To the Chairmen of the Scientific jury appointed by order of the Rector of the Medical University-Varna
№ P-109-477 /13.12.2022 г.

REVEIW

by prof. Borislav Georgiev Georgiev
Head of Cardiology department in National Cardiology Hospital
Member of the Jury for awarding the scientific and educational degree "PhD", designated by order №P-109-477 /13.12.2022 г. by the Rector of the Medical university of Varna

Regarding: Dissertation of Savi Rinaldiev Shishkov MD, full-time PhD student in the Second department of internal medicine, Endocrinology, Medical University Varna.

Topic of the dissertation: "Androgen levels in men with acute and chronic coronary syndrome"
Scientific tutor: Prof. Kiril Hristozov MD, PhD
Scientific consultant assoc. Prof. Mila Boyadzhieva DM, PhD
Higher education area 7. Healthcare and sport, National classifier code: 7.1 Medicine Scientific speciality "Endocrinology"

The documents submitted by Dr. Savi Rinaldiev Shishkov - dissertation, abstract and additional documents are in accordance with the requirements of the regulations for the acquisition of the PhD degree and the regulations of the Medical University - Varna. I find no irregularities in the submitted documentation.

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

All submitted materials are accurately arranged and described.

There is no evidence of plagiarism.

Brief biographical data:

Dr. Savi Rinaldiev Shishkov is born in 1991 in Varna. He graduated with excellent grades from the "Ioan Exarch" High School of Foreign Languages, Varna in 2010. He graduated in medicine at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. In 2022 - Master's degree in Health Management. From 2017 to 2021 he specialized in endocrinology at the University Hospital "St. Marina" In 2021 he acquired a specialty in endocrinology. In 2018 he was appointed and until now he is an assistant professor of endocrinology at the "Second Department of Internal Medicine", MU- Varna "Prof. Dr. Paraskev Stoyanov".

In August 2019 he was accepted as a PhD student at the Second Department of Internal Medicine, Board of Endocrinology and Metabolic Diseases.

He is a member of the Bulgarian Society of Endocrinology and the European Society of Endocrinology. He is a researcher in the project N 19017 under the "Science Fund" entitled: Dynamics in Serum Levels of Male Sex Steroids in Men after Acute Coronary Syndrome with the supervisor Assoc. prof. A. Angelov.

He specialized in University Medical Center Leiden, The Netherlands on Growth Hormone Treatment and transition of patients from child to adult care.

He speaks German and English.

Relevance of the topic

In recent years, a body of evidence has accumulated implicating sex hormones in the pathogenesis of CHD. Despite the empirical evidence that men are more likely to suffer from

CHD than women in the pre-climacteric period and the suggestion that androgens are one of the likely causes, no firm scientific evidence has been presented and the question remains open.

With regard to the effect of testosterone on the cardiovascular system in patients with coronary pathology, there are conflicting data. Observational studies have shown an association between low endogenous testosterone levels and mortality in CHD. On the other hand, with androgen-substitution therapy, there is no reliable evidence of positive outcomes, and even the safety issue is discussed, i.e., suspicions and a higher risk of cardiovascular events with testosterone(T) substitution.

It has been hypothesized that the decline in T levels during the acute period of acute coronary syndrome (ACS) is an adaptive mechanism providing better survival. In contrast, a number of investigators present evidence of increased mortality with low baseline testosterone during ACS.

Besides T, other androgens (DHEA-S) and estrogens are also relevant to the cardiovascular system. Their involvement in the pathogenesis of atherosclerotic cardiovascular disease has been suggested.

The topic of this dissertation is extremely interesting and topical and is among the unexplored problems in modern cardiology.

Structure of the dissertation:

The scientific work of Dr. Savi Rinaldiev Shishkov is presented in 174 pages according to the requirements and contains an introduction, literature review, aims and objectives, methods, results, discussion, conclusion, contributions and bibliography. The dissertation material is illustrated with 58 tables and 17 figures.

The **introduction** is on 2 pages. The **literature review** is presented on 37 pages demonstrating the author's good knowledge concerning the hypothalamic-pituitary-gonadal axis and the interplay between the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes; the physiological role of steroid hormones - systemic effects and effects in the myocardium. The dissertation briefly defines what coronary artery disease is and its types acute coronary syndrome and chronic coronary syndrome. He focuses on Sex Hormone Binding Globulin (SHBG) and aspects of the pathogenesis of CHD related to sex hormones - role of testosterone in CHD, and estrogens in atherosclerosis, and the effects of testosterone on coronary vascular smooth muscle, male hypogonadism and CHD, testosterone and systemic inflammation, DHEA/S and CHD, DHEA-S and CKD. Topics discussed include Androgens and Glucose Metabolism - Body Mass Index and Testosterone, DHEA-S and Glucose Metabolism, Estrogens and CVD and their impact on coronary vessel smooth musculature and on atheroma plaques. The role of estrogens in male obesity and hormonal adaptation in acute coronary syndrome - hypothalamic-pituitary-adrenal axis activation, androgens and ACS are presented. Data on Testosterone replacement therapy as cardiovascular risk and hormonal ratios - Cortisol to DHEA-S, Testosterone to Estradiol, Androgen Sensitivity Index (Testosterone to LH) are presented. The review provides an endocrinologist's perspective on cardiovascular risk. The dissertation shows a very good awareness of the topic.

Based on the literature review, the author outlines the basis of his research paper.

The **bibliography** contains 382 cited titles, of which 5 in Cyrillic and 378 in Latin. Citation 33 is not spelled correctly, probably the full text is in Cyrillic and the journal should be *Endocrinology*; the authors of citations 43, 208 and 252 are not spelled correctly.

Dr. Savi Rinaldiev Shishkov aims in his research to investigate 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 syndrome.

In order to achieve this aim, he sets the following objectives:

1. To investigate the pituitary-gonadal axis by measuring the values of total testosterone, calculated free testosterone, luteinizing and follicle-stimulating hormone in patients with acute and chronic coronary syndrome and in healthy controls.

2. To investigate differences and look for correlations in DHEA-S/cortisol; testosterone/LH; testosterone/estradiol ratios in healthy controls and in patients with acute and chronic coronary syndrome.

3. In patients with ischaemic heart disease, investigate differences in hormonal indices between subgroups with and without diabetes mellitus.

4. To follow the dynamics of sex hormones after the onset of ACS.

5. To investigate the relationship between the severity of acute coronary syndrome and other clinical, anthropometric and paraclinical indices, and steroid hormone levels in the serum of patients with acute coronary syndrome, in the acute period.

6. To screen for hypogonadism combined with anxiety and depression, and erectile dysfunction, at the onset of ACS and at the sixth month of the event.

Methods: 105 patients, mean age 56.75 years (36-70), were studied. Of these, 72 had ACS and 32 had CCS. During the study period, 35 controls with a mean age of 54.22 years were included.

The enrolled patients were divided into the following five groups:

1. Patients with acute coronary syndrome including STEMI, NSTEMI, UAP - *"ACS" group.*

2. Patients with stable ischemic heart disease - *"CHD" group.*

3. Combined ACS and CHD in one composite group - group of patients with arterial coronary disease, "ACD" (the group is composed by the common sign of the presence of arterial coronary disease, regardless of whether the coronary syndrome is acute or chronic).

4. Patients in the ACS group followed for at least 6 months after the acute cardiovascular event.

5. The *control group* consisted of patients without cardiovascular disease.

Statistical analysis includes:

- Analysis of variance - calculation of mean and standard deviation for quantitative variables;
- Parametric analysis - Student's t - criterion for comparing means of dependent (Paired t-test) and independent samples (Independent t-test) with normal distribution;
- Non-parametric analysis to compare medians (Mann-Whitney);
- Analysis of variance (ANOVA) - to determine the presence of an effect by estimating the variance associated with the effect and comparing it with the variance due to pure chance. Sheffe and Games-Howell post-hoc tests were also used;
- Kruskal-Wallis nonparametric analysis was used for data with non-normal distribution, with subsequent Mann-Whitney serial tests with Bonferroni correction;
- Spearman and Pearson correlation analysis were used to search for association between variables according to the normality of the distribution.
- Univariate and multivariate regression analysis - to study the functional relationship between two or more variables (dependent and independent variable(s)
- Graphical analysis - to illustrate the phenomena under study (line, bar, pie, pie-sector, figure diagrams). The specific analyses used are listed in the relevant sections of the thesis.

Limitations of the study:

Prospective follow-up was not performed in all intended patients due to the limitations imposed by the two-year COVID-19 epidemic situation.

Results: The results of Dr. Savi Shishkov are presented in 64 pages of the dissertation. The results are well illustrated. The results obtained meet the objectives of the study. The selection of the study subjects, the multitude of adequately applied statistical methods make the results comparable to those in the literature. This in turn makes the findings credible.

The discussion of the results is presented on 21 pages and analyzes the acquired data, where possible, with other publications on the subject.

The discussion is done competently, in proper professional language, with good knowledge of the subject at hand. Here the dissertant has skillfully used the literature data and own results. The conclusions drawn on the one hand give clarity to the issues discussed in the literature, and on the other hand offer acceptable explanations for the divergence of some of the data presented in the literature. Thus, in a contributory sense, it bridges between a number of studies seemingly contradictory to each other and allows a new reading of some points of the discussed problem of gonadal function in CHD.

The 2-page **conclusion** summarizes the results but also traces potential future research.

Conclusions: Dr. Savi Shishkov provides 23 conclusions. They are derived from the objectives and the research.

Task 1: To investigate the pituitary-gonadal axis by measuring the values of total testosterone, calculated free testosterone, luteinizing and follicle-stimulating hormone in patients with acute and chronic coronary syndrome and in healthy controls:

1. Lower values of total, free and bioavailable testosterone and a higher incidence of hypotestosteronemia are found in patients with ACS compared to controls.
2. Patients with ST-elevation ACS (STEMI) is associated with significantly lower levels of TT, FT and bioT compared to patients with non-ST-elevation ACS (NSTEMI and UAP) against a background of similar age, SHBG and BMI.
3. There are no difference in LH levels between the three groups studied (ACS, CCS, controls)

Task 2: To investigate differences and look for correlations in DHEA-S/cortisol; testosterone/LH; testosterone/estradiol ratios in healthy controls and in patients with acute and chronic coronary syndrome:

1. Aromatization index depends on both BMI and the presence of ST-elevation, it is lower in the STEMI group.
2. Testosterone to estradiol ratio, rather than the absolute androgen or estrogen levels, correlates with lipid parameters even after adjustment for statin use in the CCS group.
3. Patients with DM in both the ACS and CCS groups were found to have a lower TT/LH ratio compared with patients without impaired glucose metabolism.
4. In the ACS group, a higher TT/LH ratio is associated with a higher HDL cholesterol value even after adjustment for statin intake.
5. Higher C/D ratio was associated with worse risk profile of patients with ACS (presence of DM, older age).

Task 3: In patients with ischemic heart disease, investigate differences in hormonal indices between subgroups with and without diabetes mellitus.

1. Testosterone levels and incidence of hypotestosteronemia do not differ according to the presence of diabetes mellitus in the ACS group at different BMIs and age.
2. Lower TT levels were found in patients with CCS and diabetes mellitus compared to those without impaired glucose metabolism.

3. DHEA-S levels are not affected by the presence of DM in acute and chronic coronary syndrome.
4. In the CCS group, lower SHBG levels are associated with a worse metabolic profile of patients, namely the presence of DM2 and elevated triglycerides

Task 4: To follow the dynamics of sex hormones after the onset of ACS.
1. In mathematical modeling, we found a trend for increasing levels of free testosterone in percentage and decreasing DHEA-S over a 6-month period after the onset of ACS.
2. There was a tendency rise of TT value over time after the onset of ACS.

Task 5: To investigate the relationship between the severity of acute coronary syndrome and other clinical, anthropometric and paraclinical indices, and steroid hormone levels in the serum of patients with acute coronary syndrome, in the acute period.
1. Free and bioavailable testosterone better reflect the size of the ischemic zone (expressed by troponin) relative to total testosterone.
2. The difference between the two groups in the association of TT with BMI (absent in ACS and moderate in CCS) supports the hypothesis that there are additional factors influencing testosterone concentration in myocardial infarction.
3. The major determinants of DHEA-S were age and glomerular filtration rate in the ACS and IHD groups, whereas for the CS group it was age alone.
4. There is an association between SHBG and TT levels, liver enzymes, age and triglyceride levels in the ACS group.
5. SHBG is an appropriate parameter reflecting and complementing the risk characteristics of patients with ACS.
6. A negative correlation is found between albumin levels and patients' cardiovascular risk expressed by GRACE score.
7. By SHBG, DHEA-S and albumin values in the first 48 h after the onset of ACS, 22.9% of the variation in GRACE score could be determined.

Task 6: To screen for hypogonadism combined with anxiety and depression, and erectile dysfunction, at the onset of ACS and at the sixth month of the event.
1. In the ACS group. we a higher androtest score is associated with lower DHEA-S levels as well as at higher cortisol/DHEA-S ratio levels. Such an association was described for the first time in the literature, the significance could not be clarified with the available data.
2. The IIEF-5 score demonstrates a negative association with HADS score for anxiety and a positive association with androtest in patients with ACS.

Contributions: the contributions are 7, divided into two groups - of ***scientific and practical nature*** (4) and of ***confirmatory nature*** (3) and are important for clinical practice.

Contributions of scientific and practical nature:
1. For the first time the relationship between HDEA-S and glomerular filtration has been investigated in patients with acute coronary syndrome.
2. For the first time, the cortisol/DHEA-S ratio is investigated in patients with acute coronary syndrome.
3. For the first time, the total testosterone/LH ratio has been shown to differ in patients with acute coronary syndrome according to the presence of diabetes mellitus.
4. Based on the results, additional risk characteristics in patients with ACS were determined.

Contributions of confirmatory nature:
1. The association of DHEA-S with coronary artery disease was confirmed.
2. Confirmed the changes in total testosterone levels in the first days after the onset of acute

coronary syndrome.
3. Confirmed the higher incidence of hypotestosteronemia in patients with acute coronary syndrome compared to controls.

The thesis summary abstract is presented in Bulgarian language, contains 80 pages and reflects what is written in the thesis. It has been prepared as required.

Publication: In connection with the dissertation, the author presented 3 full-text publications in journals and 1 presentation at a scientific forum.

Conclusion: I assess the work of Dr. Savi Rinaldiev Shishkov on "*Androgen levels in men with acute and chronic coronary syndrome*" as interesting in scientific aspect and important for clinical practice. I consider the that this dissertation meets the requirements for the educational and scientific degree "Doctor" according to the Academic Staff Promotion Act and the Rules for the academic Staff promotion in Medical University Varna. I confidently recommend the members of the Honorable Scientific Jury, based on the aforesaid merits of the dissertation work of Dr. Savi Rinaldiev Shishkov, to vote positively for the awarding of the educational and scientific degree "Doctor" to Dr. Savi Rinaldiev Shishkov.

13.01.2023
Sofia

Prof. Borislav Georgiev Georgiev, MD, PhD