

Statement

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concerning dissertation for acquiring educational and scientific degree “DOCTOR”
Area of higher education 7. Healthcare and sport
National classifier code: 7.1 Medicine
Scientific speciality “Endocrinology”

With title:

**“Androgen levels in men with acute and chronic coronary syndrome”
of Savi Rinaldiev Shishkov M.D.**

full time PhD student in Second Department of Internal Medicine
Faculty of Medicine in Medical University Varna
Scientific mentor: Prof. Dr. Kiril Hristov Hristozov, PhD.

With order N: P-109-477/13.12.2022 г. of the Rector of MU-Varna I am chosen as a member of the scientific jury and according to order N: 1/20.12.2022 г. I am also required to prepare a statement on the procedure of acquiring educational degree “Doctor” with candidate Savi Rinaldiev Shishkov M.D.

Short information on the professional and qualification of the PhD student: Savi Rinaldiev Shishkov M.D. has graduated Medical University Varna in 2017. Since 2017 he works as a physician in UMHAT “St. Marina” Varna. In 2021 acquires the specialty of endocrinology. Since 2018 doctor Shishkov is assistant professor in Second Department of Internal Medicine in Medical University Varna.

Significance of the topic: With the development of genetics, biochemistry and other sciences related to medicine it has become possible to identify unknown mechanisms involved in the pathogenesis of many diseases. In recent years, evidence has accumulated for the involvement of sex hormones in the pathogenesis of ischemic heart disease (IHD). Despite empirical evidence that men are more likely to be affected than women in the pre-menopausal period and suggestions that androgens are one of the likely causes, no categorical scientific evidence has been presented and the question remains open.

Concerning the action of testosterone on the cardiovascular system in patients with coronary pathology, there is conflicting data. On the one hand, testosterone is associated with beneficial effects (reduction of infarct zone size and vasodilation), but on the other hand also with unfavorable ones (induction of inflammation, activation of signaling pathways associated with apoptosis). There are no conclusive positive outcomes with androgen replacement therapy, and even a safety issue has been

discussed - there are suspicions of a higher risk of cardiovascular events with testosterone replacement. According to some researchers, the decrease in testosterone levels during the acute period of acute coronary syndrome (ACS) is an adaptive mechanism providing better survival. According to other studies, there is increased mortality with low baseline testosterone during ACS. Besides testosterone, other androgens and estrogens are also relevant to the cardiovascular system. Their involvement in the pathogenesis of atherosclerotic cardiovascular disease has been suggested.

Given the conflicting literature on the effects of androgens on men's cardiovascular health, their study remains a topical scientific issue.

Structure of the dissertation: the dissertation of Dr. Savi Shishkov is formatted according to the requirements of 174 pages and is illustrated with 16 figures and 58 tables. The dissertation is well balanced and includes an introduction and literature review (43 pages), aim and objectives (1 page), material and method (13 pages), own results (56 pages), discussion (22 pages) and conclusions and contributions (4 pages). The bibliography contains a total of 382 sources (5 in Cyrillic), of which 25% have been published in the last 5 years. The thesis summary includes 12 figures and 25 tables.

Literature review: The literature review shows excellent awareness of the author on the topic. The physiology and pathophysiology of hypothalamic-pituitary-gonadal axis stress; the physiological role of steroid hormones and their effects on the myocardium; sex hormone binding globulin and its role in cardiometabolic syndrome are all discussed in detail. Different aspects of the pathogenesis of ischaemic heart disease involving sex hormones are discussed in a separate chapter. Currently available data on: the role of androgens in glucose metabolism; hormonal adaptation in acute coronary syndrome; and the importance of testosterone replacement therapy for cardiovascular risk are presented.

A separate chapter is devoted to so-called hormone ratios. Increasingly, a method is sought that allows simultaneous consideration of hormones that are assumed to be functionally correlated. Such an attempt to objectify hormonal interactions and balance is represented by hormone ratios (indices), which are indicators of the balance between two endocrine systems. Despite the potentially valuable information from the indices in question, the author stresses that their value in certain clinical situations, as well as the most appropriate mathematical methods for their application, have not yet been conclusively proven.

Aim and objectives: The dissertation formulates the main aim of the scientific work as a logical continuation of the literature review: to investigate the role of androgen hormones in the adaptation to acute coronary syndrome and in the development of cardiovascular disease in men with acute and chronic coronary syndrome. To achieve this aim, six objectives were formulated.

Material and method: 105 patients - 72 with ACS and 32 with chronic coronary syndrome (CCS) were included in the study in order to address the objectives. The

control group included 35 men with no evidence of IHD, hypertension, dyslipidemia and diabetes mellitus (DM).

The parameters analyzed are: demographic; risk factors for atherosclerotic vascular disease; laboratory parameters - albumin; testosterone (T), cortisol; estradiol (E); dehydroepiandrosterone sulfate (DHEA-S); sex hormone binding globulin (SHBG) and luteinizing hormone (LH). The following hormone ratios were determined: cortisol/DHEA-S; testosterone/estradiol; testosterone/LH and free Troponin/LH. Three survey methods were used in the study: androtest, HADS and IIEF-5. Advanced statistical methods were used for data interpretation.

Results and Discussion:

The following results should be noted as the most significant of those found:

- Lower total, free and bioavailable testosterone levels and higher incidence of hypotestosteronemia were found in patients with ACS compared to controls.
- Patients with ST-elevation ACS had significantly lower levels of total, free, and bioavailable testosterone compared with patients with non-ST-elevation ACS on a background of similar age, SHBG, and body mass index (BMI).
- Patients with DM in both the ACS and CCS groups had a lower total T/LH ratio compared with patients without impaired glucose metabolism.
- Aromatization index, expressed by the total T/total estradiol ratio, was better associated with cardiovascular risk factors than either parameter alone. Studying the hormonal axes in their interaction directly with each other yields more information relative to each parameter alone.
- A higher value of the C/D ratio (cortisol to DHEA-S) is associated with worse risk characteristics of patients with ACS (presence of DM, older age).
- T/E ratio, not absolute androgen or estrogen levels, correlates with lipid parameters even after adjustment for statin intake in the CCS group.
- Lower total T levels were found in patients with CCS and DM compared with those without impaired glucose metabolism.
- DHEA-S levels were not affected by the presence of DM in acute and chronic coronary syndrome.
- In the CCS group, lower SHBG levels were associated with a worse metabolic profile of patients, specifically the presence of DM and elevated triglycerides.
- Free and bioavailable testosterone better reflected the size of the infarct zone (expressed by troponin value) relative to total.
- The difference between the two groups in the association of total T with BMI (absent in ACS and moderate in CCS) supports the hypothesis that there are additional factors influencing testosterone concentration in myocardial infarction.
- The main determinants of DHEA-S were age and glomerular filtration rate in the ACS and CHD groups, whereas for the HCS group it was age alone.

Based on the results and analysis, Dr. Shishkov emphasizes that the present study clearly demonstrates the need for large prospective studies that could elucidate important aspects in the regulation of gonadal function and the extragonadal effects of steroid hormones in the context of ischemic heart disease.

Contributions: Among the suggested contribution I consider the following to be most significant:

1. Contributions of scientific and practical nature:

- For the first time, the cortisol/DHEA-S ratio is investigated in patients with acute coronary syndrome.
- 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.
- Based on the results, additional risk characteristics in patients with ACS were determined.

2. Contributions of confirmatory nature:

- The association of DHEA-S with coronary artery disease was confirmed.
- Confirmed the changes in total testosterone levels in the first days after the onset of acute coronary syndrome.
- Confirmed the higher incidence of hypotestosteronemia in patients with acute coronary syndrome compared to controls.

Publications and scientific reports in relation to the dissertation: In relation to the dissertation, Dr. Shishkov presented three publications and one participation in a scientific forum.

Critical notes:

1. Some of the references in the bibliography are not cited according to generally accepted rules.
2. Due to the use of multiple abbreviations, some reading discomfort is created at particular places.

Personal impressions: The recruitment of the study participants (patients with acute and chronic coronary syndrome) took place in the First Cardiology Clinic, University Hospital "St. Marina". I had the opportunity to closely observe the process of obtaining informed consent, patient questioning and blood sampling. All of this was done in person by the dissertant. In patients with acute myocardial infarction, this occurred within the first 1-2 days of admission. Doctor Shishkov performed these activities delicately, with care and understanding of the patients, who were not always particularly cooperative at this time. Based on the impressions from our work together over the last three years, I can say that Doctor Savi Shishkov is a young doctor who is distinguished by a serious and thorough approach to clinical work and research. The number of patients recruited to the study would certainly have been much higher, but unfortunately the pandemic of COVID-19 in the last 3 years has affected the number of hospitalizations of patients with coronary heart disease.

Conclusion. The dissertation work of Savi Shishkov M.D. fully meets the requirements for the award of the educational and scientific degree "Doctor". This gives me reason to confidently propose to the esteemed scientific jury to vote positively for the award of the scientific degree "Doctor" to Savi Shishkov M.D.

Varna, 09.01.2023

Assoc. Prof. Atanas Angelov, MD, PhD