REVIEW

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By order No. P-109-299 of 20. 08.26 of the Rector of the Medical University-Varna and by decision of the scientific jury with protocol 1 / 03. 09.2026 I have been assigned to prepare a review for the defense of a dissertation on the topic:

" CLINICAL SIGNIFICANCE OF PENTRAXIN 3 IN PATIENTS WITH ISCHEMIC HEART DISEASE WHO ARE NOT SUBJECT TO A REVASCULARIZATION PROCEDURE "

for acquiring the educational and scientific degree " **DOCTOR** "
by **Dr. Dimitar Tihomirov Hristov**

Scientific organization: Department of Cardiology and Rheumatology, Educational Sector of Cardiology, Faculty of Medicine, Medical University "Prof. Dr. P. Stoyanov" - Varna

Professional field **7.1. Medicine, Scientific specialty "Cardiology", doctoral program "Cardiology"**

Scientific supervisor: Prof. Dr. Svetoslav Georgiev, MD , Medical University "Prof. Dr. P. Stoyanov" - Varna.

Brief biographical data:

Dr. Dimitar Hristov was born in Pleven. From 2014-2020 he was a student at the Medical University "Prof. Dr. Paraskev Stoyanov"-Varna, where he obtained an educational and scientific degree Master of Medicine. He started his professional career at the Second Clinic of Cardiology-Invasive of the University Hospital "St. Marina"-Varna and at MU-Varna in 01.2021 as an physician-assistant professor at the First Department of Internal Medicine, which position he holds until 01.2026. Since 01.2026 he has been a Lecturer at the Department of Cardiology and Rheumatology, Educational Sector of Cardiology, Faculty of Medicine, MU-Varna. Along with this, he also specialized in cardiology, and in 06.2025 he acquired the specialty of Cardiology. Since 2021 he has been a full-time PhD student in the doctoral program in Cardiology at MU-Varna.

Dr. Hristov has a certificate of acquired professional qualification in highly specialized activities in echocardiography - basic level and in ultrasound vascular diagnostics.

Dr. Hristov has shown interest in scientific activity since he was a student and has participated in numerous congresses, conferences and publications.

Dr. Hristov is fluent in English, with a C1 proficiency level.

The submitted documentation for the competition for the educational and scientific degree "Doctor", announced by decision of the Faculty Council of the Faculty of Medicine, MU-Varna with protocol N63/17.08.2026, is in accordance with the requirements of the Law for development of academic staff in Bulgaria ZRASRB and Practical Regulations for development of academic staff of the Medical University-Varna.

Topic relevance

Biomarkers are measurable quantitative biological parameters that can have a significant impact on certain clinical conditions. The use of various biomarkers in medicine is increasing. The ideal biomarker should be related to the outcome of the disease and, sometimes, even be a surrogate end point, must be a factor in the development of the disease, but be independent of other established risk factors, to have a clear physiological manifestation, which can be used in interpreting the results in different populations, and have prognostic value beyond the known traditional risk factors, with the availability of standardized testing methods, and at a relatively low cost.

Ischemic heart disease (IHD) is one of the most common cardiovascular diseases worldwide. It is a major cause of death and disability in the world, and for Bulgaria it is one of the most fatal diseases. The clinical course is diverse and may be asymptomatic for a long time. IHD is the most common cause of premature morbidity and mortality, which has its adverse economic and social consequences.

Inflammation has a primary, foundational and supporting role in the development and progression of atherosclerosis and its complications. This process is potentiated by various risk factors, such as dyslipidemia, arterial hypertension, smoking, etc., with the basic step being endothelial dysfunction. Inflammation itself is a complex interaction of cellular and humoral factors, with the active participation of oxidized cholesterol, oxygen radicals and smooth muscle cells of the vascular wall. As a result, atherosclerotic plaques develop, which can obstruct the vascular lumen to varying degrees, but also lead to remodeling of the arteries and to the clinical manifestation of chronic coronary syndrome. In case of complication (rupture, hemorrhage, etc.), instability occurs in the plaque itself and as a result, acute coronary syndrome follows.

In recent years, scientific research has sought and found various biomarkers that can show us when there is an increased risk of developing IHD or that are precursors of an unfavorable prognosis. A number of them, such as C-reactive protein, homocysteine, peroxidases, etc., have established themselves as useful in daily clinical practice or in research. Regardless of their usefulness, the residual risk of developing IHD remains relatively high, which opens the opportunity to search for other and new biomarkers that can improve the definition of individuals with high cardiovascular risk or patients with a potentially unfavorable prognosis in an already existing disease. One such promising biomarker is pentraxin 3 (PTX3). As a representative of the family of inflammatory peptides pentraxins, it has the potential to provide additional insight into the process of atherosclerosis in relation to known risk factors for IHD, and also to predict unfavorable clinical development in chronic forms of the disease.

All this makes the problem examined in this dissertation significant and dissertable.

Structure of the dissertation

The dissertation consists of 126 standard typewritten pages. The bibliography includes a total of 211 literary sources, all in Latin. It is richly illustrated with 30 figures and 23 tables, which provide sufficient information about the issues under consideration and are easy to understand.

The dissertation is structured as follows:

Introduction – 2 pages
Literature review – 38 pages (30 % of the dissertation);
Aim and objectives – 1 page;
Participants and methods – 6 pages (5%);
Results and discussion – 48 pages (38%);
Pharmacoeconomic analysis – 2 pages (0.2%);
Conclusions – 1 page;

Contributions of scientific work – 1 page;
Conclusion – 1 page;
Disadvantages and future perspectives – 2 pages in total;
List of publications – 1 page;
Appendices – 2 pages;
Book article – 14 pages.

I believe that the structure of the dissertation meets generally accepted requirements.

Literature review

It is written competently and shows excellent knowledge of the available information on the problem. The bibliography includes 211 titles, all in Latin. ***Recommendation:*** *It was possible to search for sources in Cyrillic, although there are no similar studies on this topic in the available Bulgarian scientific literature.*

The first part provides a detailed analysis of the role of inflammation in the development of atherosclerosis in humans. The initial studies are outlined in historical terms and current achievements in scientific research are highlighted. The complex interactions between cellular, genetic and humoral factors in the development of the low-level inflammatory process, at the core of which is endothelial dysfunction, are examined. A reasonable conclusion is made that inflammation has a primary, fundamental and supporting role in the development and progression of atherosclerosis and its complications, and the measurement of inflammation using biomarkers may have clinical application for assessing the prognosis in patients with ischemic heart disease and be a target for treatment and slowing the development of the atherosclerotic process.

The second part of the literature review focuses on the pathogenesis of obstructive coronary atherosclerosis. According to modern understanding, it is the basis of chronic coronary syndrome in its various forms. Myocardial ischemia can be due to changes in large resistance arteries or in the microcirculation. Hemodynamic and neurohumoral mechanisms play an important role in the occurrence of ischemia, but remodeling of the coronary vessels as a result of atherosclerotic changes in the vascular wall is also of great importance. Two types of remodeling have been described - positive and negative, and both types of remodeling can exist simultaneously in one patient . With the increase in the atherosclerotic load coronary reserve is reduced, and this leads to exhaustion of compensatory autoregulatory mechanisms and to clinical manifestations of IHD.

The third part is devoted to the clinical assessment of the severity of anginal symptoms. The main types of angina are indicated. Along with the known classification systems, which have their limitations, various questionnaires are used today to assess the severity of angina pectoris. The main types of questionnaires that are currently used are briefly reviewed. The presentation emphasizes that the Seattle Angina Questionnaire (SAQ) is disease-specific, considers the manifestation of symptoms in several domains, is sensitive to minimal changes in the patient's condition during follow-up and has been validated in various studies, including the abbreviated 7-point version.

The fourth part of the review discusses biomarkers of inflammation with a role in IHD. The main characteristics of the biomarker as a secretin in the circulation, a sign of inflammation, are indicated. As an advantage of the dissertation, I would point out the differentiation of the potential role of each biomarker as a risk factor for the development of the disease or only as a marker of risk and a mediator of this risk. The main markers of inflammation known so far in IHD, such as C -reactive protein, interleukins, fibrinogen, metalloperoxidase, etc., are briefly reviewed, with their positive sides and disadvantages. Special attention in the review is paid to the role of pentraxin-3 as a more vascular-specific representative of the family of long-chain pentraxins. It is emphasized that it is a specific marker for vascular inflammation and does not

only passively reflect vascular inflammation in the course of the development of atherosclerosis, but has an active role in its progression and is probably protective in terms of the development of atherosclerosis. Evidence is provided that PTX3 is involved in three different independent and self-contained pathogenetic mechanisms in the development of cardiovascular pathology (endothelial dysfunction, inhibition of neoangiogenesis, oxidative stress and inflammation). Other potential benefits of PTX3 are also indicated, such as participation in immunity, neoangiogenesis and oncogenesis, as well as the presence of extreme elevations in other serious inflammatory conditions, such as rheumatoid arthritis, sepsis, inflammatory bowel diseases, kidney damage and many others. Different normal values of PTX3 depending on age and gender are also discussed in this section.

Finally, the evidence for a relationship between PTX3 and major risk factors for CVD and with major clinical forms of CVD – acute coronary syndrome with and without ST - elevation and chronic coronary syndrome – is reviewed. In all these cases, there is an increase in the biomarker, but with different evidence for a relationship and prognostic value. An interesting aspect of the dissertation work is the study of the effects of established anti-atherosclerotic drugs on PTX3, such as statins, and their significance.

The logical summary of the unknowns and contradictions in the role of PTX3 is a natural justification for the study of the problem in the present dissertation.

The rich literary material is purposefully synthesized, interpreted with skill, maturity, and criticality.

Aim and objectives

The main goal of the dissertation is clearly defined: To define the risk of rehospitalization in patients with ischemic heart disease, in whom revascularization procedures are not an option, by examining pentraxin-3 levels.

In connection with the main objectives, 5 specific tasks are also presented, related to the establishment of PTX3 levels, the correlation of the biomarker with the clinical manifestation of stable angina, with rehospitalizations and new invasive examinations due to worsening of IHD, as well as their relationship with PTX3 levels at baseline. As a result of the analyses, the final task is to search for patients with increased risk based on the conducted examinations.

Participants and methods

The dissertation is based on a prospectively follow-up total of 80 patients up to the age of 70, in whom the existing coronary atherosclerosis was proven by selective coronary angiography, but in whom revascularization was not required due to established < 70% stenosis of the lumen in at least one coronary artery or due to diffuse coronary atherosclerosis. All patients were examined in the period from December 2022 to January 2025, with the follow-up of the last patient until September 2025. ***As a note, no method of calculating the required number of subjects was described, which is a prerequisite for obtaining valid and practically statistically significant results.***

The methods section is well and in detail presented by Dr. Hristov. The design of the study with the individual steps is clearly described graphically. The questionnaire used for the severity of angina, the indicators of clinical examination, invasive and non-invasive instrumental examinations, laboratory examinations are listed in detail. Of the latter, the methodology for the study of PTX3 by the ELISA method is indicated in particular detail.

The obtained data were statistically analyzed using a very rich set of statistical methods, including correlation techniques, univariate and multivariate logistic regression analysis techniques for assessing the correctness of the hypotheses.

Results

The results are presented sequentially in several sections:

- Baseline characteristics of the patients. The patients studied have a mean age of just under 60 years and a slight male predominance. The patients are overweight or obese in 88% of cases. They have in the large percentage of cases echocardiographycally preserved dimensions and systolic function of the left ventricle, but without optimal lipid control. The antianginal therapy used is dominated by beta-blockers and dihydropyridine calcium channel blockers, alone or in combination. Forty one percent of the individuals studied used combination therapy, with prevailing double and less often triple combination of medications. The risk profile is typical for patients with IHD. The clinical severity of IHD and the distribution of symptoms according to the different domains of the questionnaire used are examined in detail. The use of non-parametric analyses when using clinical variables is justified by the lack of normal distribution. Rest ECG changes are found in about half of patients, and they correlate, as expected, with increased left ventricular muscle mass.
- Risk profile of patients. The profile is typical for patients with IHD – over 90% have hypertension, every fifth has diabetes mellitus, 42% are smokers and a large part have dyslipidemia. The poor control of LDL-cholesterol is striking, regardless of the use of moderate- or high-intensity statin therapy. There are few patients with previous interventions, and almost equally proportion of patients currently have single-, double- and triple-vessel coronary involvement.
- Baseline PTX3 levels. In the study group, 11 (14%) had elevated PTX3 levels above 2.419 ng/mL. Women have nominally higher mean levels of the biomarker, which cannot be explained only by the difference in age between the sexes. There is no statistically significant correlation between traditional risk factors and PTX3 levels, with the exception of patients with dyslipidemia. This suggests a probable independent mechanism of increased risk with increased PTX3 and is confirmed by other authors. In this direction of thought is the lack of a relationship between PTX3 and CRP, another marker of inflammation. On the other hand, a trend of decreasing biomarker levels was observed in the studied group depending on the presence and intensity of statin therapy, although statistically insignificant. The pleiotropic effects of statins are known and the decrease in PTX3 can be used as a marker of their anti-inflammatory effect, beyond CRP.
- PTX3 levels are higher in patients with severe clinical disease. These are most pronounced in patients with physical activity limitation according to the SAQ, with a weak significant negative correlation observed with physical limitation indicators. A threshold value above 1.1025 ng/mL has the best predictive value for distinguishing mild from severe angina pectoris, with an AUC of 0.654 (95%CI 0.51–0.80, p =0.028) . Similar results were found for the section of SAQ "Frequency of angina symptoms". Here the predictive value of PTX3 has a greater sensitivity, AUC 0.72 (95%CI 0.56–0.88, p=0.01). The relationship of the biomarker with the other sections for assessing the severity of the disease - "Quality of life, "Stability on angina symptomatology" and "Satisfaction from treatment". This is probably related to the fact that PTX3 rather reflects the severity of symptoms and the limitation of physical capacity.
- Pentraxin-3 and clinical follow-up. After a mean of 16 months of follow-up, a total of 11 rehospitalizations for repeated angiography (14%) were recorded, 9 of which were accompanied by intervention. Rehospitalizations were more frequent in men and in those with higher baseline PTX3 values. The correlation between the biomarkers was weakly positive and statistically significant (p=0.017). The AUC was 0.72 and showed a significant moderate discrimination ability on PTX3 to differentiate patients with increased risk from rehospitalizations for acceleration of angina symptoms. Determination of the value of PTX3 $\geq$ 0.9205 ng/mL was found as optimal threshold value for prediction

on the risk from rehospitalizations. There was no difference between the two groups with high and low PTX3 and the distribution of traditional risk factors. Multivariate logistic regression analysis showed a more than 5-fold higher risk of re-catheterization, with or without intervention, after adjusting for smoking, dyslipidemia, and type 2 diabetes. Interestingly, optimization of drug treatment in those with disease progression was done in only slightly more than a third. The addition of PTX3 improved the prognostic value for predicting new events over traditional risk factors, with an AUC of 0.754 (95% CI 0.62–0.89, p=0.007).

A positive feature of this dissertation is ***the pharmacoeconomic analysis.*** It shows that a strategy with routine PTX3 testing leads to **only one less rehospitalization to achieve a favorable cost-benefit ratio.**

As ***a recommendation****, I would point out avoiding presenting the same results in tabular and graphical form*.

The discussion looks at the detailed methodology used, the comparison of the demographic characteristics of those included in the present study with those from other studies, shows the closeness of the obtained results for PTX3 with similar studies conducted among different types of populations. It is pointed out that the prognostic value found in the present study is high and exceeds the indicative risk values obtained so far, although only for non-fatal cardiovascular outcome. Other studies also show this relationship with mortality and morbidity, although the results in them are contradictory.

The limitations of the study are correctly indicated and are related to the small number of subjects studied, the single-point examination of PTX3 and the lack of monitoring of the biomarker over time, the short period of follow-up of the patients, the insufficient use of functional methods for objective detection of ischemia as an end-point, as well as the comparison with hsCRP. In connection with the indicated limitations, **future perspectives for the development of the study** are also outlined .

There are **7 conclusions in total** and they are in line with the set aim and objectives.

The conclusion summarizes the overall work on the dissertation and the conclusions drawn as a result of the work done and the results obtained. Pentraxin-3 is a vascular-specific biomarker that can be integrated into clinical practice. The biomarker can be used to support the process of initial assessment of the severity and frequency of anginal symptoms. In patients with coronary atherosclerosis, who are not eligible for revascularization procedure can correctly differentiate patients with a high risk for subsequent rehospitalizations on basis on the output values on pentraxin-3.

There are **4 contributions – 3 of them are original and 1 of a confirmatory nature**, which are of scientific and practical value. I fully accept the contributions.

The bibliography covers the problem of researching IHD and the role of biomarkers, and in particular PTX3, in a multifaceted manner. It is striking that the majority of titles are from recent years. The cited sources are arranged according to the scientific requirements. **A disadvantage is the lack of citing research in Cyrillic.**

The well-written text, with a precise scientific style and good layout, should be noted. The dissertation is well illustrated, which complements the main text.

The abstract is 72 pages in total, is prepared according to standards and correctly presents the main problems and results discussed in the dissertation.

In connection with the dissertation work, 2 articles have been published in Bulgarian publications, 1 of which is referenced in international databases. Dr. Hristov is the first and sole author in the publications.

In conclusion, I believe that the dissertation presented by **Dr. Dimitar Tihomirov Hristov,** on the topic **"CLINICAL SIGNIFICANCE OF PENTRAXIN 3 IN PATIENTS WITH ISCHEMIC HEART DISEASE WHO ARE NOT ELIGIBLE FOR**

REVASCULARIZATION PROCEDURE ", is an original scientific study that presents a new biomarker for assessing the clinical progression of atherosclerotic ischemic disease. This study demonstrates that PTX3 upgrades over traditional risk factors in assessing worsening angina. It also has prognostic value for predicting short-term worsening of the atherosclerotic process with a view to new rehospitalizations and invasive procedures in patients with known coronary artery disease, but with an initial lack of indications for invasive treatment. The dissertation has significant scientific and practical value in daily clinical work and shows a good economic result of including PTX3 in the algorithm for monitoring patients with an appropriate coronary artery disease profile. It possesses all the qualities and **MEETS THE REQUIREMENTS** of the law on the development of the academic staff in the Republic of Bulgaria and the regulations for its implementation at MU-Varna, **FOR AWARDING AN EDUCATIONAL AND SCIENTIFIC DEGREE "DOCTOR"**, which is why I confidently give her **a POSITIVE rating.**

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

Varna
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