To
Chairman of the Scientific Jury,
Order No. R-109-477/13.12.2022
of the Rector of the University of Varna
Prof. Valentin Ignatov, MD, PhD

Review of dissertation
by Prof. Maria Mitkova Orbetzova, MD, PhD,
Scientific specialty "Endocrinology and metabolic diseases"
Head of the Clinic of Endocrinology and Metabolic Diseases, "Sv. Georgy" University hospital,
Head of the Department of Endocrinology, MF, MU - Plovdiv

of a dissertation for awarding the educational and scientific degree "Doctor of philosophy"
of **Dr. Savi Rinaldiev Shishkov**,
full-time doctoral student at the Second Department of Internal Medicine,
Faculty of Medicine, MU "Prof. Dr. Paraskev Stoyanov" - Varna

on: **"Androgen levels in men with acute and chronic coronary syndrome"**
in scientific specialty **"Endocrinology"**
with academic supervisor: **Prof. Kiril Hristov Hristozov, MD, PhD**

I. Procedural requirements

I was elected as an external member of a Scientific Jury by Order No. R-109-477/ 13.12.2022 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna based on the Report of the Head of the Second Department of Internal Medicine with an entry No. 102-2873/23.11.2022 and decision of the Faculty Council of the Faculty of Medicine - Protocol No. 77/05.12.2022. The review was prepared in accordance with the requirements of the Regulations for the structure and activity of the Medical University of Varna. The full-time doctoral student **Dr. Savi Rinaldiev Shishkov** in the doctoral program **"Endocrinology"** in professional direction **7.1. Medicine**, from the field of higher education **7. Health care and sports** at the Second Department of Internal Medicine, MF, MU "Prof. Dr. Paraskev Stoyanov" - Varna submits all the necessary materials for review in accordance with the Regulation for awarding the educational and scientific degree "Doctor of philosophy", incl. dissertation, abstract, curriculum vitae, administrative data, publications.

II. Biographical data

Dr. Savi Rinaldiev Shishkov graduated from "Ioan Ekzarch" foreign languages high school in Varna with a German language profile in 2020. He completed his higher education in medicine in 2017 at "Prof. Dr. Paraskev Stoyanov" Medical University of Varna.

From 2017 to 2021, he has conducted a specialization in endocrinology at "St. Marina" University Hospital in Varna, working until now as a doctor in the Clinic for Endocrinology and Metabolic Diseases. Since 2018, he is currently an assistant in Endocrinology at the Second Department of Internal Medicine, Medical faculty, MU-Varna. In August 2019, he was enrolled for full-time doctoral studies at the same department.

Dr. Savi Shishkov has conducted specialized training at University Medical Center Leiden, Netherlands on the topic "Growth hormone treatment, transition of patients from pediatric care to adult care".

Dr. Savi Shishkov has a total of 8 full-text publications and 8 participations in national and international scientific forums. Has participated in 1 scientific research project at the "Science" Fund of MU-Varna on the topic: "Dynamics in serum levels of male sex steroids in men after acute coronary syndrome" with the supervisor Assoc. prof. A. Angelov.

He is fluent in German and English.

He is a member of the Bulgarian Society of Endocrinology and the European Society of Endocrinology.

III. Relevance of the topic

The dissertation work is devoted to an interesting, important and still debatable problem of the relationship between androgen levels in men and cardiovascular diseases (CVD), in particular ischemic heart disease (CHD).

In recent years, more and more data have been accumulated on the role of sex steroids in the pathogenesis of CHD. Despite observations that men are affected more often than women in the premenopausal period and suggestions that androgens are one of the possible causes, the question still remains open because there is a combination of a number of other cardiovascular risk factors and pathophysiological mechanisms that appear complex. Thus, huge observational studies and meta-analyses provide a broad spectrum of negative health consequences of testosterone deficiency with an emphasis on the cardiovascular system, with a number of prospective studies reporting an inverse relationship between low testosterone levels and cardiovascular mortality. But the question of whether changes in gonadal status are a cause of CVD or a consequence of the latter still remains unresolved. There is also some conflicting opinion regarding testosterone replacement therapy and its overall benefits and risks.

In this sense, any national study, especially a prospective one, with the investigation of certain biomarkers of a diagnostic and prognostic nature is also valuable on a global scale. For the endocrinological community, the hormonal changes - adaptive and subsequent, seen in specific acute coronary events and in chronic coronary syndrome are less known and their clarification is of great clinical benefit.

Based on the above, I assess the topic of the dissertation work as relevant and modern, with a certain theoretical and clinical significance.

IV. Structure of the dissertation; aim and objectives; material and methods; results, discussion, conclusions.

The dissertation work covers 255 standard typewritten pages and is properly structured in terms of required components. The different chapters and sub-chapters are described in details in the table of contents, which brings clarity and comprehensibility to the entire material.

The literature review (49 pages) is purposeful and presents physiological and pathological mechanisms of regulation of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenocortical (HPA) axes and their interactions, as well as the physiological role of steroid hormones and their systemic effects in the myocardium in the context of the objectives of the thesis and the investigated parameters.

The manifestations of arterial coronary disease - acute and chronic coronary syndromes (ACS and CCS), subtypes of ACS and hormonal adaptation in acute events are described in detail. The review in this aspect includes specific data on the pathogenesis of CHD, related to the action of sex steroids in men - androgens in view of hypotestosteronaemia and estrogens in view of hyperestrogenaemia seen in obesity and metabolic syndrome. Current information regarding the effects of gonadal and adrenal steroids specifically on the low-reactive inflammation, the atherosclerotic process, the vascular smooth muscles and also the interrelationship of their alterations with anthropometric parameters and carbohydrate metabolism is reviewed. A place is also devoted to sex hormone-binding globulin (SHBG) and its involvement in the pathophysiological mechanisms of the cardiometabolic syndrome development. Considerations regarding the relationship between testosterone hormone replacement therapy and cardiovascular risk, for which the data are the most contradictory, are also presented.

In view of the idea and design of the dissertation work, more or less popular hormonal ratios and indices were also considered, giving targeted and useful information contributing to the assessment of the relationship between hypogonadism in men and CHD, as well as cardiovascular risk in general and risk of mortality.

In summary, the literature review is purposefully constructed according to the conceptual design of the dissertation work and provides basis for interpretation of the results. It is also valuable as up-to-date information on the issue.

The ***aim*** of the dissertation work is clearly formulated and concerns the study of the role of androgen hormones in the adaptation to acute coronary syndrome, as well as the development of cardiovascular disease in men with acute and chronic coronary syndromes.

The ***tasks*** are formed as 6 in number, arising from the main objective and containing specifically the separate aspects of the analyzes - research of the HPG axis through certain hormones, their ratios and indices, as well as various correlations in men with acute and chronic coronary syndromes in comparison with clinically healthy controls; analyzing differences in the studied parameters in subgroups with and without diabetes; follow-up of sex hormone changes over time after ACS; study of the relationship between the severity of ACS and other clinical and biochemical, incl. hormonal variables in the acute period; screening for hypogonadism combined with anxiety, depression and erectile dysfunction at the onset of ACS and after 6 months.

The approaches used in the construction of the ***design*** of the clinical study, the ***material and methods*** are adequate to the aim and the tasks of the thesis, their description occupies 13 pages.

To fulfill the thesis goals, a *cross-sectional study* was performed on 105 patients, mean age of 56.75 years (36-70 years) – n=72 with ACS and n=32 with CKD and on 35 controls, mean age of 54.22 years (44-68 years). The included patients were divided into subgroups: with ACS, including STEMI, NSTEMI, NAP; with stable CHD; combined ACS and CCS in a pooled group with coronary artery disease (CAD).

Additionally, a *prospective study* was performed in some patients from the ACS group for a 6-month post-event period.

The characteristics of the patients in the individual subgroups are presented in great detail and with precision from a cardiological point of view, and inclusion and exclusion criteria are defined for each subgroup, as well as for the controls. This shows additional knowledge, acquired skills and a very good understanding of the material by the dissertation student.

Applied methods: questionnaire (2 completed validated questionnaires - IIEF-5 shortened version of the International Index of Erectile Function IIEF-15; HADS for anxiety and depression and a structured interview - Androtest), physical and anthropometric methods, as well as a wide range of laboratory tests, including hormonal ones.

A very good attestation for a clinical thesis with both endocrinological and cardiological aspects is the designation of specific definitions regarding myocardial damage, ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), time in ischemia; and also the applied criteria for hypotestosteronaemia.

The *statistical processing* is at a high state of the art and all the various analyzes (parametric, non-parametric, variance, correlation, regression, graphical, etc.) are properly described. They were performed with a modern statistical package SPSS 19.

The ***results*** are presented on 57 pages. They are illustrated with 54 tables and 12 figures, which help to present the information from the statistical analyzes in a synthesized form, and enrich the presentation of the material.

The results are presented according to the specific tasks, which shows that the goals and tasks of the thesis have been fulfilled in full and are clearly a personal contribution of the PhD student.

The ***discussion*** (24 pages) is built logically and is structured according to the presentation of the results, showing a very good knowledge of the selected topic by the PhD student, a deep insight into the underlying ideas and a purposeful analysis of the investigated variables in the dissertation work with the possibility of their interpretation. Some limitations regarding the prospective study in relation to the pandemic setting are also indicated.

The ***conclusions*** are 23 in number - they are presented separately for each of the 6 tasks and represent a generalized analysis of the results regarding the main aspects of the thesis.

The ***bibliography*** is comprehensive, meets the requirements - it includes 382 basic and modern literary sources, of which 4 are in Cyrillic and 378 are in Latin.

V. Contributions

With the results of the current dissertation work, the overall knowledge about the levels of androgens and the changes in the HPG and HPA axes in patients with ACS and CCS is significantly enriched, with an outline of certain changes, tendencies and correlations of the investigated hormonal parameters, while the association of hypotestosteronaemia with ACS is categorically confirmed.

The present study is a pioneer one for our country in terms of examining the parameters of the HPG and HPA axes in ACS and CCS, with the establishment of lower values of total (tT), free (fT) and bioavailable (bioT) testosterone and a higher frequency of hypotestosteronaemia in patients with ACS compared to controls. The patients with ST-elevation ACS (STEMI) had significantly lower levels of testosterone fractions compared to non-ST-elevation patients (NSTEMI and NAP) on the background of similar age, SHBG and BMI, while there were no differences in LH levels between ACS, CCS and controls.

Interesting and very important for clinical practice regarding prevention and identifying timely measures are the facts that in the group with ACS the largest share is occupied by patients with ST-elevation myocardial infarction (72.2%), while those with NSTEMI and NAP are significantly less – 12.5%, resp. 15.8%. The percentage of persons aware of suffering from CAD is significantly higher in CCS than in ACS (78.1% vs. 38.9%, $p<0.001$), and the opposite applies to the presence of DM (22.5% vs. 34.3%, $p=0.003$). On the other hand, all patients included in the groups with ACS and CCS had dyslipidemia, with total and LDL-cholesterol being higher in ACS ($p<0.001$), and arterial hypertension was found in 93.8% in CCS and in 58.3% in OCS. Regarding the other biochemical parameters, including those of whole blood count and levels of HDL-cholesterol and triglycerides, no significant difference was observed between the ACS group and the CCS group, with the exception of leukocytes – higher in ACS ($p<0.001$), which is logical. The T/E ratio, but not the absolute levels of androgens or estrogens, correlated with the lipid parameters even after adjustment for statin use in the CCS group.

An important aspect of the thesis as regards the clinical practice of internists and with a scientific-applied contribution is the established fact that fT and bioT reflect better than tT the size of the infarct area (expressed by troponin value). The difference in the association of tT with BMI (absent in ACS and moderate in CCS) supports the hypothesis that additional factors affecting testosterone concentration play role in myocardial infarction.

For the first time, the cortisol/DHEA-S ratio as well as the relationship between DHEA-S and estimated glomerular filtration rate (eGFR) were also investigated in patients with ACS. Main determinants of DHEA-S were age and eGFR in the ACS and CAD groups (while for the CCS group such factor was only the age). In the present study, a negative correlation was also established between albumin and the risk characteristics of the patients, expressed through the GRACE score. The values of SHBG, DHEA-S and albumin in the first 48 hours after the onset of ACS could account for 22.9% of the variations in the GRACE score.

Data on patients with CHD and DM are also fundamentally valuable. Thus, testosterone levels and the frequency of hypogonadism did not differ depending on the presence of DM in the ACS group at different BMI and age in the ACS and CCS groups, but lower levels of tT were found in the combination of CCS and DM compared to individuals without disorders in glucose metabolism. For a first time, a difference in the tT/LH ratio in patients with ACS, depending on the presence or absence of DM, was also demonstrated. With a confirmatory contribution are the established association of DHEA-S with presence of DM, in addition to that with presence of CAD, and the fact that the higher value of the cortisol/DHEA-S ratio was associated with a worse risk profile of patients with ACS (presence of DM, and higher age).

Similarly, the dissertation work provides valuable information on SHBG as a suitable marker reflecting and complementing the risk characteristics of patients with CAD. Thus, in the group with ACS, a correlation of SHBG with age, tT, liver enzymes and triglycerides was established. At the same time, in the CCS group, the lower SHBG levels were associated with a worse metabolic profile - presence of type 2 DM and elevated triglycerides.

A definite clinical-applied contribution is also the established data in the *prospective study* that there was a tendency towards an increase in the tT levels with time, and a trend to increasing of the fT and DHEA-S levels for a period of 6 months after the onset of an OCS.

With both scientific-theoretical and practical contributions are the pioneering results and conclusions regarding screening for hypogonadism combined with anxiety and depression and erectile dysfunction at the onset of an ACS and on the 6^{-th} month after the event. Thus, in the ACS group the Androtest result is higher in patients with lower DHEA-S, as well as with higher cortisol/DHEA-S ratio, and the IIEF-5 score demonstrated a negative association with the HADS anxiety score and a positive one with the Androtest. The data of the present study can serve as a basis for future prospective studies in this aspect of endocrine and mental disturbances in ACS, for which there is no data in the available literature.

In summary, the dissertation work provides valuable scientific-theoretical results and judgments, as well as specific data and conclusions for the clinical practice.

I accept the self-assessment of Dr. Savi Shishkov regarding the contributions of the dissertation work, framed as scientific-applied and confirmatory in nature, although contributions are usually categorized as original and confirmatory in nature, as well as primarily scientific-theoretical and scientific-practical. I think that the dissertation student could expand the description of the actual contributions of his work.

VI. Publication activity

In connection with the dissertation work, Dr. Savi Shishkov provides 3 publications, of which 2 are in Latin; he is the first author of all of them. He has 2 participations in national scientific forums as first author. He also participates in a scientific project at the "Science" Fund of MU-Varna: No. 19017 of 2020 on the topic: "Dynamics in serum levels of male sex steroids in men after acute coronary syndrome".

I recommend increasing publication activity and publishing materials in scientific journals with an impact factor.

VII. Abstract

The submitted abstract is properly structured, contains all the required details and reflects the most important aspects of the thesis, thereby meeting the requirements of the Regulations of the MU-Varna.

VIII. Conclusion

Based on the above, I consider that the dissertation work of Dr. Savi Rinaldiev Shishkov is actual and relevant, with a contributing character, it represents a completed scientific research work, meeting all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation and the Regulations for the organization and operation at the Medical University of Varna for the acquisition of the educational and scientific degree "Doctor of philosophy" (PhD) in the doctoral program "Endocrinology".

I give a positive review and strongly recommend to the members of the esteemed Scientific Jury to give a positive vote for the implementation of the procedure for awarding a PhD degree to Dr. Savi Rinaldiev Shishkov on the basis of the dissertation work presented by him.

Plovdiv, January 15th 2023

Signature: .....

/Prof. Maria Orbetzova/