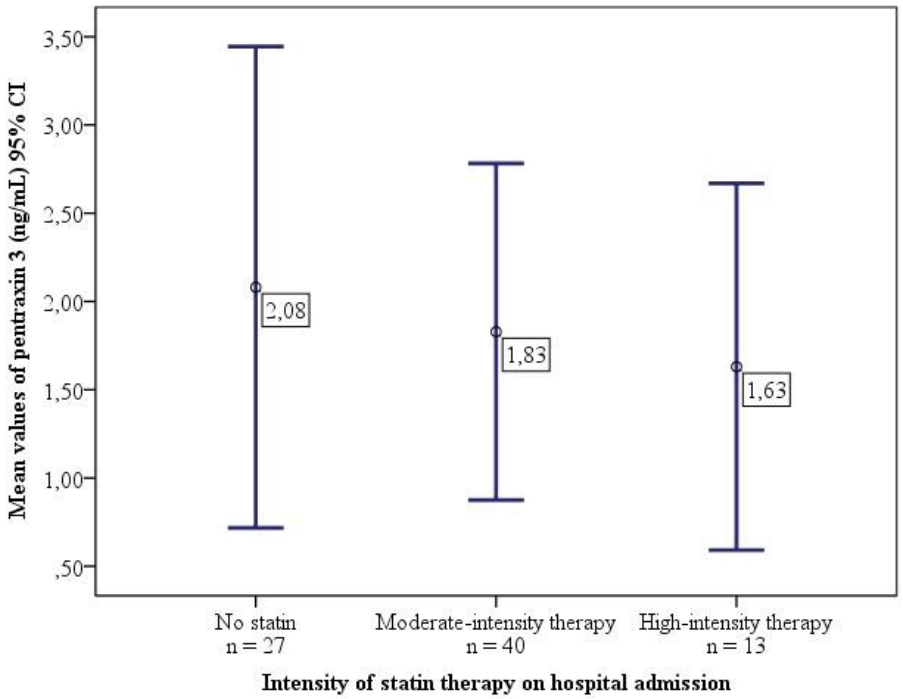


Figure 8. Error bars, distribution of mean pentraxin 3 values according to statin intensity treatment on hospital admission.

4.3.7. Discussion

Statin therapy is a cornerstone in the treatment of patients with ischemic heart disease. In addition to their lipid-lowering effects, statins exert pleiotropic actions, one of which is the upregulation of endothelial nitric oxide synthase, which is important for vasodilation, platelet aggregation and smooth muscle proliferation (Oesterle et al., 2017). Statins were the first proven medications to reduce baseline systemic inflammation, as assessed by the reduction in hsCRP levels. There is an ongoing debate as to whether the reduction in hsCRP levels is mediated by LDL-C lowering or represents an independent effect. The possibility of

pharmacological impact on systemic inflammation was initially suggested by the results of the AFCAPS/TexCAPS and CARE trials, where in patients with relatively low LDL-C but elevated CRP levels, treatment with pravastatin and lovastatin reduces adverse cardiovascular events (Ridker et al., 2001; Ridker et al., 1999).

As shown in figure 8, patients receiving high-intensity statin therapy visually exhibit lower baseline PTX3 levels compared with the other two groups. This difference does not reach statistical significance, most likely due to the relatively small sample size of the study cohort. In addition, selective coronary angiography did not provide evidence of unstable atherosclerotic plaques. In the literature, unstable plaques have been associated with elevated PTX3 levels and statin-induced plaque stabilization has been shown to reduce PTX3 concentrations. In this context, it is also possible that the pharmacological effect of statins, through plaque stabilization, does not exert an additional lowering effect on baseline PTX3 levels. Akaike et al. compare the possibility of PTX3 to serve as a biomarker of atherosclerotic plaque instability, similarly to how B-type natriuretic peptide is used as a marker of heart failure severity (Akaike et al., 2014). In our cohort, statin therapy, regardless of intensity, significantly reduced total cholesterol and LDL-C levels compared with the non-statin group, as demonstrated by parametric ANOVA and post hoc Tukey HSD analysis. However, no statistically significant difference in pentraxin 3 levels were observed, indicating that PTX3 remains independent of the pleiotropic anti-inflammatory effects of statins in our cohort of patients with coronary atherosclerosis who did not require revascularization. In the PRINCE study, pravastatin exerted its anti-inflammatory effect through a reduction in low-grade systemic inflammation, as measured by CRP levels (Albert et al., 2001). These findings further support the concept that CRP and PTX3 reflect different aspects of the inflammatory process. Statins reduce systemic inflammation as measured by hsCRP, independently of the presence of unstable atherosclerotic plaques, whereas PTX3 appears to decrease only up to the point of plaque stabilization, without evidence of further reduction thereafter. This raises the hypothesis that baseline vascular inflammation measured by PTX3 may be genetically determined, with limited potential for further pharmacological reduction, and that it may increase primarily in the setting of plaque instability and acute coronary syndrome. Large-scale studies addressing this question are still lacking, however, different polymorphisms in genes encoding PTX3 have been reported in the literature, some of which are associated with increased cardiovascular risk in the affected individuals (Dharma et al., 2020).

4.4. Pentraxin 3 and Seattle Angina Questionnaire

The mean results from the „Physical Limitation“ and „Angina Frequency“ scales in our study were 57,2 ± 17,3% and 76,7 ± 17,0%, respectively. For the „Quality of Life“ scale, the mean score was 47,9 ± 20,4%, while for the „Angina Stability“ and „Treatment Satisfaction“ scales the mean scores were 42,4 ± 32% and 78,3 ± 14,8%, respectively. In our cohort, a statistically significant correlation was found between pentraxin 3 levels and the „Physical Limitation“ scale of SAQ. Spearman's correlation analysis showed a coefficient of $\rho = -0,258$, $p = 0,021$, indicating a statistically significant weak negative correlation, where higher PTX3 levels were associated with lower „Physical Limitation“ scores, i.e., more severe angina limiting physical activity. A weak negative correlation was also observed between PTX3 levels and the „Angina Frequency“ scale of SAQ. The trend suggests more frequent angina episodes in these patients, with results close to the threshold of statistical significance: Spearman's correlation coefficient $\rho = -0,219$, $p = 0,051$. A statistically significant weak negative correlation was observed between higher PTX3 values and the „Quality of Life“ scale of SAQ, with Spearman's correlation coefficient $\rho = -0,319$, $p = 0,004$. For the remaining two SAQ scales, „Angina Stability“ and „Treatment Satisfaction“, no statistically significant correlations were found, with Spearman's correlation coefficients of $\rho = 0,015$, $p = 0,893$ and $\rho = -0,085$, $p = 0,455$, respectively. Pentraxin 3 also showed a statistically significant weak negative correlation with the summary SAQ score, which includes the „Physical Limitation“, „Angina Frequency“ and „Quality of Life“ scales: Spearman's correlation coefficient $\rho = -0,311$, $p = 0,005$.

4.4.1. Pentraxin 3 and The Physical Limitation scale

We divided the SAQ „Physical Limitation“ scores into quartiles. In literature the „Physical Limitation“ and „Quality of Life“ scales are divided as follows: 0–24%, 25–49%, 50–74%, and 75–100% (Thomas et al., 2021). Table 8 presents PTX3 values according to the different groups of the SAQ „Physical Limitation“ scale. It is noteworthy that higher mean PTX3 values were associated with more severe physical limitation and vice versa. However, this relationship between groups did not reach statistical significance. Using the non-parametric Kruskal–Wallis test, only a trend was observed: Kruskal–Wallis $\chi^2 = 6,894$, $p = 0,075$. The described correlation is illustrated in figure 9 using a scatter plot.

Physical Limitation (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–24	4,02	-30,02–38,07	4,03	3,79	2
25–49	2,99	1,18–4,80	1,16	4,18	23
50–74	1,46	0,68–2,25	0,88	2,45	40
75–100	1,01	0,74–1,27	0,81	0,48	15

Table 8. PTX3 values according to the SAQ „Physical Limitation“ scale divided into quartiles in the study cohort.

Tables 9 and 10 present PTX3 values according to the different quartiles of the SAQ „Physical Limitation“ scale, stratified by sex.

Physical Limitation (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–24	6,71	–	–	–	1
25–49	1,92	0,43–3,40	1,16	2,21	11
50–74	1,09	0,74–1,45	0,86	0,78	21
75–100	1,17	0,73–1,60	1,04	0,57	9

Table 9. PTX3 values according to the SAQ „Physical Limitation“ scale divided into quartiles in men.

Physical Limitation (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–24	1,35	–	–	–	1
25–49	3,97	0,59–7,35	1,20	5,32	12
50–74	1,88	0,20–3,55	0,90	3,47	19
75–100	0,78	0,68–0,87	0,75	0,09	6

Table 10. PTX3 values according to the SAQ „Physical Limitation“ scale divided into quartiles in women.

Table 11 presents PTX3 values according to the quartiles of the SAQ „Physical Limitation“ scale after exclusion of outlier values from the statistical analysis. PTX3 values ≥ 2.491 ng/mL were excluded from the analysis.

Physical Limitation (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–24	1,35	–	–	–	1
25–49	1,00	0,79–1,20	1,12	0,39	17
50–74	0,92	0,83–1,01	0,85	0,27	36
75–100	1,01	0,74–1,27	0,81	0,48	15

Table 11. PTX3 values and the quartiles of the SAQ „Physical Limitation“ scale after exclusion of patients with outlier PTX3 values.

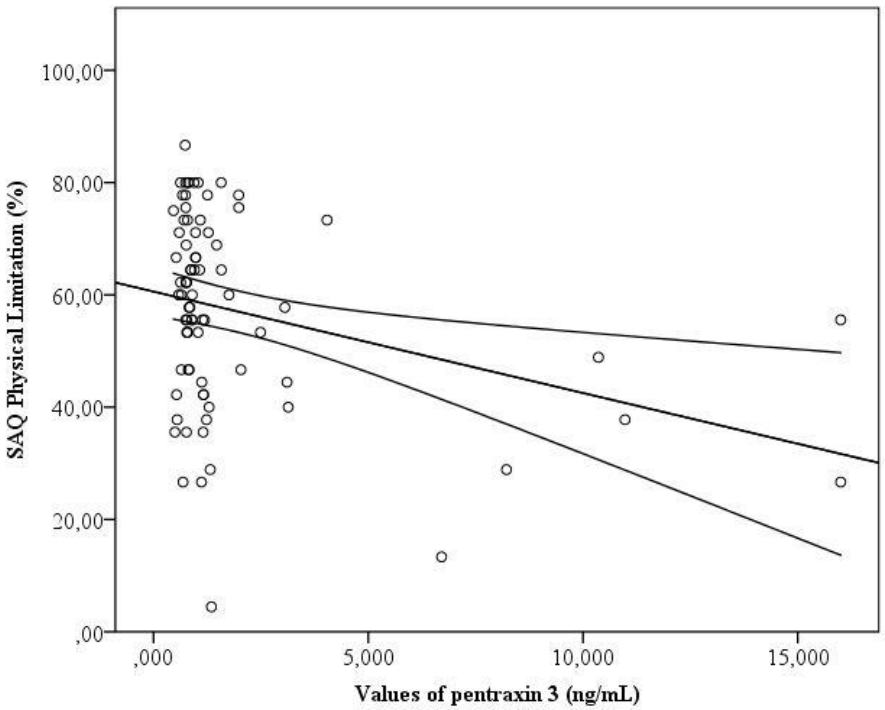


Figure 9. Scatter plot illustrating the change in values of pentraxin 3 according to daily physical activity limit assessed with the SAQ „Physical Limitation“ scale.

The cohort was divided into two groups according to the severity of angina symptoms. Mild angina was defined as SAQ „Physical Limitation“ scores of 50–100% (n = 55), while severe angina was defined as scores of 0–49% (n = 25). After dichotomization of the variable, the statistically significant difference in PTX3 levels between the two groups was further confirmed: Mann–Whitney U = 475,5, Z = -2,201, p = 0,028. Receiver Operating Characteristic (ROC) analysis, presented in figure 10, demonstrates that pentraxin 3 levels alone have a statistically significant weak ability to discriminate between patients with severe angina symptoms and those with mild symptoms. The area under the curve (AUC) from the ROC analysis is 0,654 (95% CI 0,51–0,80, p = 0,028). The optimal cut-off value determined by the Youden index is 1,1025 ng/mL, at which PTX3 demonstrates 68% sensitivity and 74,5% specificity in differentiating patients with severe angina symptoms from those with mild symptoms. To further illustrate the described results and associations for the SAQ “Physical Limitation” scale, we created a boxplot graph in which patients were divided into two groups according to the optimal cut-off value determined by the Youden index, figure 11. First, it can be visualized that the median score of the respective SAQ scale was higher in the group with low PTX3 values. In addition, the group with high PTX3 levels showed a greater range of variability. An isolated outlier value for the SAQ “Physical Limitation” scale was observed in the low PTX3 group.

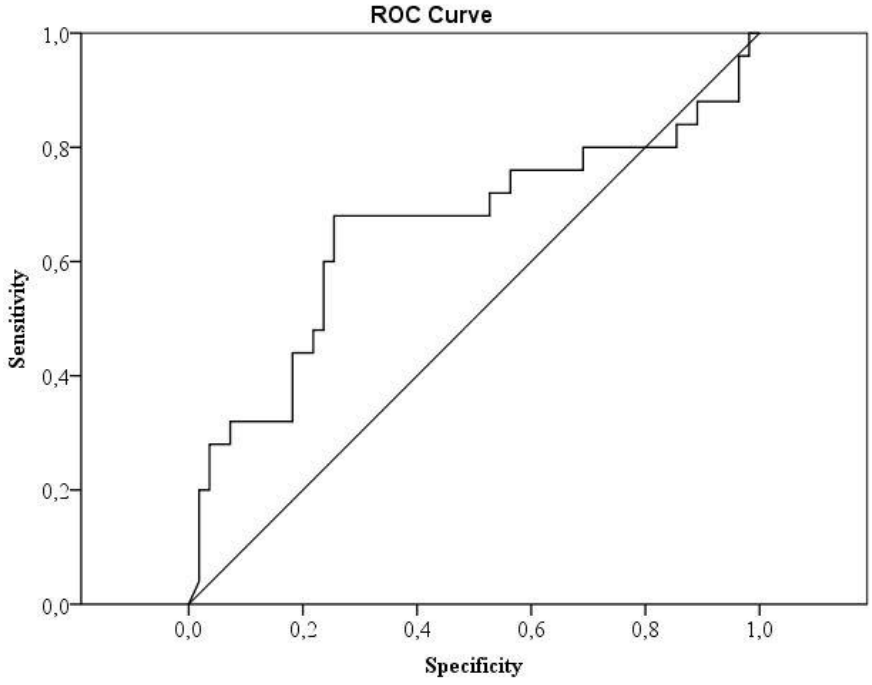


Figure 10. ROC curve of PTX3 for discrimination of angina severity.

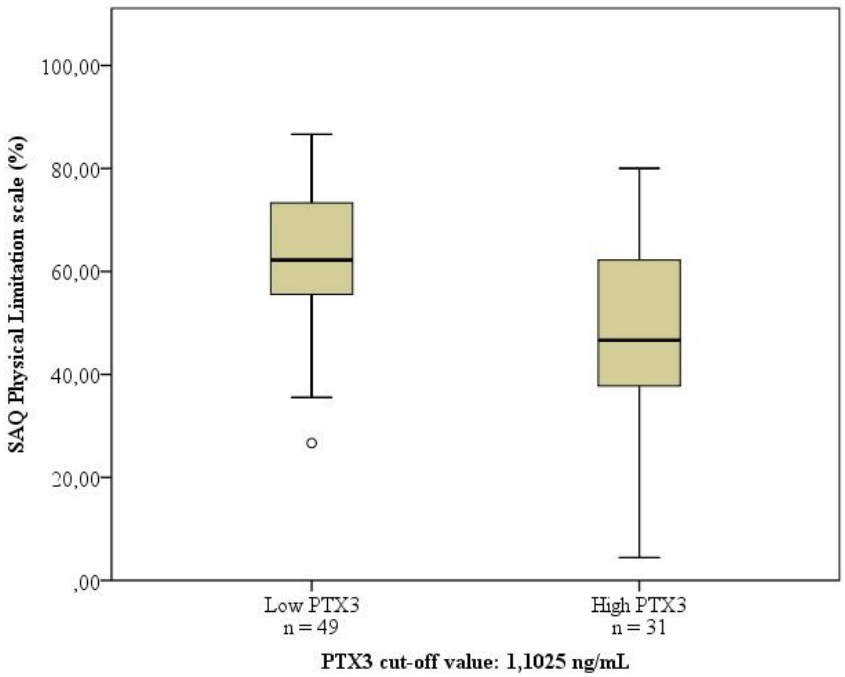


Figure 11. Boxplot showing the distribution of patients according to the determined PTX3 cut-off value for the SAQ „Physical Limitation“ scale.

4.4.2. Pentraxin 3 and The Angina Frequency scale

We divided the entire cohort into two groups according to the SAQ „Angina Frequency“ scale. The first group included patients with scores of 0–60% (n = 14) with daily to weekly angina symptoms, while the second group included patients with scores of 61–100% (n = 66), corresponding from monthly symptoms to absence of angina. After dichotomization of the variable, a statistically significant difference in PTX3 levels between the two groups was observed: Mann–Whitney U = 259, Z = -2,571, p = 0,01. Pentraxin 3 has a statistically significant moderate ability to independently discriminate patients with more frequent angina symptoms: AUC = 0,720 (95% CI 0,56–0,88, p = 0,01). The ROC analysis is illustrated in figure 12. The optimal cut-off value determined by Youden index is 1,01 ng/mL, yielding a sensitivity of 78,6% and a specificity of 63,6% for PTX3 to distinguish patients with more frequent angina. The described association is graphically depicted in figure 13 with a scatter plot.

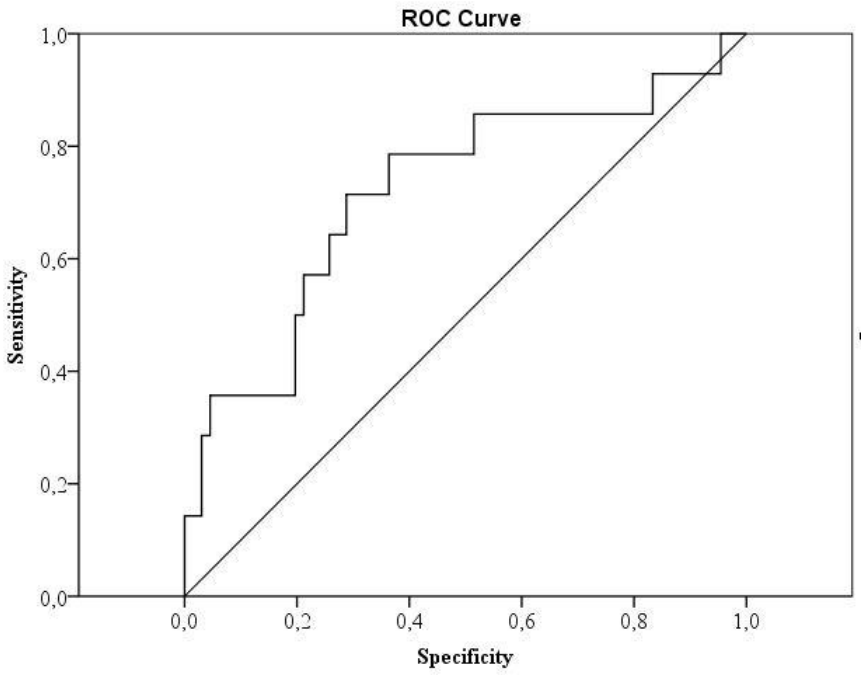


Figure 12. ROC curve of PTX3 to differentiate the frequency of angina symptoms.

Tables 12, 13 and 14 present PTX3 values in the cohort and according to the SAQ „Angina Frequency“ scale, stratified into two groups and by sex. It is noteworthy that higher mean PTX3 values were associated with a higher frequency of daily angina symptoms. A statistically significant difference between the two groups was observed in men: Mann–Whitney U = 84, Z = -1,977, p = 0,048, but not in women: Mann–Whitney U = 44, Z = -1,663, p = 0,096. Table 15 presents PTX3 values after exclusion of outlier patients from the analysis. Using the non-parametric Mann–Whitney U test, no statistically significant difference was found between the two groups: Mann–Whitney U = 198, Z = -1,283, p = 0,200. Table 16 shows the distribution of patients with outlier PTX3 values across the SAQ „Angina Frequency“ categories.

Angina Frequency (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–60	4,24	1,07–7,42	1,30	5,50	14
61–100	1,38	0,94–1,82	0,87	1,78	66

Table 12. PTX3 values and the SAQ „Angina Frequency“ scale scores in the study cohort.

Angina Frequency (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–60	2,69	0,52–4,86	1,28	2,83	9
61–100	1,12	0,87–1,37	0,86	0,71	33

Table 13. PTX3 values and the SAQ „Angina Frequency“ scale scores in men.

Angina Frequency (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–60	7,04	-3,12–17,20	1,35	8,18	5
61–100	1,64	0,79–1,20	0,89	2,41	33

Table 14. PTX3 values and the SAQ „Angina Frequency“ scale scores in women.

Angina Frequency (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–60	1,04	0,82–1,26	1,14	0,29	9
61–100	0,95	0,86–1,04	0,82	0,36	60

Table 15. PTX3 values and the SAQ „Angina Frequency“ scale scores after excluding the outlier patients.

Angina Frequency (%)	Mean value of PTX3 (ng/mL)	95% Confidence interval	Median	Standard deviation	N
0–60	10,01	2,85–17,18	8,22	5,77	5
61–100	5,67	1,57–9,77	3,57	3,91	6

Table 16. PTX3 values and the SAQ „Angina Frequency“ scale scores in outlier patients.

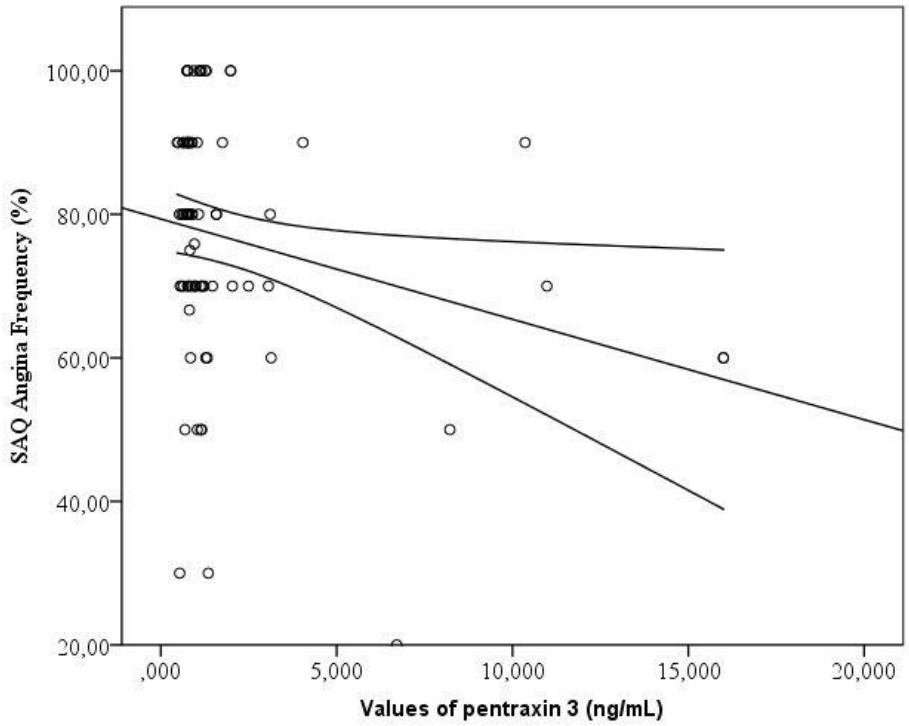


Figure 13. Scatter plot illustrating the change in values of pentraxin 3 according to angina frequency assessed with the SAQ „Angina Frequency“ scale.

The described results and associations for the SAQ „Angina Frequency“ scale are illustrated using a boxplot, in which patients were divided into two groups according to the optimal cut-off value determined by the Youden index, figure 14. An isolated outlier value was observed in the low PTX3 group.

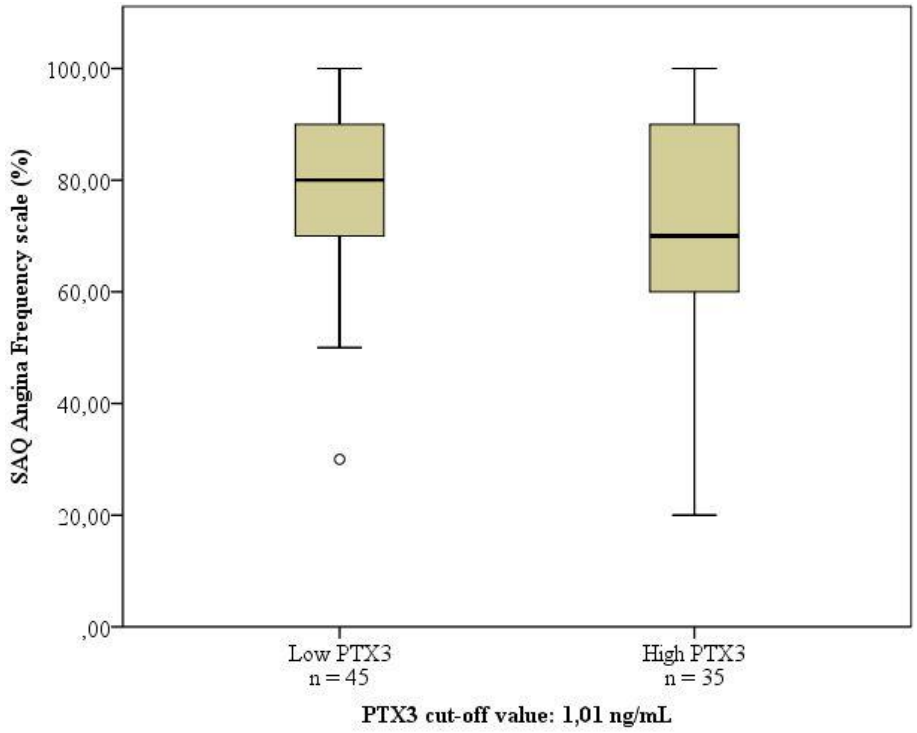


Figure 14. Boxplot showing the distribution of patients according to the determined PTX3 cut-off value for the SAQ „Angina Frequency“ scale.

4.4.3. Pentraxin 3 and The Quality of Life scale

The third SAQ scale showing a statistically significant association with PTX3 levels based on Spearman correlation coefficient is the „Quality of Life“ scale. We dichotomized the patient results from this scale by defining two groups. The group with poorer quality of life were with scores 0–49% (n = 38) and the group with better quality of life were with scores 50–100% (n = 42). A statistically significant difference in PTX3 values between the two groups was observed: Mann–Whitney U = 567, Z = -2,226, p = 0,026. Pentraxin 3 has a statistically significant weak ability to distinguish patients with poorer quality of life with an AUC = 0,645 (95% CI 0,52–0,77, p = 0,026). The optimal PTX3 cut-off value determined by the Youden index was 0,821 ng/mL, yielding a sensitivity of 73.7% and a specificity of 52.4% for identifying patients with poorer quality of life, figure 15.

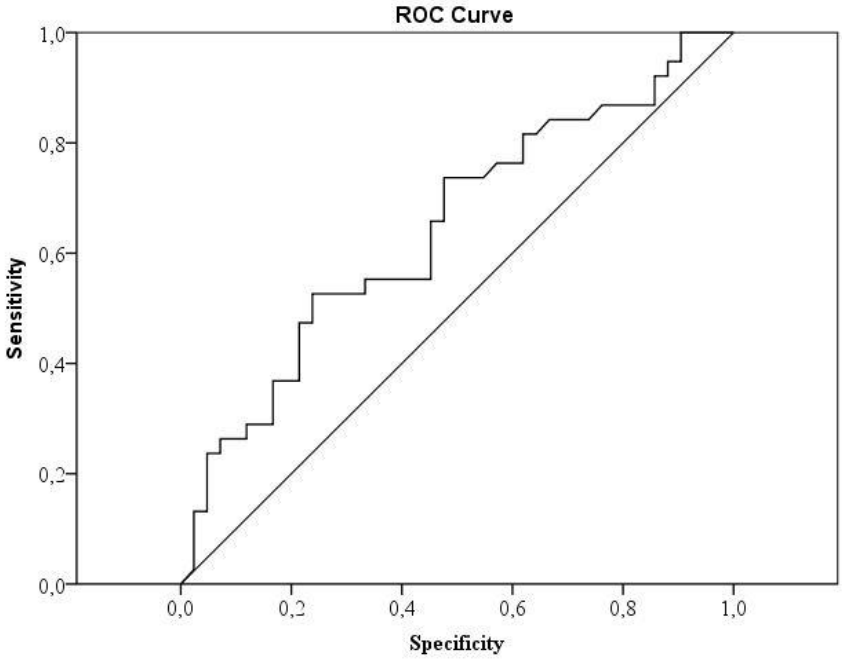


Figure 15. ROC curve of PTX3 to identify patients with worse quality of life.

To illustrate the distribution of SAQ „Quality of Life“ scores across groups with low and high PTX3 levels determined by the Youden index, a boxplot was created, figure 16.

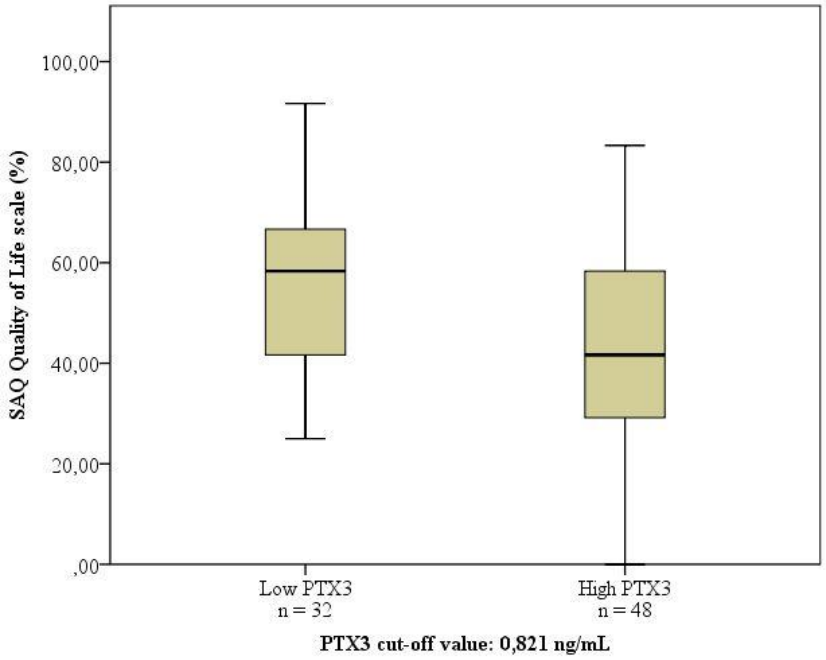


Figure 16. Boxplot showing the distribution of patients according to the determined PTX3 cut-off value for the SAQ „Quality of Life“ scale.

4.4.4. Discussion

Recognition of angina symptoms in everyday clinical practice may be challenging, as it is largely subjective and dependent on the clinician’s experience. According to data reported by Arnold et al., there is a tendency to underestimate the severity of angina symptoms when assessment is based solely on medical history interview. In order to increase objectivity in the evaluation of patients’ angina symptoms, we used the validated Seattle Angina Questionnaire (SAQ). The questionnaire is sensitive to clinical changes in angina functional class and patient symptoms. The difficulties in analyzing the questionnaire results arise from the lack of clearly standardized reference values and cut-off limits for the individual scales. Nevertheless, the use of a validated questionnaire with standardized, equal and comprehensible questions at each assessment provides a more accurate evaluation of patients’ functional capacity than a medical history interview conducted by the clinician during examination. For the assessment of the individual scales, we used the formulas proposed by the developers of the questionnaire, obtaining scores expressed as percentages from 0 to 100% with higher scores reflecting a more favourable patient status depending on the respective scale, i.e., less physical limitation in daily activities, greater treatment satisfaction, reduction of angina symptoms during the performance of everyday activities and better quality of life.

The results of our study support the hypothesis that patients with elevated baseline pentraxin 3 levels experience more frequent angina episodes, greater physical limitation in daily activities and poorer quality of life. The individual domains of the questionnaire have different predictive values. The SAQ „Physical Limitation“ scale has been reported as a strong predictor of rehospitalizations, myocardial infarction and mortality. The SAQ „Angina Frequency“ scale has the strongest predictive value regarding subsequent hospital admissions, thus, low scores in these two questionnaire domains are associated with a fourfold higher risk of subsequent rehospitalizations (Thomas et al., 2021). The mean score for the „Angina Frequency“ scale in our cohort is 76.7 ± 17.0%, which corresponds to findings from large registries such as COURAGE and ISCHEMIA, where daily angina is observed only in a small proportion of patients (Ferraro et al., 2020). Among women with daily to weekly angina symptoms, marked variability in PTX3 values was observed, with a tendency toward higher biomarker levels and consequently higher baseline vascular inflammation. Reynolds et al. reported that angina symptoms are more pronounced in women than in men, even in the presence of a similar degree of coronary atherosclerotic involvement. This may partly be explained by psychological stress and lower endorphin levels (Reynolds et al., 2020).

Regarding the independent prognostic value of the biomarker, despite the statistically significant weak negative correlation with the SAQ „Physical Limitation“ scale and the significant difference between groups with different angina frequency, the biomarker alone has limited ability to discriminate the severity and frequency of angina symptoms. In both sexes, pentraxin 3 may be used in clinical practice as an adjunct to clinical assessment of the severity and frequency of angina symptoms.

From a pathophysiological perspective, it can be summarized that acceleration of angina symptoms, even in the absence of elevated markers of myocardial injury, leads to increased levels of local vascular inflammation, which in turn promotes ulceration of atherosclerotic plaques and enhances thrombogenicity. The lack of a statistically significant correlation between PTX3 and the SAQ scales „Angina Stability“ and „Treatment Satisfaction“ is likely due to the fact that the former consists of only a single item, which compares the patient’s subjective perception of angina during the most strenuous activity with their health status four weeks earlier. In their daily lives, patients tend to limit their physical activity until the onset of angina, without necessarily recognizing that their overall functional capacity is reduced. For this reason, symptoms may be underreported and a statistically significant association is not observed in this scale. In addition to limiting daily activities,

some patients may not be aware that their treatment could potentially be further improved to achieve complete relief from angina. Consequently, no statistically significant correlation with PTX3 is observed in the „Treatment Satisfaction“ scale as well.

Our data indicate that pentraxin 3 is associated with patients' current symptoms and the severity of their clinical presentation, as assessed by the SAQ scales „Physical Limitation“, „Angina Frequency“ and „Quality of Life“.

Patients with stable angina represent a low-risk group for adverse cardiovascular events. Douglas et al. reported a 3,1% event rate during follow-up, while results from the Euro Heart Survey of Stable Angina shows a 1,4% rate of non-fatal myocardial infarction in the overall cohort and 3,2% in the subgroup with angiographically documented coronary atherosclerosis (Daly et al., 2006; Douglas et al., 2015). The low incidence of adverse cardiovascular events in this population further emphasizes the need for more precise and careful evaluation of the indications for performing selective coronary angiography. In our study, we find that pentraxin 3 has functional relevance in patients with angina pectoris, showing a consistent relationship with the individual scales of the SAQ questionnaire. Jenny et al. describe an association between PTX3 levels and coronary artery calcium score in the MESA study, linking the biomarker to the anatomical substrate of angina. PTX3 is associated with the presence of coronary calcium but not with its burden as quantified by the Agatston score (Jenny et al., 2014).

Our data and the available literature suggest that the biomarker may be used in addition to other imaging or functional methods in cardiology in the assessment of coronary atherosclerosis, thus providing additional clinical value. This is of particular importance given the increasing role in clinical practice of functional tests for the assessment of myocardial ischemia and for refining the indications for subsequent selective coronary angiography in patients with chronic coronary syndrome.

4.5. Pentraxin 3 and distribution of coronary atherosclerosis

We divided the patients according to the number of coronary arteries with < 70% luminal stenosis. The non-parametric Kruskal–Wallis test was used to assess difference in PTX3 levels across groups with different numbers of diseased coronary arteries. The results showed no statistically significant difference between the groups: Kruskal–Wallis $\chi^2 = 2{,}409$, $p = 0{,}300$. Nevertheless, figure 17 presents a bar chart illustrating a visual trend toward decreasing mean PTX3 values with the increasing number of coronary arteries affected by atherosclerosis. It should again be emphasized that this visual association did not reach statistical significance in the non-parametric analysis, as confirmed by Spearman's correlation coefficient: $\rho = -0{,}106$, $p = 0{,}348$.

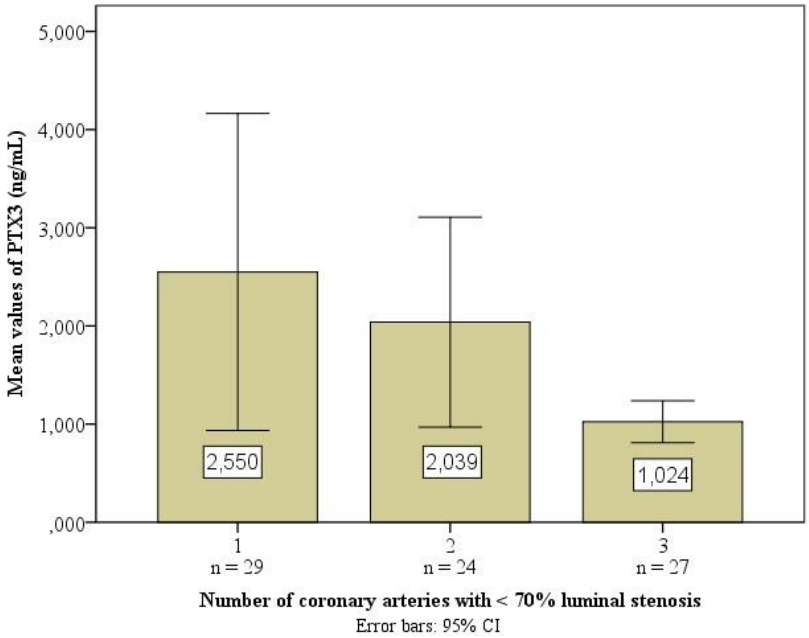


Figure 17. Bar chart with error bars (95% CI) of mean plasma PTX3 values according to the number of coronary arteries with < 70% luminal stenosis.

To determine whether there is a difference between patient groups divided according to the number of coronary arteries affected by intermediate-grade stenoses and the SAQ „Physical Limitation" scale, given that the Shapiro–Wilk test ($p > 0.05$) indicated normal distribution of the data and Levene's test ($p > 0.05$) showed no significant differences in variances, a one-way analysis of variance (ANOVA) was performed. The results demonstrated no statistically significant difference between the three groups ($F = 1{,}123$, $p = 0{,}331$).

Non-normal distribution of the data in the SAQ „Angina Frequency“ scale was assumed following the Shapiro–Wilk test ($p < 0.05$) and the non-parametric Kruskal–Wallis test was applied. No significant difference was found between the patient groups classified according to the number of affected coronary arteries by intermediate-grade stenoses and the SAQ „Angina Frequency“ scale: Kruskal–Wallis $\chi^2 = 2{,}013$, $p = 0{,}365$.

Following sample processing and acquisition of PTX3 results, it was observed that some of the patients in whom antianginal therapy was intensified with increased number of medications at discharge were, in fact, those with higher baseline PTX3 values. This correlation shows a weak positive trend without reaching statistical significance: Spearman’s correlation coefficient $\rho = 0{,}117$, $p = 0{,}303$. It should be noted that this finding may be influenced by therapeutic clinical pragmatism and the absence of a conducted exercise stress test in our study, which could have objectively assessed exertional angina symptoms and guided the prescription of antianginal therapy. Table 17 and figure 18 present the groups and the percentage of antianginal medications prescribed at discharge. The change in the frequency of use was assessed using McNemar’s test for paired binary variables.

Medication	Frequency on admission	Frequency at discharge	p
Beta-blocker	60%	73,8%	p = 0,007
Dihydropyridine calcium channel blockers	42,5%	55%	p = 0,013
Nitrate	13,8%	7,5%	p = 0,332
Ivabradine	3,8%	1,3%	p = 0,500
Trimetazidine	7,5%	10%	p = 0,687
Ranolazine	0%	0%	–

Table 17. Frequency of antianginal medication classes on admission and at discharge.

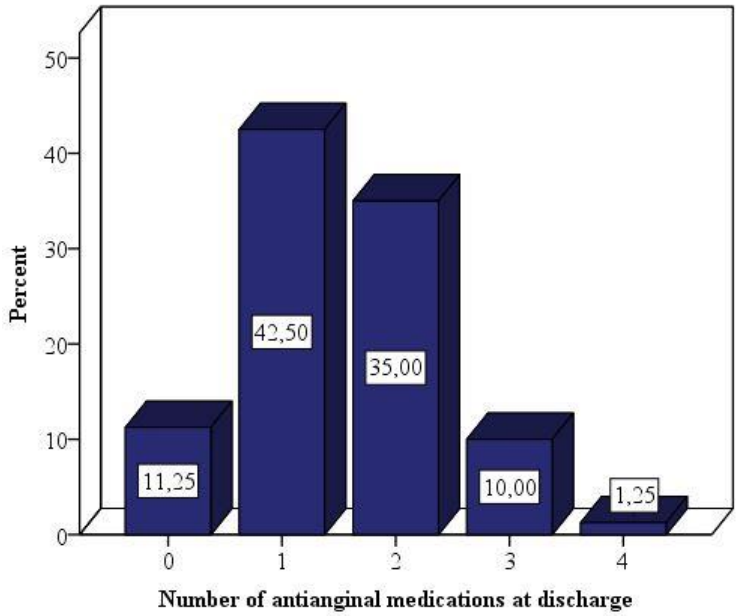


Figure 18. Bar chart illustrating the number of antianginal medications at discharge in percentage.

4.5.1. Discussion

The results show no statistically significant difference between plasma PTX3 levels and the distribution of groups according to the number of coronary arteries affected by intermediate-grade stenoses. In our study, PTX3 did not correlate with the anatomical substrate for angina and the number of coronary arteries affected by atherosclerosis. Contrary to expectations, PTX3 values do not elevate with the increasing number of diseased coronary arteries. These findings, together with the previously described weak but statistically

significant negative correlation between PTX3 levels and the SAQ „Physical Limitation“ scale, suggest that some patients experience angina not solely due to epicardial coronary atherosclerosis, but possibly also due to overlapping microvascular dysfunction. The results in our study are most likely attributable to the complex and multifactorial role of the biomarker, reflecting local vascular inflammation. Previous studies have demonstrated increased systemic and local levels of PTX3 in the development of complicated atherosclerotic plaques (ulceration, thrombosis). However, in the context of stable atherosclerotic plaques, PTX3 may exert an additional stabilizing effect by reducing smooth muscle cell proliferation and thrombosis (Otani et al., 2024). Conte et al. reported elevated PTX3 levels in patients with high-risk low-attenuation atherosclerotic plaques identified by computed tomography angiography, as part of the CAPIRE study (Conte et al., 2021). A discrepancy between the severity of coronary atherosclerosis and PTX3 levels has also been described by Nakamura et al., who performed computed tomography angiography in patients either undergoing screening or presenting with angina symptoms (Nakamura et al., 2015). Interestingly, Norata et al. demonstrated in experimental models that increased HDL-C levels are associated with upregulation of PTX3 expression, but not of CRP or SAP (Norata et al., 2008). These findings support the notion that PTX3 is not a unidirectional molecule. It has diverse biological functions and, in relation to atherosclerosis, likely exerts a primarily protective effect in its early stages, while potentially contributing to the amplification of an already established plaque destabilization. PTX3 secretion by vascular endothelial cells is the result of the multifactorial interplay between pro-inflammatory and anti-inflammatory mechanisms. The biomarker appears to have a balanced and likely disease-dependent role in maintaining vascular homeostasis, modulating the acute inflammatory response and contributing either to stabilization or destabilization of atherosclerotic plaques.

4.6. Pentraxin 3 and clinical follow-up

All patients were followed after discharge with an individual follow-up visit. The median follow-up period was 16 months (IQR 9–23). We kept track of the onset and the number of rehospitalizations due to acceleration of angina symptoms (unstable angina pectoris, fatal and non-fatal myocardial infarction) and the following selective coronary angiographies performed, and subsequent percutaneous coronary interventions up to September 2025. No cardiovascular deaths were observed in our cohort during the follow-up period. During the clinical follow-up, one-time rehospitalizations due to worsening of angina symptoms requiring repeat selective coronary angiography, based on indications established

at the respective cardiology department, were documented in 13,8% (n = 11) of patients. Percutaneous coronary intervention (PCI) was performed in 11,8% (n = 9) of patients. When stratified by sex, 72,7% (n = 8) of rehospitalizations occurred in men, while 27,3% (n = 3) occurred in women. Interestingly, patients with higher baseline biomarker values were at increased risk of rehospitalization due to acceleration of angina symptoms, with baseline PTX3 levels in the cohort demonstrating a weak but statistically significant positive correlation: Spearman's correlation coefficient $\rho = 0{,}266$, $p = 0{,}017$. A ROC analysis was performed to determine the optimal prognostic value of the biomarker in the cohort. The ROC curve is presented in figure 19.

The area under the curve is 0.723 (95% CI 0,59–0,86, $p = 0{,}018$), indicating a statistically significant moderate discriminative ability of PTX3 to identify patients at increased risk of rehospitalizations due to worsening of angina symptoms during the clinical follow-up.

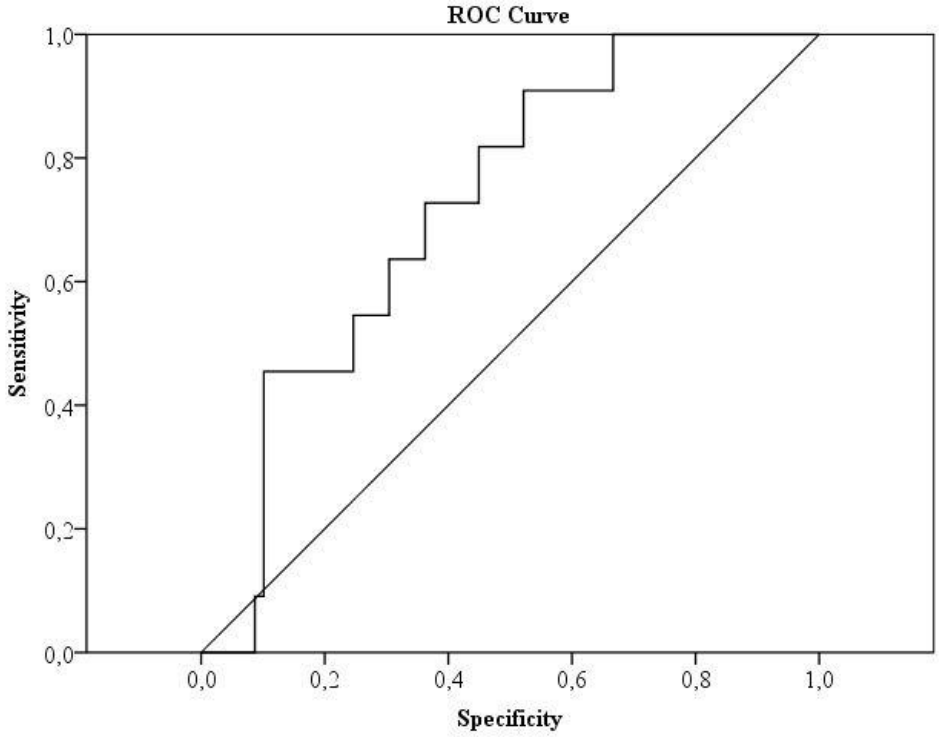


Figure 19. ROC curve of PTX3 as a predictor of rehospitalizations due to worsening of angina symptoms.

We identified a PTX3 value ≥ 0,9205 ng/mL as the optimal cut-off threshold for predicting the risk of rehospitalizations using the Youden index. This threshold demonstrated a sensitivity of 81,8% and a specificity of 55,1%. In comparison, the same value used to determine the severity of angina symptoms in daily life through the SAQ „Physical Limitation“ scale showed a sensitivity of 68% and a specificity of 58,2%. As an absolute value, the value of 0,9205 ng/mL is close to the previously determined optimal PTX3 cut-off of 1,1025 ng/mL for the respective SAQ domain. When this threshold was applied to assess the risk of rehospitalizations due to worsening of angina symptoms, specificity increased to 65,2% but at the expense of lower sensitivity of 63,6%. Table 18 summarizes the proposed from us PTX3 cut-off values together with their corresponding sensitivity and specificity according to the respective endpoint. Both the optimal PTX3 thresholds (with bold) and those determined using the Youden index are presented, with the sensitivity and specificity of each biomarker value additionally evaluated in relation to the other study endpoints.

	PTX3 value (ng/mL)	**Acceleration of angina symptoms (unstable angina pectoris, fatal and non-fatal myocardial infarction)**	**SAQ „Physical Limitation“ scale**	**SAQ „Angina Frequency“ scale**	**SAQ „Quality of Life“ scale**
Clinical equivalent		Worsening of clinical condition	Physical capacity	Frequency of complaints	Quality of life
Sensitivity	0,9205	**81,8%**	68%	78,6%	55,3%
Specificity		**55,1%**	58,2%	56,1%	54,8%
Sensitivity	1,1025	63,6%	**68%**	71,4%	52,6%
Specificity		65,2%	**74,5%**	68,2%	73,8%
Sensitivity	1,01	72,7%	68%	**78,6%**	55,3%
Specificity		60,9%	67,3%	**63,6%**	66,7%
Sensitivity	0,821	90,9%	72%	85,7%	**73,7%**
Specificity		44,9%	45,5%	45,5%	**52,4%**

Table 18. Summary of the optimal cut-off values recommended from us based on our study that can be applied in clinical practice.

Following the determination of a PTX3 cut-off value ≥ 0,9205 ng/mL as the optimal threshold for predicting the risk of rehospitalizations, the patient cohort was divided into groups with high and low PTX3 levels according to the predefined cut-off and median value of 0,9205 ng/mL. Given the binary nature of the primary endpoint: rehospitalizations due to worsening of angina symptoms - a univariate logistic regression analysis was performed with pentraxin 3 as a predictor. The analysis demonstrated that patients with PTX3 values ≥ 0,9205 ng/mL had a 5,5-fold higher likelihood of rehospitalizations compared with those with levels < 0,9205 ng/mL: Odds Ratio (OR) = 5,516. The regression coefficient (B = 1,708) indicated a positive association between elevated PTX3 values and the risk of rehospitalizations due to

worsening of angina symptoms. The statistical significance of the results was further supported by the Wald statistic: Wald = 4,355, p = 0,037. The model constant (B = −2,944) indicates a low probability of rehospitalizations among patients with PTX3 levels < 0,9205 ng/mL. These findings have important clinical implications, enabling the stratification of patients into two groups based on PTX3 levels: a low-risk and a high-risk group, figure 20.

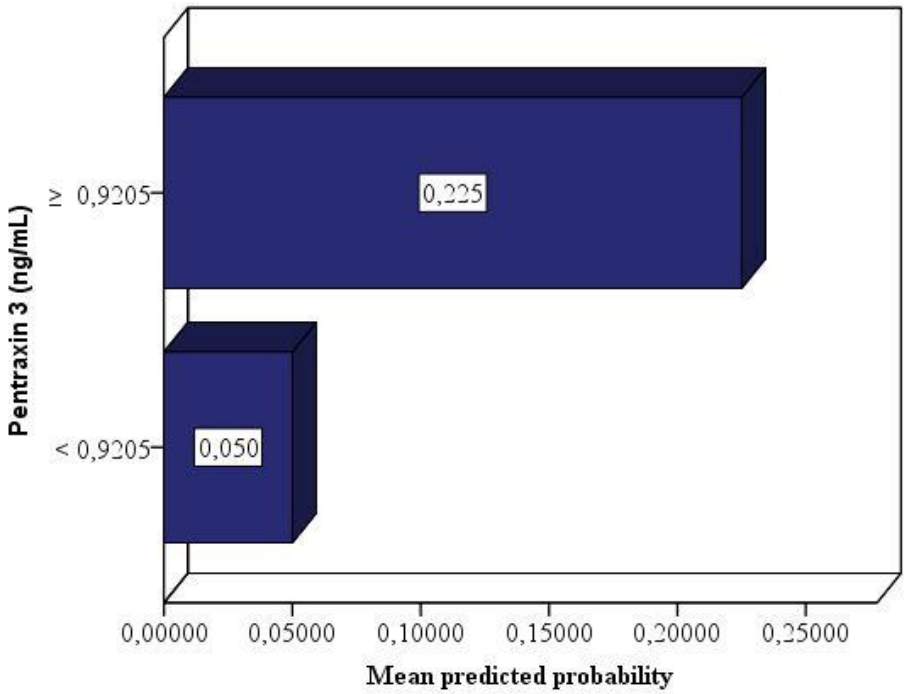


Figure 20. Bar chart illustrating the increased likelihood for rehospitalizations due to worsening of angina symptoms in patients with elevated values of PTX3 during the follow-up.

The results from the logistic regression analysis indicate that elevated baseline PTX3 levels have informative value regarding the occurrence of rehospitalizations during follow-up. The mean predicted probability for rehospitalizations estimated by the logistic model in our study was 22,5% in the high PTX3 group, compared with 5% in the low PTX3 group. Figure 20 illustrates this relation. These findings were further confirmed using the non-parametric Pearson Chi-square test, which again demonstrated a statistically significant association between elevated PTX3 values ≥ 0,9205 ng/mL and rehospitalizations due to worsening of angina symptoms during clinical follow-up: Pearson Chi-square $\chi^2 = 5,165$, df = 1, p = 0,023. This further supports the robustness of the observed dependence, indicating that PTX3 may represent a clinically relevant biomarker for the stratification of high-risk patients. Table 19

presents the difference in baseline characteristics between the two groups stratified by PTX3 ≥ 0,9205 ng/mL.

Variable	**Low PTX3 Mean ± 1 SD**	**High PTX3 Mean ± 1 SD**	**p**
N	40	40	
Age	58,23 ± 7,31	59,60 ± 7,14	0,311
BMI (kg/m^2)	30,82 ± 5,66	30,04 ± 5,73	0,433
LVEDD (mm)	49,40 ± 5,22	48,93 ± 5,79	0,517
FS (%)	32,10 ± 6,10	29,43 ± 4,99	0,035
Creatinine (mmol/L)	73,80 ± 16,44	76,20 ± 14,82	0,495
eGFR (mL/min/1,73m^2)	91,02 ± 20,26	86,90 ± 17,36	0,319
Total cholesterol (mmol/L)	4,94 ± 1,56	4,87 ± 1,19	0,969
LDL-C (mmol/L)	2,85 ± 1,44	2,77 ± 1,0	0,897
HDL-C (mmol/L)	1,28 ± 0,39	1,31 ± 0,37	0,707
Triglycerides (mmol/L)	1,95 ± 1,26	1,74 ± 1,17	0,416
CRP (mg/L)	3,10 ± 3,86	3,29 ± 4,38	0,889

Table 19. Difference in baseline characteristics of the study cohort stratified by PTX3 ≥ 0,9205 ng/mL.

Table 20 presents the presence of traditional risk factors in the two groups stratified by PTX3 ≥ 0,9205 ng/mL.

Risk factor	Low PTX3	High PTX3	p
N	40	40	
Smoking	45% (n = 18)	40% (n = 16)	0,651
Arterial hypertension	95% (n = 38)	87,5% (n = 35)	0,432
Type 2 diabetes mellitus	27,5% (n = 11)	25% (n = 10)	0,799
Dyslipidemia	67,5% (n = 27)	57,5% (n = 23)	0,356

Table 20. Presence of traditional risk factors in the groups stratified by PTX3 ≥ 0,9205 ng/mL.

Table 21 presents the mean number of coronary arteries affected by intermediate-grade stenoses in the groups stratified by PTX3 ≥ 0,9205 ng/mL.

	Low PTX3	High PTX3	p
N	40	40	
Number of affected coronary arteries by intermediate-grade stenoses Mean ± 1 SD	2,03 ± 0,86	1,93 ± 0,83	0,599

Table 21. Mean number of coronary arteries affected by intermediate-grade stenoses ± 1 SD, stratified by PTX3 ≥ 0,9205 ng/mL.

The median time to rehospitalization due to worsening of angina symptoms, as determined by Kaplan–Meier analysis, was 10 months (95% CI 5,68–14,32). The study shows that after all registered rehospitalizations during the follow-up, only 36,4% (n = 4) of them were not by the end of the first year, figure 21.

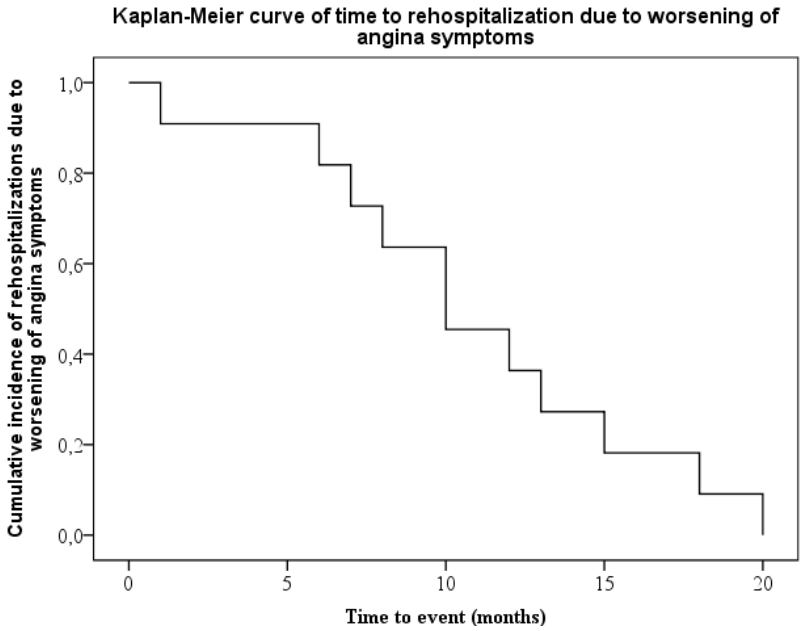


Figure 21. Time to rehospitalization due to worsening of angina symptoms in patients with coronary atherosclerosis who are not eligible for a revascularization procedure.

Although the number of repeat hospital admissions and selective coronary angiographies performed during follow-up in the cohort is only 11, it is noteworthy that intensification of antianginal therapy is observed in only 36,4% (n = 4) of patients, while 63,6% (n = 7) remained on the same number of medications at discharge. Regarding statin therapy, treatment was intensified in 54,6% (n = 6) of patients, whereas 45,5% (n = 5) remained on the same or a lower equivalent statin dose. These data suggest that not all patients at increased risk of rehospitalizations due to worsening of angina symptoms receive optimal antianginal therapy or intensification of statin treatment.

A multivariate logistic regression analysis was performed to assess whether the binary PTX3 variable retains its prognostic value for rehospitalizations due to worsening of angina symptoms in the presence of traditional risk factors, which were added in the following order: smoking, type 2 diabetes mellitus and dyslipidemia. Due to the high prevalence of arterial hypertension in the study cohort (91,3%), it was not included in the model. Given the limited number of events n = 11, a stepwise approach was used for the sequential inclusion of variables, while monitoring the statistical significance of the primary study predictor: PTX3. With smoking included in the model, PTX3 with a cut-off value ≥ 0,9205 ng/mL remained a statistically significant predictor for future rehospitalizations (B = 1.693, standard error (SE) = 0,822, p = 0,039), OR = 5,44 (95% CI 1,09–27,24). We then added type 2 diabetes mellitus to the model: (B = 1,68, SE = 0,82, p = 0,041), OR = 5,38 (95% CI 1.07–27.01). Finally,

dyslipidemia was included in the model: (B = 1,685, SE = 0,824, p = 0,041), OR = 5,39 (95% CI 1,07–27,12). In the multivariate logistic regression analysis, after adjusting for traditional risk factors, PTX3 remained a statistically significant predictor of subsequent rehospitalizations due to worsening of angina symptoms. Across all models, a consistent five-fold increase in the risk was observed. PTX3 values ≥ 0.9205 ng/mL can be used in combination with traditional risk factors to assess the risk for future rehospitalizations due to worsening of angina symptoms in patients with coronary atherosclerosis who are not eligible for a revascularization procedure. Figure 22 presents the data graphically as a forest plot with 95% confidence intervals.

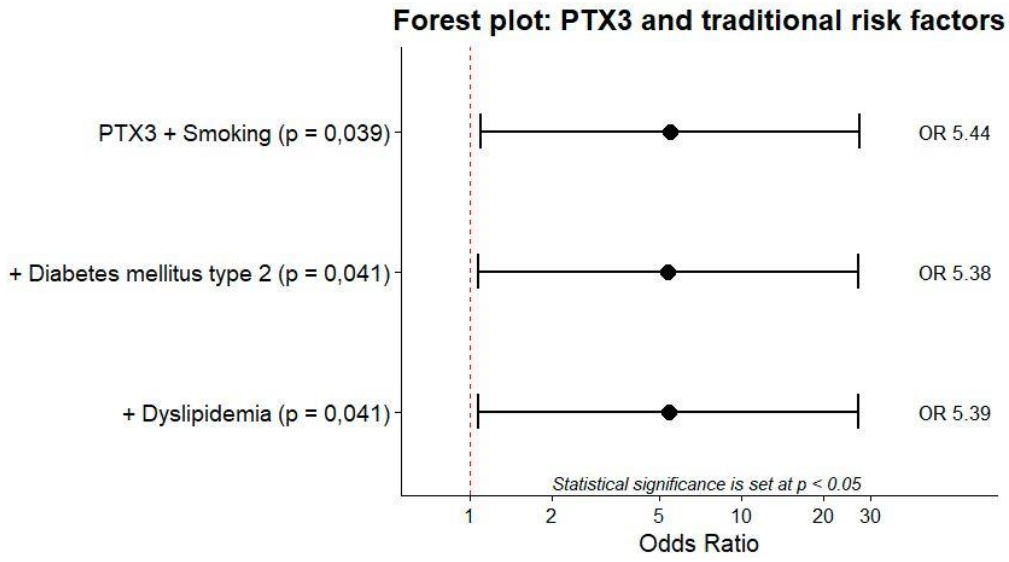


Figure 22. Forest plot of the multivariate logistic regression analysis with stepwise inclusion of traditional risk factors into the model including PTX3.

To assess the discriminative ability of the models in evaluating prognostic value in regard to rehospitalizations due to worsening of angina symptoms, ROC curves and AUC values were calculated and compared in two separate models. The first model included the following risk factors: smoking, type 2 diabetes mellitus, dyslipidemia and arterial hypertension, yielding weak discriminative ability that did not reach statistical significance: AUC = 0,636 (95% CI 0,43–0,84, p = 0,150). In the second model, pentraxin 3 was added to the traditional risk factors, resulting in a statistically significant moderate discriminative ability of the model: AUC = 0,754 (95% CI 0,62–0,89, p = 0,007). The results clearly show

that the addition of pentraxin 3 improves the discriminative ability of the model by ΔAUC = 0,118, rendering the model statistically significant and highlighting the independent and additive prognostic value of PTX3 beyond the established traditional risk factors, figure 23.

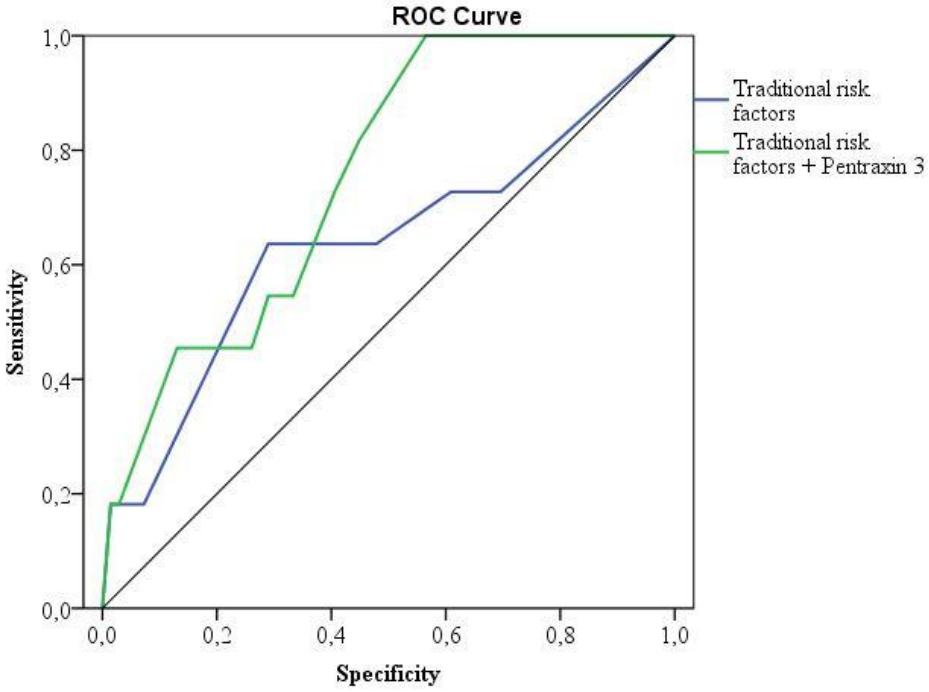


Figure 23. ROC curves illustrating the prognostic values of two models regarding rehospitalizations due to worsening of angina symptoms. The first model (blue) includes only the traditional risk factors, while the second model (green) is after the addition of pentraxin 3.

4.6.1. Discussion

The prognostic significance of pentraxin 3 for major adverse cardiovascular events has been established in the literature mainly in studies following patients after acute coronary syndrome. However, the currently available data regarding the association of the biomarker with rehospitalizations due to worsening of angina symptoms in patients with chronic coronary syndrome remain limited. Only a few reports have evaluated patients with chronic coronary syndrome. Dubin et al. reported the prognostic value of pentraxin 3 for MACE in patients with chronic coronary syndrome, demonstrating that PTX3 levels in range of 0,809–9,770 ng/mL were associated with an increased risk of non-fatal myocardial infarction, stroke, cardiovascular death or new-onset heart failure. Noteworthy is that after including renal function in the model, assessed by glomerular filtration rate, the prognostic value of the

biomarker decreased but nevertheless remained significant (Dubin et al., 2012). These findings are consistent with our data, demonstrating that elevated pentraxin 3 levels have high sensitivity for identifying high-risk patients for cardiovascular events, even in the absence of chronic kidney disease. In our study, all patients have an eGFR > 60 mL/min/1,73m² and pentraxin 3 retained independent prognostic value with respect to rehospitalizations due to worsening of angina symptoms. In our cohort, univariate logistic regression analysis demonstrated that the mean predicted probability of rehospitalizations due to worsening of angina symptoms is 5,5 times higher in the group with PTX3 levels ≥ 0,9205 ng/mL compared with the group with PTX3 < 0,9205 ng/mL. Comparison of the two subgroups stratified by the median value of pentraxin 3 showed no statistically significant differences in laboratory parameters (CRP, creatinine, total cholesterol, LDL-C, HDL-C, and triglycerides) or in the distribution of traditional risk factors (smoking, hypertension, type 2 diabetes mellitus and dyslipidemia). Notably, no statistically significant difference was observed between the groups regarding the number of coronary arteries affected by atherosclerosis. A statistically significant difference among the examined parameters was observed only in the fractional shortening. Thus, dividing the patients by the median value of pentraxin 3 in our study provides additional information in two groups that are otherwise comparable in terms of demographic, clinical and risk factor profiles.

A meta-analysis conducted by Chu et al., including 5 174 patients across the full spectrum of ischemic heart disease, further confirmed the role of pentraxin 3 as a predictor of MACE, particularly in patients after acute coronary syndrome. This is explained by the pronounced local vascular inflammatory response, with marked elevation of biomarker levels during the acute phase (Chu et al., 2019). Among the reported studies, Miyazaki et al. did not find a prognostic role of PTX3 in patients enrolled at least 3 months after myocardial infarction who had experienced recurrent myocardial infarction or cardiovascular death during follow-up (Miyazaki et al., 2014). Nevertheless, despite the limited number of studies in patients with chronic coronary syndrome, pentraxin 3 appears to retain a positive predictive value for MACE, although to a lesser extent compared with patients after acute coronary syndrome. It remains unclear whether persistently elevated plasma PTX3 levels are associated with a proportionally increased cardiovascular risk. In our cohort, 11 outlier values were identified. In 4 patients PTX3 levels were exceeding 10 ng/mL. Persistently elevated baseline PTX3 levels in the absence of an acute inflammatory process may present as a predictor of high cardiovascular risk. Similar findings have been reported for hsCRP in the Women's

Health Study, where cardiovascular risk was shown to increase proportionally with rising of the absolute hsCRP levels (Ridker et al., 2004).

In our study, an optimal cut-off value for predicting rehospitalizations due to worsening of angina symptoms was identified at ≥ 0,9205 ng/mL. This threshold differs from the values corresponding to the SAQ scales „Physical Limitation“ and „Angina Frequency“, where a weak negative correlation was observed using Spearman’s method, which was statistically significant for the „Physical Limitation“ scale and showed a trend for the „Angina Frequency“ scale. These findings suggest that PTX3 provides additional informative value to clinical assessment by helping to differentiate ischemic chest pain and by drawing attention to earlier and more comprehensive optimization of medical therapy, including the use of multiple medications with different pathogenetic mechanisms of action, as well as early intensification of statin therapy in high-risk patients for subsequent rehospitalizations due to worsening of angina symptoms. Each rehospitalization due to worsening of angina symptoms is associated with additional healthcare expenditure and often requires invasive procedures such as selective coronary angiography, which, despite being routinely performed in clinical practice, carry inherent risks for potential complications. In real-world clinical settings, the use of a unified cut-off value is often more practical, however, our data indicate that small differences in absolute PTX3 values result in substantial variations in sensitivity and specificity depending on the clinical endpoint. Therefore, different biomarker cut-off values should be used depending on the clinical context. It should also be acknowledged that the observed differences may be attributable to methodological errors, pre-analytical variability in sample processing or subjectivity in patient-reported data.

Our findings demonstrate that very low baseline PTX3 levels and consequently lower vascular inflammation are associated with more favorable clinical outcomes compared with patients exhibiting elevated PTX3 levels. In this context, baseline PTX3 assessment in patients with coronary atherosclerosis not requiring a revascularization procedure can help overcome clinical inertia and support the discharge of high-risk patients on optimal medical therapy, including medications targeting different pathogenetic mechanisms, thereby reducing the risk of recurrent rehospitalizations.

In order to be used in clinical practice, pentraxin 3 should provide better prognostic value than hsCRP when added to models with the traditional risk factors. In clinical settings, there is a need for a biomarker that improves the predictive value of existing risk models

based on traditional risk factors, as these explain only a proprotion of major adverse cardiovascular events. It is reasonable to believe that a vascular-specific biomarker would offer superior predictive value compared with a systemic, non-specific inflammatory biomarker. In the Rotterdam Study, which included 7 983 participants aged > 55 years, hsCRP has shown no improvement in the prognostic performance of models incorporating traditional risk factors (van der Meer et al., 2003). Although this study was conducted in an older population, these findings were confirmed in the ARIC study, which included 15 792 participants aged 45–74 years, where the addition of hsCRP resulted in only a minimal improvement of the model with traditional risk factors, with a statistically non-significant increase in AUC of 0.003 (Folsom et al., 2006). High-sensitivity C-reactive protein has good predictive value as a standalone marker, however, when added to models including traditional risk factors, it does not improve significantly the predictive values of the models for subsequent major adverse cardiovascular events.

In our study, the addition of PTX3 to the prognostic model improved AUC by 0,118 compared with the use of traditional risk factors alone. It should be noted that long-term follow-up of patients, including additional atherosclerotic cardiovascular endpoints (stroke, peripheral artery disease), may further improve the predictive value of the model. The Cardiovascular Health Study included 1 583 participants aged ≥ 65 years without cardiovascular disease at baseline. During follow-up, the recorded endpoints included incident angina, myocardial infarction, stroke, cardiovascular mortality and all-cause mortality. The results of the study demonstrated that pentraxin 3 improved the model when added to traditional risk factors with respect to two endpoints: cardiovascular mortality and all-cause mortality: HR 1,14 (95% CI 1,04–1,26) and HR 1,10 (95% CI 1,04–1,16), respectively. However, no statistically significant correlation was observed for incident angina and myocardial infarction: HR 1,06 (95% CI 0,96–1,18) and HR 0,94 (95% CI 0,79–1,13), respectively (Jenny et al., 2009).

In summary, the biomarker pentraxin 3 presents a promising opportunity to improve the prognostic value of models based on traditional cardiovascular risk factors. It differs from hsCRP in being independent of traditional risk factors, which enables it to serve as a prognostic biomarker in patients with coronary atherosclerosis who are not eligible for a revascularization procedure. A key clinical advantage is the documented correlation between locally measured vascular PTX3 levels and circulating systemic concentrations, making it an easily accessible biomarker for clinical use.

5. PHARMACOECONOMIC ANALYSIS

Selective coronary angiography is an invasive procedure performed in specialized catheterization laboratories. According to ESC data from 2022, Bulgaria has 8,88 hospitals (public and private) equipped with catheterization laboratories per million population (https://eatlas.escardio.org/Atlas/ESC-Atlas-in-Interventional-Cardiology/Interventional-cardiology-infrastructure/ccr_cath_1m_r-hospital-with-cath-labs-per-million-people). It is important to note that the presence of more than one catheterization laboratory within a single hospital is not additionally accounted for, therefore, the actual number of available catheterization laboratories is higher. The number of performed selective coronary angiographies and/or diagnostic (left and/or right) cardiac catheterizations per million population in 2022 is 4 397,6 (https://eatlas.escardio.org/Atlas/ESC-Atlas-in-Interventional-Cardiology/Diagnostic-coronary-angiography/sipcp_diag_1m_r-coronary-angiography-and-diagnostic-cardiac-catheterization-per?utm_source=chatgpt.com). The number of performed coronary interventions (balloon angioplasty with or without stent implantation) or other coronary interventions per million population in 2022 is 4 914,6 (https://eatlas.escardio.org/Atlas/ESC-Atlas-in-Interventional-Cardiology/Interventional-cardiology-procedures/sipcp_pci_1m_r-percutaneous-coronary-interventions-pci-per-million-people?utm_source=chatgpt.com). Compared with other European countries, Bulgaria performs the highest number of interventional coronary procedures. Table 22 presents ESC data from 2022 for Western European countries and countries sharing a border with Bulgaria. Coronary interventions performed in the settings of acute coronary syndrome with ST-elevation are not included in this analysis.

Country	Number of coronary interventions per million population in 2022
Bulgaria	4 914,6
Germany	4 008,8
Turkey	3 822,2
France	3 164,8
Italy	2 493,3
Greece	2 287,4
Spain	1 572,9
Romania	1 038,8

Table 22. Number of performed coronary interventrions per million population in 2022 in countries from Western Europe and countries sharing a border with Bulgaria, ESC.

Despite the high number of selective coronary angiographies and percutaneous coronary interventions performed in Bulgaria, the country continues to report the highest premature cardiovascular mortality in both men and women (https://eatlas.escardio.org/Data/Cardiovascular-disease mortality/hs_dth_cvd_prem_100k_m_r-prenature-deaths-due-to-cardiovascular-diseases-male;https://eatlas.escardio.org/Data/Cardiovascular-disease-mortality/hs_dth_cvd_prem_100k_f_r-premature-deaths-due-to-cardiovascular-diseases-female). This statistic suggests that in Bulgaria a substantial proportion of healthcare resources is allocated to high-specialty diagnostic and interventional procedures such as SCA and PCI, without a substantial impact on overall premature cardiovascular mortality compared with countries where fewer financial resources are invested in this sector.

We performed a cost-effectiveness analysis to evaluate two clinical strategies: a standard strategy involving a follow-up SCA ± PCI in patients with known coronary atherosclerosis who are not eligible for a revascularization procedure and a strategy incorporating pentraxin 3 evaluation after performing a SCA in this patient population.

The health outcome was defined as the number of avoidable SCA procedures in patients, attributable to intensification of medical therapy and a close clinical follow-up.

We calculated a cost-effectiveness ratio, with costs expressed in monetary units (euros). In the first strategy, costs were based on National Health Insurance Fund reimbursement tariffs for clinical pathways 25 and 26, whereas in the second strategy costs

were derived from the average price of the ELISA kit required for pentraxin 3 measurement in 80 patients, related to the number of preventable SCA ± PCI procedures. In our study, the median time to rehospitalization due to worsening of angina symptoms leading to a SCA ± PCI is 10 months. In routine clinical assessment of pentraxin 3, the prevention of only one rehospitalization in this patient population would be sufficient to achieve a favourable cost–effectiveness ratio. Additionally, widespread use of this biomarker is expected to reduce its cost as a consumable, further improving its cost-effectiveness ratio.

6. IMPLICATIONS

1. Mean pentraxin 3 levels are higher in women compared to men.

2. There is no correlation between pentraxin 3 and C-reactive protein values in patients without clinical and laboratory evidence of inflammatory disease.

3. Pentraxin 3 has the potential to be used in clinical practice as a functional biomarker for angina symptom severity, helping clinical assessment and patient selection for SCA.

4. In our study, patients with pentraxin 3 values < 0,9205 ng/mL have a 5% probability of rehospitalizations due to worsening of angina symptoms during clinical follow-up: median 16 months (IQR 9–23).

5. In our study, pentraxin 3 values ≥ 0,9205 ng/mL demonstrate a sensitivity of 81,8% and a specificity of 55,1% for predicting rehospitalizations due to worsening of angina symptoms during clinical follow-up: median 16 months (IQR 9–23).

6. Measuring pentraxin 3 after SCA in patients who are not eligible for a revascularization procedure may contribute to differentiating a high-risk group according to the risk of subsequent rehospitalizations due to worsening of angina symptoms.

7. The high-risk group requires intensification of statin and antianginal therapy, as well as close medical follow-up.

7. CONTRIBUTIONS

7.1. Fundamental contributions

1. This is one of the first studies in Bulgaria to investigate pentraxin 3 in patients with ischemic heart disease.

2. To the best of our knowledge, this is the first time a biomarker with possible functional significance in patients with stable angina pectoris is described, supporting the clinical assessment of angina symptoms.

3. Pentraxin 3 may contribute to identifying high- and low-risk groups of patients for subsequent rehospitalizations due to worsening of angina symptoms and a cut-off value that can be integrated into clinical practice is proposed.

7.2. Confirmatory contributions

1. The complex and multifactorial role of the biomarker pentraxin 3 in patients with ischemic heart disease is confirmed.

8. CONCLUSION

Pentraxin 3 is a vascular-specific biomarker that can be integrated into clinical practice. It may be useful in helping the initial assessment of the severity and frequency of angina symptoms, which in routine clinical practice largely depends on the clinician's experience. In patients with coronary atherosclerosis who are not eligible for a revascularization procedure, the baseline pentraxin 3 values establish two groups: high- and low-risk group for subsequent rehospitalizations due to worsening of angina symptoms. This approach supports individualized therapeutic strategy for each patient, including intensification of antianginal and lipid-lowering therapy, as well as recommendations for close medical follow-up. The implementation of such measures in high-risk patients can reduce healthcare system expenditure.

9. FUTURE PERSPECTIVES

The study provides an opportunity for future research related to the vascular inflammatory biomarker pentraxin 3 in the field of chronic coronary syndrome. Chronic coronary syndrome is a part of ischemic heart disease in which no clinically useful biomarkers are currently available. The association of pentraxin 3 with angina symptoms suggests its potential use for distinguishing patients with anginal from non-anginal chest pain, as well as for supporting the interpretation of exercise electrocardiography or other functional cardiac tests, with the aim of eliminating false-positive and false-negative results. Large-scale studies with long-term follow-up are needed to confirm our findings. In addition, future research in the field of genetics is warranted to clarify the genetic determinants of baseline levels of vascular inflammation and its growing clinical relevance.

10. STUDY LIMITATIONS

1. We acknowledge the limitation of a single measurement of pentraxin 3 without repeat sampling, which could have helped to eliminate potential measurement error in baseline biomarker levels and to confirm stable basal levels of vascular inflammation over time.

2. The duration of clinical follow-up is limited, which reduces the likelihood of cardiovascular mortality as well as all-cause mortality.

3. Some of the rehospitalizations are reported based on patient medical history.

4. A non-invasive functional testing is not performed prior to SCA to assess for myocardial ischemia.

5. Invasive functional assessment (FFR) for evaluation of myocardial ischemia is not performed in all patients.

6. HsCRP is not measured, preventing the possibility for a direct comparison of the prognostic value of the two biomarkers.

7. Relatively small study cohort.

11. LIST OF PUBLICATIONS RELATED TO THE THESIS TOPIC

1. **Hristov D.** Inflammation as a risk factor in patients with ischemic heart disease. Heart-Lung (Varna), 2023;29:37-42.

2. **Hristov D.** The role of the inflammatory protein Pentraxin 3 as a biomarker in patients with ischemic heart disease. Bulgarian Cardiology. 2024;30(3):36-44.