

**To the Chairman of the Scientific Jury
appointed by Order P-109-581/17.12.2021
by the Rector of the Medical University,
prof. Valentin Ignatov, PhD,
Varna**

REVIEW

By **Assoc. Prof. Dr. Katya Nikolova Todorova MD, Ph.D.**, external member of the scientific jury

"Department of Cardiology, Pulmonology, Endocrinology and Rheumatology", Sector "Endocrinology", Medical Faculty, Medical University-Pleven,

Head of the Clinic of Endocrinology at the University Hospital "dr. G. Stransky" - Pleven

In connection with a dissertation for the award of a degree of education and science

"DOCTOR" on the topic:

"METABOLIC PROFILE OF PATIENTS WITH HORMONALLY INACTIVE ADENOMAS"

Author: **EVELINA BOYKOVA ZLATANOVA**

Doctoral student of independent study

Professional field: **Medicine 7.1**

Doctoral programme, **ENDOCRINOLOGY code& 01.03.03**

BASIS. Second Department of Internal Medicine

Scientific supervisor:

Assoc. Prof. Dr. Mira Siderova, MD, PhD, Medical Faculty, Medical University "Prof. Dr. Paraskev Stoyanov", Varna,

1. General presentation of the procedure and the PhD student

For the preparation of the "Review" of the dissertation of Dr. Evelina Boikova Zlatanova, I was selected as an external member of the scientific jury for the dissertation defense procedure, according to the Order No. R-109-581/17.12.2021 of the Rector of MU-Varna. In this regard, I was presented with the dissertation on paper, the abstract of the dissertation, an electronic medium with publications and all scientific materials, references and documents related to its defense. The documents have been submitted within the required time limit, are properly arranged and contain well-formed evidence. I believe that the documentation provided is in accordance with the requirements of the procedure for the defence for the award of the PhD degree at MU - Varna, the Regulations of MU-Varna (15.09.2020) and is in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria (LADAB) (<http://mon.bg>, dated 05.05.2018).

The peer review has been prepared in accordance with the requirements of the LADAB in the Republic of Bulgaria / LADABRB/ Chapter II, Section I of the LADAB RB.

Dr. Evelina Boykova Zlatanova was born in 1987 in Sofia. She completed her secondary education at the National Natural and Mathematical High School, Sofia with full honors. In 2011 she completed her higher education, specialty "Medicine" at MU-Varna and graduated again with full honors, being awarded the prestigious "Golden Hippocrates" for excellent grades during her studies. Dr. Zlatanova immediately began her working career as a doctor in the team of the Endocrinology Clinic at the University Hospital "St. Peter". Marina" Varna, since 2011. In 2011, she started working at the University of Medical Sciences in Marina Marina, where she has been working ever since. Two years later, in 2013, after a successful competition, she was appointed as a regular assistant in the same clinic. She has a specialty in Endocrinology. Dr. Zlatanova has a Master's degree in Health Management.

In 2018, by Order No. R-109-568/25.09.2018 of the Rector of MU-Varna, she was enrolled as an independent doctoral student in the doctoral program "Endocrinology" at the Second Department of Internal Medicine of MU-Varna.

She is a member of the Bulgarian Society of Endocrinology, the Bulgarian Medical Association and the European Society of Endocrinology.

He speaks and writes English at a high level.

2. Evaluation of the doctoral student's personal participation in the dissertation work

Dr. Zlatanova has successfully implemented the tasks set in the individual and group study plan, has received the required number of credits and has developed and completed the scientific study in time. She has successfully passed the required examination for "Doctoral Minimum".

Dr. Zlatanova has independently developed the steps of conducting the research. She participated in the collection of anthropometric data and in the collection of biological samples for the study of specific laboratory parameters.

Writing the thesis, collecting and processing the data, analyzing, illustrating, discussing and summarizing them are the author's personal work. Particularly valuable is the creative approach to building independent hypotheses and analyzing results for which there is no convincing evidence in the available literature.

The dissertation is written in 126 standard typewritten pages and contains the following sections: 'Abbreviations used' - 1-page, 'Introduction'-3-pages, 'Literature review'-47-pages, 'Aim and objectives'-1-page, 'Material and methods'-5-pages, 'Results and discussion'-60-pages, "Discussion"-2 p. "Conclusion"-3 p., "Contributions"-1 p., "List of publications"-1 p., "List of and scientific contributions"-1 p., "References used"-25 p. "Appendices"-1 p. The bibliography includes 250 references, of which 13 in Cyrillic and 237 in Latin. Of the references used by the author, 16 (6.4%) are from the last 6 years and 108 (43.2%) are from the period 2005-2015. All cited publications are relevant to the problem under study.

The dissertation is written in clear, precise and easily readable professional and grammatically correct language. The ratio: overview: methodological: result-analysis part is optimal and is 40:10:50 respectively.

The text includes **47** figures and **31** tables arranged in a logical sequence.

The layout of the dissertation as a whole, as well as of the attached abstract, is in full compliance with the requirements of the Regulations of MU-Varna.

3. Presentation of the thesis

Topic Relevance. The topic chosen for the dissertation is aptly chosen in terms of the importance of the problem and sounds contemporary and topical, due to the fact that in the last ten years there has been an increased scientific interest in the study of the causal relationship of "benign adrenocortical incidentalomas (BCI) and metabolic syndrome (MetS)". The analysis of the clinical implications of this combination provides a basis for assuming the existence of an equal and bidirectional relationship between adrenal incidentalomas and progressive cardio-metabolic risk over time, on the one hand, and an increased risk of adenoma malignancy on the other. Options for vascular-metabolic and mitogenic risk reduction are limited and are limited to prevention of modifiable cardio-metabolic risk factors, lifestyle modification and surgical removal of the adenoma. The issue of choice of therapy remains controversial and requires further studies. In our country, there are almost no studies on the problem of metabolic disturbances in non-secreting incidentalomas, and internationally there is conflicting or incomplete data regarding causation and its clinical implications. These facts determine the need for the present research study.

The title of the dissertation is specific and reflects the nature of the study and the importance of the research problem at the present time, but also with a possible projection into the future.

The literature review is very well structured and is organized in 54 pages. It includes up-to-date information that is entirely focused on the problem. The topics of incidentalomas in terms of epidemiology, clinico-hormonal characteristics and imaging, the metabolic syndrome and its components, and the relationship between 'non-functioning adrenocortical adenomas and metabolic syndrome' are covered in turn. In this part of the review, Dr. Zlatanova presents in detail the existing controversies and knowledge gaps regarding vascular-metabolic health in non-functioning incidentalomas /N I/. She summarizes and analyzes data from multiple studies that consider benign adrenocortical adenomas as the primary cause of vascular-metabolic dysfunction, the basis of which is endogenous hyperinsulinemia conditioned by clinically unmanifest hyperglucocorticism. Dr. Zlatanova also presents another perspective, seeking to answer the question of whether metabolic syndrome-associated hyperinsulinemia *per se* can lead to the development of an adrenal

tumor. The analysis of the clinical consequences of this combination provides a basis for assuming the existence of an interrelated relationship between N I and the progression over time of metabolic and mitogenic risk. In the light of these two foci, Dr Zlatanova commented on the issue of the choice of clinical management regarding the possibilities for reduction of expected adverse complications, in the benefit/risk direction. As evidence, she presents the results of multiple interventional trials showing significant improvement in metabolic and vascular prognosis after surgical removal of nonfunctioning incidentalomas.

In the concluding part of the literature review, Dr. Zlatanova outlines in summary form the characteristics of N I, the bidirectionality of the relationship "benign nonfunctioning adrenocortical adenomas - hyperinsulinemia and metabolic syndrome or metabolic syndrome - hyperinsulinemia - adrenal tumor development," emphasizing that the issue of choice of management remains controversial and requires rigorous judgment regarding the application of appropriate therapy to the underlying and comorbidities.

This analytical approach shows that Dr Zlatanova not only knows the situation very well but has also brought creativity to her interpretation of the problem.

The aim of the dissertation is clearly and precisely stated, namely to analyze the diagnostic, metabolic, biochemical and hormonal aspects of patients with hormonally inactive adrenal adenomas. It is consistent with the title and the study's capabilities.

To achieve it, Dr. Zlatanova has set six **tasks** concerning: 1). determination of the frequency of Met C, its components and their relationship with anthropometric indices, 2). study of serum cortisol, rhythm, urinary cortisol, express blockade with 1 mg. Dexamethasone, aldosterone/renin ratio, metanephrines and their relationship with adenoma size, 3). Comparison of the incidence of metabolic disorders between patients with NI and the general Bulgarian population, 4). Determination of the relative proportion of newly detected glycemic disorders and the role of insulin resistance in N I, 5) Investigation of fatty liver index, liver enzymes and their correlation with adenoma size, 6) Development of a diagnostic algorithm to search for metabolic disorders in patients with NF I.

The design and sequence of the tasks reflects Dr. Zlatanov's logistical skill in structuring and conducting the research.

Material and Methods. Ethical requirements were met by signing informed consent to participate in the study and approval by an ethical committee. The characteristics and

methodology of the anthropometric, clinical-laboratory and hormonal studies performed are described. All the conditions for blood collection for hormonal analysis were observed, allowing correct interpretation of the results obtained. The nutritional status was stratified into categories according to the WHO classification. Imaging diagnosis by computed tomography (CT) is very accurately described and an assessment of image density as a diagnostic marker of the benign nature of the adenoma is given. Statistical analysis: the reliability of the obtained results was proved using modern statistical processing methods such as comparative, variance, correlation, regression, risk assessment and ROC curve analysis, the data were processed using statistical package SPSS v.20. 0

4. Contributions and significance of the development for science and practice

The analysis of the data shows that all patients with N I have metabolic dysfunction and the components of MetS are expressed in different relative proportions. The predominant ones were: arterial hypertension (AH), which was found in 93.3% of Met S patients, increased waist circumference, which was above normal in 90% of men and 68.3% of women and carbohydrate disturbances. Of the metabolic parameters, elevated fasting blood glucose above 5.6 mmol/L was the most common, recorded in 76.2%, and atherogenic dyslipidemia with predominant hypertriglyceridemia was found in 68.6%. Obesity occurred in 49.5%, and overweight in 35.2% of patients. There was a statistically significant difference in the prevalence of obesity between men and women, assessed in terms of waist circumference, which has not been found before.

Changes in the metabolic profile allow interpretation in two directions. The first relates to increased cardiovascular risk and is associated with each of the components of MetS. The second line of reasoning relates to obesity and overweight, which are characterized by insulin resistance. Endogenous hyperinsulinemia could be a factor in the development of benign or malignant adrenal adenomas. Furthermore, obesity is an independent cause of cardiovascular mortality or cancer-related mortality.

To be reliable, the inference of metabolic disturbances and the assessment of the associated risk needed to demonstrate that adenomas were nonfunctional in all patients. For this purpose, the functional hormonal characterization of adrenal adenomas was determined. By measuring the 24-hour serum total cortisol, the presence of a preserved circadian rhythm of serum cortisol and its suppression in the course of the suppression test with 1 mg. Dexamethasone, autonomous glucocorticoid hypersecretion was excluded in all patients with

adrenal incidentalomas. A normal aldosterone/renin ratio was measured. Plasma metanephrine and normetanephrine levels were also normal, thus definitively demonstrating that the sample consisted of only hormonally inactive HI.

Despite the fact that evening cortisol values were normal, there was a direct positive relationship between evening cortisol and adenoma size. Evening cortisol, even upper-limit cortisol, is shown to play an important role in the onset of glucose and metabolic disorders.

Despite the lack of functional activity of the adenomas, all patients had an increased cardiovascular risk, defined by significantly higher BMI and worse metabolic parameters, compared with the general population. This suggests the involvement of steroidogenesis precursors because adenomas express all steroidogenesis enzymes. Therefore, Dr. Zlatanova proposed future investigation of some of the more significant steroid precursors should be considered to identify subclinically expressed pathological secretory activity of steroidogenesis in patients with HI.

In order to clarify the percentage distribution of MetS constituents in the cohort of patients with hormone-non-secreting adrenal adenomas compared to the general Bulgarian population, a comparison of the frequency of the observed metabolic disorders was made. In the population of individuals with NI are predominantly patients with Met S diagnosed by three features, the most common of which are increased waist circumference, obesity and atherogenic dyslipidaemia. It is likely that N I, although nonsecreting, has an adverse effect on body weight, as confirmed by the significant difference in the proportion of obese individuals with a BMI greater than 30 kg/m² (49.5% vs. 33.2% in the general population). Lipid abnormalities were shown to be more prevalent in persons with NI , characterized by low HDL cholesterol (in 66.8%) and high triglycerides (in 33.7%) compared to the general population, indicating that persons with NI are more likely to develop MetS.

The results of the study on carbohydrate disorders and insulin resistance were particularly significant. After using the harmonized criteria for the diagnosis of MetS, with a level of blood glucose above 5.6 mmol /L, it was shown that 76.2% of the individuals studied met these criteria. After performing an oral glucose tolerance test (OGTT), it was found that only 10.5% of subjects with NI had normal glucose tolerance, and newly diagnosed type 2 diabetes mellitus (DMT2) was detected in 38.1%. Significantly the highest number of carbohydrate disorders (64.5%) were diagnosed in individuals who had a combination of impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT). The prevalence of

newly diagnosed DMT2 was significantly higher than in the general population. This makes it possible to generalize that HI are associated with a higher prevalence of NGT and DMT2. All patients with carbohydrate disorders had an elevated BMI.

Tracking of insulin levels during the course of OGTT showed that there was no significant difference in basal insulin levels measured at 0 min between subjects with Normal OGTT and those with newly diagnosed DMT2. A significant difference was observed when comparing subjects with IGT and those with IFG. Tracking of stimulated insulin levels measured at 120 min showed evidence of hyperinsulinaemia in the subgroups of individuals with Normal OGTT and the combination of IFG and IGT, with over 67.6% of all having a greater than 5-fold increase in insulin levels within 2 hours. No correlations were found with adenoma size and blood glucose and insulin levels. There are currently no detailed data on insulin levels during OGTT in hormonally inactive adenomas in the world literature. hyperinsulinaemia, with an emphasis on 120 min, should be the subject of clinical search and early detection, in order to prevent the mitogenic effects of insulin that are associated with a malignant transformation claim.

Although speculative, the data from recorded overstimulated hyperinsulinaemia give reason to consider the possibility that some of the effects of insulin may alter adenoma size due to activation of the mitogenic potential of insulin in the setting of chronic hyperinsulinaemia. This is a basis on which dr Zlatanova made offer to warrants laying the groundwork for a future prospective study right now, in which it would be interesting to investigate the role of insulin at 120 min in correlation with insulin-like growth factor-1 (IGF-1) and subclinical inflammation.

The data from determining the threshold values of blood glucose and insulin at 0 and 120 min, above which there is a risk of increasing adenoma size, are extremely informative. Precision ROC curve analysis found that the threshold value for insulin at 0 min was 6.5 IU and the threshold value for insulin at 120 min was 54.5 IU, with a proven reliability, sensitivity of 54.1% and specificity of 58.1%. The threshold bloodglucose at 0 min. is 6,35 mmol/l., and for 120 min. - 8.25 mmol/l., (with a test sensitivity of 52.7% and specificity of 54.8%). The threshold value of the HOMA- IR index was 1.85 (with a sensitivity of 50.0% and a specificity of 54.8%). For QUICKI index, the threshold value was 0.34 (with a test sensitivity of 51.6% and specificity of 52.7%).

Interest in early liver injury in Met C has warranted investigation of liver transaminases and calculation of the fatty liver index (FLI). Therefore, it is not surprising to find that 32.4% of NI patients had upper limit serum liver transaminase levels and FLI was high in about 50% of them, which is evidence of hepatic steatosis. Also, high FLI levels above 57% can reliably predict the risk of recurrence of nonalcoholic steatotic disease and adenoma growth.

Based on these studies, two algorithms were developed. The development of the first algorithm for the management of persons with NI based on international recommendations is a substantial contribution, with practical relevance. It directs the steps of hormone activity testing of adenoma as screening and confirmatory and defines specificities in the management of positive and negative tests. It gives recommendations for the follow-up of patients with the determination of the frequency of radiographic and hormonal control and criteria for the judgment of surgical treatment.

Also valuable and of great practical value is the second algorithm presented, which is based on the metabolic profile of patients not amenable to surgical treatment. These individuals should be mandatorily monitored for cardiovascular risk factors and dynamically reassessed according to cardio-metabolic risk after a defined period of conservative therapy or after surgical removal of NI.

Valuable and of great practical value is also the presented second algorithm, which is built on the basis of own results and tailored to the metabolic profile of patients who have no indications for surgical treatment. These individuals should be mandatorily monitored for cardiovascular risk factors and dynamically reassessed according to cardio-metabolic risk after a period of conservative therapy due to an expected worsening of metabolic status, regardless of the treatment administered.

The **discussion** made after each result obtained is presented as a competent and thorough interpretation, with the author's opinion incorporated, which is compared with the data of different author's teams, and where these are not available, independent reasoning is attempted, which gives authenticity and individuality to the work. Dr. Zlatanova's self-assessment is evident in the contributions she has presented, which reflect the data from the conclusions.

Conclusions. The most significant and original for our country are conclusions 1, 2, 8, 9, 11 and 13.

Contributions. I take the liberty to divide the eight contributions into four of a scientific-theoretical and four of a scientific-applied nature. Of these, the most significant are: Studying the relationship between N I and MetS components, proving the high incidence of metabolic disorders among N I patients compared to the general population, analyzing the role of different glucose tolerance disorders and high insulin levels in adenoma growth, applying new non-invasive methods to screen for hepatic steatosis and building an extended algorithm with the addition of metabolic markers assessing cardio-metabolic risk.

Recommendations are given for active follow-up of individuals with hormonally inactive adrenocortical adenomas with disturbances in carbohydrate metabolism to prevent diabetes mellitus and metabolic syndrome. The innovative results of this work can serve to build a registry to follow these patients and report the efficacy of therapy. The work may form the basis for future prospective studies to demonstrate the mitogenic role of insulin in the genesis of NI.

Bibliography: The bibliographic reference confirms the high awareness of the PhD student on the thesis. 13 Bulgarian sources are cited. There are very few publications on the topic in the Bulgarian scientific literature. The worldwide interest in the problem is extremely high and this explains the multiplicity of foreign authors cited.

5. Abstract:

An abstract of the dissertation is submitted with the defense materials. It contains in abbreviated form the text of the relevant sections and summarizes the most relevant data of the methodology, the results analyzed, the conclusions and the contributions.

6. Critical Notes.

I have a minor critical note pertaining to the "Material and Methods" section. The characteristics of the study are not described and the time period for collection and observation of the patients is not indicated.

7. Assessment of publications on the thesis

Dr. Zlatanova has published 3 scientific original articles on the topic of her dissertation, accepted and printed as full-text publications with bibliography in two Bulgarian (in the journal "Endocrinology" 3/2020 and "MEDINFO" 5/2021) and one foreign journal ("General Medicine" 5/2020). Fragments of the thesis have been presented at five Bulgarian

scientific forums. She is the presenting author of all presentations. On this basis, the scientific activity during the period of the thesis development is assessed as optimal.

8. Conclusion.

In it, she presents the existing controversies and the incompleteness of knowledge regarding vascular-metabolic disorders in non-functioning NI. In the process of completing the set tasks, Dr. Evelina Zlatanova has increased her knowledge in the field of endocrinology, gastroenterology and imaging diagnostics. The results achieved, conclusions, contributions and recommendations made have important practical significance for our country and they warrant NI to be considered as a public health problem, if only because of the proportion of carbohydrate disorders.

The dissertation work is detailed and precise and deserves high praise. It contains clinically significant scientific, scientific and applied results, some of which represent an original contribution to science and meet all the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDA), the Regulations for the Implementation of the ASDA and the Regulations of MU - Varna. The submitted materials and dissertation results fully comply with the specific requirements adopted in connection with the Regulations of MU - Varna for the application of the LADAB RB.

.With conviction, I express my **positive assessment** of the dissertation work **of Dr. Evelina Boykova Zlatanova** and propose to the scientific jury to confer on Dr. Zlatanova the educational and scientific degree of „**DOCTOR**” in the subprofessional field of Medicine in the scientific specialty of Endocrinology.

01.02.2022.

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Prepared the review:

Assoc. Prof. Dr. Katya Todorava MD, PhD

