To the Chairman of the Scientific Jury,
appointed by Order № P-109-479/20.11.2025
by the Rector of the Medical University-Varna

STATEMENT

by Prof. Borislav Georgiev Georgiev
Head of the Cardiology Clinic at the National Heart Hospital
Member of the Scientific Jury for awarding the educational and scientific degree "Doctor" (PhD),
determined by order of the Rector of the Medical University-Varna
№ R-109-479/ 20.11.2025

Regarding: Thesis of Dr. Rozen Krasimirov Grigorov, PhD student in full-time education, Medical University-Varna, with the topic of the dissertation work

"***Time for contrast to pass through the myocardium in patients with non-obstructive coronary artery disease***"
Scientific supervisor**:** Prof. Dr. Svetoslav Georgiev, PhD

field of higher education 7. Health and Sports, professional field 7.1. Medicine,
under the doctoral program "Cardiology".

The documents submitted by Dr. Rozen Krasimirov Grigorov - dissertation, Thesis summary in Bulgarian and English and additional documents are in accordance with the requirements of the regulation for acquiring the educational and scientific degree "Doctor" (PhD) and the regulations of the Medical University, Varna. I do not find any gaps in the submitted documentation.

I declare that I have no conflict of interest with the candidate.

All submitted materials are precisely arranged and described.

There is no evidence of plagiarism.

Significance of the topic

The topic of the dissertation is extremely relevant, especially after the introduction of the terms ANOCA (angina in non-obstructive coronary artery disease) and INOCA (ischemia in non-obstructive coronary artery disease), which describe, respectively, symptomatic patients and/or patients with proven ischemia in the absence of obstructive stenoses >50% in the epicardial arteries and the in-depth scientific work to clarify the significance of this pathology in the context of morbidity and mortality in the presence of insignificant coronary disease. The significant number of patients with angina referred for coronary angiography, in whom obstructive epicardial coronary disease is absent, as well as the worse prognosis of these patients compared to the general population and the lack of a reliable, easily applicable and accessible method for examining the coronary microcirculation are a prerequisite for conducting the scientific study.

Structure of the dissertation:

The scientific work of Dr. Rozen Krasimirov Grigorov is structured on 158 pages according to the requirements and contains an introduction, a literature review, aims and objectives of the study, materials and methods, results and discussion, conclusions, contributions, applications and bibliography. The thesis is illustrated with 31 tables and 28 figures.

The **literature review** is presented on 55 pages and is structured in several points - 1. Angina/ischemia in non-obstructive coronary disease (with *an Algorithm for invasive diagnostics in ANOCA/INOCA*); 2. Focus on coronary microvascular dysfunction (MVD); 3. Fluoroscopic methods for assessing coronary blood flow; 4. Treatment of patients with angina/ischemia in non-obstructive coronary disease (ANOCA/INOCA). The clear statement and concise presentation of the data show a thorough knowledge of the data discussed.

The **bibliography** contains 332 cited titles, all in Latin. All citations are spelled correctly. Dr. Rozen Grigorov sets the aim of his research work to define a new, easily reproducible method for fluoroscopic assessment of the microcirculation that is as free as possible from external influences, referred to as the indexed time for contrast to pass through the myocardium (iTCPM), in patients without significant epicardial coronary artery disease, and to analyse its association with the severity of anginal symptoms in these patients

To achieve the goal, he sets the following **tasks**:

1. To standardize the methodology for measuring TCPM, including frame rate of recording, type of contrast agent used, parameters of the automatic injection system (rate and volume), and standard angiographic projections for visualization of the coronary sinus.
2. To determine the mean TCPM value in the study cohort.
3. To assess the severity of anginal symptoms using a standardized questionnaire and a clinical evaluation.
4. To record variables that may influence the TCPM value, including myocardial mass and hemodynamic conditions during the examination.
5. To index TCPM relative to myocardial mass, heart rate, and mean aortic pressure at the ostium of the coronary artery during the examination.
6. To analyse the relationship between indexed TCPM and the severity of anginal symptoms, assessed by CCS class and a standardized angina questionnaire.
7. To identify factors that may predict the value of indexed TCPM, such as classical cardiovascular risk factors and concurrent pharmacological therapy.

Methodological approach: The study was conducted as a cross-sectional study with prospective inclusion of patients, observational and single-center, conducted in real clinical conditions.

The study included 102 patients meeting the inclusion criteria, with a mean age of 61.5 9.9 years, of whom 59% were women (n = 60). The initial assessment included clinical and demographic data. Information was collected on cardiovascular risk factors: age, body mass index (BMI), presence of diabetes mellitus, arterial hypertension, dyslipidemia, and smoking. Additionally, concomitant diseases and previous coronary angiographies were documented. During rest, a complete clinical examination and ECG recording were performed with analysis for signs of ischemia. Blood samples were analysed for levels of total, LDL- and HDL-cholesterol, serum creatinine, complete blood count and coagulation status. Transthoracic echocardiography (TTE) was performed to assess left ventricular dimensions, myocardial mass, systolic and diastolic function, valvular apparatus and estimated pulmonary artery pressure. The most common cardiovascular risk factors were arterial hypertension and dyslipidemia (94% for both variables), smoking (57%), type 2 diabetes mellitus (22%), mean body mass index was 30.5±6.3 kg/m2, eGFR – 86.5±14.8 ml/min/1.73m2. 97% had preserved left ventricular systolic function (over 50%). After coronary angiography and in the absence of obstructive coronary disease, myocardial contrast transit time (TCPM) was measured in the patients. The invasive examination, clinical assessment and questionnaire completion were performed against the background of continuous anti-ischemic therapy, if available. The end point is the determination of the mean indexed time for contrast to pass through the myocardium (iTCPM) in patients with nonstenotic coronary artery disease and the study of the correlation of iTCPM with the severity and frequency of anginal

symptoms, assessed by the CCS functional class and the standardized angina questionnaire – Seattle Angina Questionnaire (SAQ) and variables that may affect the value of iTCPM, such as classical cardiovascular risk factors and the type and number of medications taken, were analysed.

Statistical analyses were performed using Python using Pandas for data manipulation, NumPy for numerical operations, SciPy for statistical tests and Matplotlib/Seaborn for data visualization. Data from angiographies, echocardiographies, questionnaires and laboratory parameters were entered and pre-validated in a structured electronic database. Preliminary analysis of the main continuous variables included a check for normality of distribution using the Shapiro–Wilk test, as well as visual assessment using Q-Q plots and histograms. In order to maintain consistency and avoid assumptions of normality in correlation and comparative analysis, non-parametric statistical methods were used in the study. Continuous variables were presented as mean and standard deviation or median and interquartile range (IQR), and categorical variables were presented as absolute and relative proportion (%).

Spearman's rank correlation coefficient was used to assess the relationship between iTCPM and CCS angina functional class. The analysis included separately the Spearman correlation of iTCPM with each of the five components of the SAQ.

An intergroup comparative analysis was performed on participants divided into two groups according to the mean iTCPM - patients with iTCPM below the mean value ("fast" group), and patients with iTCPM above the mean value ("slow" group). Given the non-normal distribution of SAQ results and the ordinal nature of CCS results, the Mann–Whitney U-test was used for statistical comparison. Two separate multivariate linear regression analyses were conducted to identify independent predictors of the iTCPM value.

Results: The results obtained by Dr. Rozen Grigorov are presented in detail and are well illustrated.

Myocardial mass, mean arterial pressure (meanBP) and heart rate (HR) affect the rate of contrast passage through the coronary arteries. In order to account for the influence of these physiological factors on the studied TCPM, two indexing approaches were considered: Indexing by relative correlation coefficients (used for the study) and Indexing by regression coefficients.

The assessment of the relationship between iTCPM and the severity of anginal symptoms was based on a correlation analysis between iTCPM values and the functional class of the patients according to the CCS classification. All patients in the study were included and a moderate positive correlation between iTCPM and CCS class with high statistical significance was established.

Of interest is the correlation analysis using the Spearman coefficient between iTCPM and the five subcategories of the Seattle Angina Questionnaire (SAQ), which established a statistically significant moderate negative correlation between iTCPM and the category "Physical limitation", a moderate negative correlation with "Frequency of anginal symptoms", a weak but statistically significant negative correlation was established in the category "Illness perception", and there was no statistical significance in the categories "Stability of anginal symptoms" and "Satisfaction with treatment".

The dissertation is very rich in various analyses of the assigned tasks, some of which are so well presented and interpreted in the literature for the first time.

The **discussion of the results** is separated into a separate chapter and compares the results obtained by the author with publications on the topics.

The study provides a new potential opportunity for assessing the coronary microcirculation in patients with angina without significant epicardial coronary disease by using the indexed myocardial contrast transit time (iTCPM). The method is fast and technically easy to perform during conventional coronary angiography, is not associated with additional risks for the patient

and additional costs. The main results emphasize the relationship between the prolonged myocardial contrast transit time and the severity of anginal symptoms, assessed by the CCS functional class and the Seattle Angina Questionnaire.

The study applied an innovative and standardized angiographic approach to assess myocardial contrast dynamics by measuring myocardial contrast transit time (TCPM) followed by indexing (iTCPM) accounting for the three main physiological variables that influence perfusion – mean arterial pressure (meanBP), heart rate (HR), and myocardial mass.

Unlike previously published studies using various fluoroscopic methods for assessing microcirculation with manual injection of contrast via a syringe - an approach associated with significant variability in terms of applied force, volume and injection speed - the study used an automated injection system (Acist CVi) with precisely set volume (6 ml), speed (2 ml/s) and time to reach peak velocity - rise time (0.5 s), which eliminates the heterogeneity arising from the individual characteristics of manual injection and the associated variations in the studied parameter, thus ensuring a higher degree of standardization and reproducibility compared to earlier methods. The dissertation proposes a methodological solution to a number of shortcomings of previous studies by standardizing both contrast administration and interpretation, with transit time indexed to meanBP, HR and myocardial mass. Indexing limits the influence of individual variations related to hypo- or hyperdynamic circulation and anatomical differences, and provides a more objective basis for comparison between individual patients.

To date, no data have been published in the available literature on myocardial contrast transit time (TCPM), determined in the manner described in the dissertation. This study provides the first-of-its-kind quantitative characterization of this parameter. It also outlines guidelines for future stratification of patients based on microvascular function. iTCPM aims to exclude individual variability as much as possible and to reflect the real efficiency of the microcirculation. The proposed methodology builds on established approaches by combining technical accessibility, standardized contrast injection, and physiologically based correction – both clinically applicable and scientifically valid.

After indexing according to meanBP, HR, and myocardial mass, the mean iTCPM value is 4.97 seconds, with a standard deviation of ±1.02 seconds. The Shapiro–Wilk test confirmed the absence of a statistically significant deviation from normality ($p = 0.390$; $W = 0.9865$), and the Q-Q plot demonstrated a good fit between the empirical and theoretical values. The shape of the f iTCPM distribution remained similar to that of the iTCPM – the indexing process achieved the desired physiological correction without introducing data distortion, while improving the possibility of interindividual comparison and analytical applicability.

The intergroup differences complement and extend the established correlation between iTCPM and the severity of anginal symptoms, demonstrating that even when using a simplified threshold value; it is possible to statistically and clinically meaningfully distinguish between patients with different symptom manifestations. This gives additional practical value to iTCPM – not only as a continuous physiological measure, but also as a potential tool for confirming the clinical severity in patients with ANOCA. The observation that patients with more severe symptoms also have a longer contrast transit time supports the hypothesis of underlying microcirculatory dysfunction and could open up opportunities for better phenotyping and individualized treatment in the future.

An additional comparative analysis between patients stratified by the mean iTCPM value (“fast” and “slow” group) confirmed the lack of significant differences between the groups with respect to classical risk factors and anatomical variations. None of the categorical variables, including gender, hypertension, diabetes mellitus, dyslipidemia, smoking or the presence of coronary features, showed a statistically significant association with belonging to the respective group. No significant differences were found for continuous variables (age, BMI, eGFR, LDL), except for age, where a borderline trend towards higher values in the “slow” group was observed without statistical significance.

It is important to note that a similar weak association with traditional risk factors was also observed in large-scale studies in patients with ANOCA, in which the frequency of hypertension, dyslipidemia and diabetes was systematically lower compared to that in obstructive CHD.

The presented study found a significant burden of cardiometabolic risk factors, which distinguishes the cohort with a high-risk profile even in the absence of angiographically significant stenoses. This finding has important pathophysiological implications, as hypertension, insulin resistance, dyslipidemia, and chronic inflammation - conditions often associated with increased vascular risk—are thought to contribute to the development of endothelial dysfunction and microvascular remodeling. This supports the hypothesis that microvascular angina in this population is not a benign or "functional" manifestation, but rather reflects significant structural and/or functional changes in the vascular wall that require a targeted diagnostic and therapeutic strategy.

The study data highlight the therapeutic heterogeneity in patients presenting with angina and as yet undetermined coronary status. In routine practice, treatment is often based on symptoms and comorbidities, without a clear understanding of the underlying mechanism. Even after exclusion of obstructive coronary artery disease by angiography, it remains unclear why many of these patients remain symptomatic. This creates a need for additional, easily applicable methods for assessing microcirculation, which can point to possible microvascular dysfunction or ANOCA. In this context, iTCPM could be a potentially useful tool to identify these patients, help individualize therapy or point to the need for a more in-depth functional analysis of the coronary circulation.

Dr. Rozen Grigorov is critical of the results obtained, which he formulates in the limitations of his study, but also clearly outlines future research on the topic.

Conclusions: Dr. Rozen Grigorov offers 6 conclusions. They arise from the tasks set and the conducted study.

1. The methodology for measuring the time for contrast to pass through the myocardium (TCPM) can be standardized and applied in clinical practice. The study demonstrates that TCPM measurement is technically feasible, quantitatively reproducible, and amenable to standardization. Implementing a unified protocol, including fixed parameters of the automatic injection system, recording speed, angiographic projections, and strictly defined criteria for identifying the first and last frame ensures high reliability of the measurement.

2. The mean indexed time for contrast to pass through the myocardium (iTCPM) in the studied cohort was 4.96±1.12 seconds. The distribution of values showed a clear central tendency, with minimum and maximum values of 2.40 s and 7.70 s, respectively. This is the first study to describe this parameter in patients with angina and non-obstructive coronary anatomy.

3. The severity of anginal symptoms in the cohort was clinically significant despite the absence of anatomically proven obstruction. The mean CCS functional class and Seattle Angina Questionnaire (SAQ) scores demonstrated clinically relevant symptom burden, comparable to or even greater than that observed in patients with obstructive coronary artery disease. This emphasizes that the absence of angiographic stenoses should not lead to underestimation of symptoms.

4. TCPM is influenced by certain physiological and anatomical variables. Correlation analysis revealed a weak but statistically significant positive relationship between TCPM and myocardial mass, and a negative association with mean arterial pressure. The relationship with heart rate was weak and statistically non-significant, yet heart rate was included in the indexation process for physiological reasons, given its well-established effect on myocardial perfusion. The combined indexation against these three parameters aimed to eliminate physiological variability and improve comparability between patients.

5. There is a statistically significant relationship between indexed TCPM and the severity of anginal symptoms assessed by CCS and SAQ. Patients with higher iTCPM values exhibited

higher CCS class and lower SAQ scores – particularly for physical limitation and angina frequency, supporting the applicability of iTCPM as an objective marker of symptom burden.

6. No classical cardiovascular risk factors or pharmacological therapies predicted iTCPM values. Despite including sex, age, traditional risk factors (hypertension, diabetes, dyslipidemia), and medication use in the regression model, none demonstrated a statistically significant predictive effect on iTCPM.

Contributions: There are 8 contributions, divided into three groups - of fundamental nature (1), of original nature (4) and of confirmatory nature (2).

Fundamental contributions:

1. A novel angiographic index has been developed and theoretically substantiated – the indexed time for contrast to pass through the myocardium (iTCPM). The index integrates three key physiological parameters: myocardial mass, mean arterial pressure, and heart rate. This method aims to eliminate individual hemodynamic variability and offers a quantitative, standardized, and potentially reproducible approach for assessing myocardial contrast dynamics, with potential applications in the diagnosis and management of patients with microvascular dysfunction

Scientific contributions:

1. A standardized methodology for measuring the time for contrast to pass through the myocardium (TCPM) has been applied in patients with angina and non-obstructive coronary anatomy for the first time. The protocol includes the use of an automated contrast injection system with fixed parameters, frame rate, and recording speed.
2. For the first time, an average TCPM value has been reported in a cohort of patients with angina and no significant epicardial stenoses, establishing a basis for its use as a quantitative variable in both clinical and research settings.
3. An indexed time for contrast to pass through the myocardium (iTCPM) has been developed, incorporating myocardial mass, arterial pressure, and heart rate to account for physiological variability in contrast dynamics. This indexing improves the objectivity and comparability of results between individual patients.
4. A statistically significant relationship between higher iTCPM values and more severe anginal symptoms has been demonstrated, supporting the applicability of iTCPM as a marker of microvascular dysfunction severity in patients with ANOCA.
5. A new conceptual approach has been proposed for using iTCPM as a diagnostic tool in patients with ANOCA and suspected microvascular dysfunction. The method allows quantitative assessment of myocardial contrast dynamics without requiring additional equipment, intracoronary guidewires, pharmacological provocation, or prolongation of the angiographic procedure. The approach does not increase procedural risk, examination time, radiation exposure for either patient or operator, and entails no additional costs.

Confirmatory contributions:

1. The absence of a clear association between classical cardiovascular risk factors (age, sex,arterial hypertension, type 2 diabetes, dyslipidemia) and the severity of symptoms in patients with ANOCA has been confirmed, consistent with published data from large studies in patients with angina and no obstructive coronary artery disease.
2. It has been confirmed that patients with ANOCA frequently present with pronounced symptoms and often need to be treated with intensive pharmacological therapy despite the absence of angiographically significant obstruction.

The **Thesis summary** is presented in Bulgarian and English, the Bulgarian version contains 72 pages, the English version – 69 pages and reflects what is written in the dissertation. It has been passed according to the requirements.

Publications: In connection with the dissertation, the author presents 3 publications in journals and 4 presentations at scientific forums.

Conclusion: I evaluate the work of Dr. Rozen Krasimirov Grigorov on the topic "*Time for contrast to pass through the myocardium in patients with non-obstructive coronary artery disease*" as interesting in scientific terms, innovative in concept and important for invasive coronary diagnostics. I believe that this dissertation meets the requirements for awarding the educational and scientific degree "Doctor" (PhD) stated by the Development of Academic Staff in the Republic of Bulgaria Act (DASRBA) and the Rules and Regulations for Development of the Academic Staff at the Medical University-Varna. Based on the above-mentioned merits of the dissertation work of Dr. Rozen Grigorov, I strongly recommend that the members of the esteemed Scientific Jury vote positively and award Dr. Rozen Krasimirov Grigorov the educational and scientific degree "Philosophy doctor".

08.01.2026
Sofia

Prepared by:

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