

## STATEMENT OF OPINION

By Prof. Dr. Milena Staneva Staneva, PhD  
Head of Angiology Clinic,  
Acibadem City Clinic University Hospital Tokuda

for dissertation paper for acquisition of educational and scientific degree **“PhD”**  
in the field of higher education 7. Healthcare and sport, professional direction 7.1. Medicine, doctoral program “Internal Medicine”

**Author:** Dr. Stanislava Lyubomirova Ilieva

**Form of doctoral program:** self-tuition

**Research unit:** Clinic of Internal Medicine,

**Topic:** "Role of some pathogenetic and genetic factors in the development of diabetic nephropathy in patients with type 2 diabetes mellitus"

**Scientific supervisor:** Assoc. Prof. Dr. Lachezar Boyanov Lozanov, PhD

### 1. General presentation of the procedure and the doctoral student

The dissertation thesis has been discussed and proposed for defense by the extended Scientific Collegium of the Clinic of Internal Medicine at "Acibadem City Clinic University Hospital Tokuda" EAD. At a meeting of the Scientific Council of "Acibadem City Clinic University Hospital Tokuda" EAD (Minutes 54/06.11.2024) and with order No. 15-05-147/20.11.2024 and supplement order No. 15-05-147#1/03.12.2024 of the Executive Director and the Procurator of the hospital, I have been appointed as an internal member of the Scientific Jury in connection with the dissertation of Dr. Stanislava Ilieva. I have been appointed to present an opinion. There are no omissions in the documentation submitted by Dr. Ilieva, which is in compliance with the requirements of the LDASRB, RILDASRB and the Rules for the terms and and the Regulations for the Development of Academic positions in "Acibadem City Clinic University Hospital Tokuda" EAD.

No plagiarism is found from the submitted StrikePlagiarism.com Metadata reference.

I declare that I have no conflict of interest with the author of the dissertation thesis.

Dr. Stanislava Ilieva graduated from the Medical University of Sofia in 1997. In 1997, she started working at the SHATENG "Acad. Ivan Penchev", later - at the University Hospital "Tsaritsa Ioanna - ISUL", Sofia. Since September 2007, she has been a doctor in the Nephrology Department, Clinic of Internal Medicine at Acibadem City Clinic University Hospital Tokuda. In 2004, she acquired a specialty in "Internal Medicine", and in 2013 - a specialty in "Nephrology" from the Medical University of Sofia. She has specialized in Bulgaria and conducted training courses in Bulgaria and abroad.

### 2. Relevance of the topic

Diabetes mellitus (DM) is a widespread, socially significant disease, leading to early disability and increased mortality due to cardiovascular, neurological and renal complications. In recent decades, diabetic nephropathy (DN) has been the leading

cause of end-stage renal disease and initiation of renal replacement therapy worldwide, as well as a significant risk factor for cardiovascular complications. This provokes clinicians to early diagnosis of carbohydrate disorders, and researchers to search for biomarkers that could be used as early predictors of diabetic kidney damage, even at the preclinical stage. This would lead to the development of strategies for early diagnosis, prevention and treatment of diabetic kidney damage, which is a prerequisite for conducting the present study. The topic is relevant from a practical point of view.

### **3. Knowledge of the problem**

The doctoral student knows the state of the problem and creatively evaluates the literary material.

### **4. Research methodology**

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation thesis.

### **5. Characteristics and evaluation of the dissertation thesis and contributions**

Dr. Ilieva's dissertation is written in 102 pages and is illustrated with 33 tables and 15 figures. It is structured correctly and includes: Table of Contents – 2 pages, Abbreviations and Symbols – 1 page, "Introduction" – 2 pages, Chapter One. "Literature Review" - 22 pages, Chapter Two "Research Methodology" - 10 pages, Chapter Three "Results of the Study" - 34 pages, Chapter Four "Discussion of the Results" - 15 pages, Chapter Five "Conclusions and Recommendations" - 3 pages, Contributions – 1 page, Bibliography - 13 pages,

The bibliography includes 148 sources, of which 15 in Cyrillic and 133 in Latin.

**The introduction** well formulates the problem and justifies the purpose of the dissertation.

**The literature review** is well structured in accordance with the topic being developed. It is written competently and shows good knowledge of the problem under consideration in its various aspects.

**The purpose** of the dissertation is well formulated in accordance with the topic of the dissertation and the presented literature review. The present work aims to establish a relationship between specific clinical and laboratory biomarkers and the manifestation of diabetic nephropathy in patients with type 2 diabetes mellitus, in order to establish predictors for its development and optimize the diagnosis of diabetic kidney damage. The tasks set, a total of 7, are fully sufficient and have been implemented in the dissertation.

The section **"Materials and Methods"** describes 91 patients with CKD and histologically proven nephropathies. They are between 22 and 76 years old, 55 (60.4%) of them are men and 36 (39.6%) are women. They were studied for the period January 2018 - February 2024. The patients were divided into 3 groups, depending on the presence of diabetes and diabetic nephropathy (DN): Group I: Patients with type 2 diabetes and histologically proven diabetic nephropathy (30 patients); Group II: Patients with type 2 diabetes, in whom other, non-diabetic kidney damage is present in the renal histology (25 patients); Group III (control): Patients with histologically proven nephropathies, without diabetes (36 patients). The following methods were applied to all patients: General assessment of the clinical status by organs and systems; presence of

hypertension; presence of complications of diabetes; obesity; laboratory indicators of renal function (cystatin C, serum creatinine, estimated glomerular filtration rate (e-GFR); laboratory indicators of metabolic control (glycated hemoglobin, lipid profile, uric acid); excretion of total protein and albumin in 24-hour diuresis; laboratory markers of inflammation and vascular damage in the blood (C-reactive protein (CRP), interleukin 6 (IL-6), D-dimer, fibrinogen); study of the polymorphism of the methylenetetrahydrofolate reductase gene (MTHFR) gene, in particular the variants A1289C and C677T; study of the levels of homocysteine and folic acid in serum; study of indicators of thyroid function (TSH, FT4, TAT, MAT).

3a Appropriate methods and modern statistical software program (SPSS, version 22) were used for statistical processing of the data, which guarantees the reliability of the results obtained.

**Results:** The presented results are shown correctly. The most important of them are the following. The doctoral student found that the histological results of the PKB performed in the patients show that tubulointerstitial and vascular-hypertensive changes are present, alone or in combination, in 64% of diabetics without DN and in 26.7% of diabetics with DN. Male gender can be considered a risk factor for DN in diabetics. The manifestation of DN is not related to the age of the patients and the duration of DM. The presence of arterial hypertension, obesity, poor glycemic control, dyslipidemia and hyperuricemia are not pathogenetic factors leading to the manifestation of DN, but are associated with the accelerated progression of existing CKD in diabetics. The manifestation of DN is not associated with increased inflammatory (CRP, IL-6) and coagulation (D-dimer, fibrinogen) markers.

**Discussion:** The obtained results are discussed and compared with the known data in the medical literature. The doctoral student freely handles scientific facts and shows competence on the issue.

**Conclusions and contributions:** Based on the obtained results, Dr. Ilieva logically makes 9 conclusions and 3 recommendations for clinical practice, which correspond to the tasks set.

I accept 6 of the **proposed contributions**. I do not accept the first contribution. The relationship between diabetic nephropathy and thyroid dysfunction has been studied with clinical significance, and a high frequency of thyroid disorders and autoimmune thyroid diseases in diabetics has been established.

**6. The abstract** reflects what is written in the dissertation. In accordance with the academic requirements, a list of publications related to the dissertation is attached.

## **7. Assessment of the publications and personal contribution of the doctoral student**

The doctoral student has submitted 2 publications (1 in a national and 1 international journal) and 4 scientific reports, 1 of which at an international scientific forum. This scientific activity is sufficient to cover the national minimum requirements for the educational and scientific degree "PhD".

## **8. Critical remarks and recommendations.**

The following remarks can be made, without reducing the value of the scientific work:

- The type of study (prospective or retro-prospective) is not specified

- There is duplication of information in the "Results" section. The many tables are also presented in graphical form.
- In most graphs, the % of patients with the relevant indicator should be used, rather than an absolute value, since the total number of those studied in the three groups is different.
- I recommend conducting a correlation analysis and regression analysis of the study data in the future to establish the presence and type of relationship between the studied parameters and diabetic nephropathy, which would improve clinical recommendations.

## CONCLUSION

The dissertation contains scientific and applied results that represent a contribution to science by expanding our knowledge in the field of nephrology. It is written in a strictly scientific style. It meets all the requirements of the Law on the development of academic staff in the republic of Bulgaria (LDASRB), the regulations for the implementation of LDASRB and the regulations of "Acibadem City Clinic UMHAT Tokuda" EAD.

Dr. Stanislava Lyubomirova Ilieva is a well-established physician - clinician, specialist in internal diseases and nephrology with extensive work experience. The dissertation shows that the doctoral thesis possesses theoretical knowledge and professional skills in the scientific specialty "Internal diseases", demonstrating qualities and skills for independent conduct of scientific research.

**In view of the above I give my *positive assessment* and recommend in confidence to the members of the Scientific jury to grant positive evaluation to the dissertation thesis "Role of some pathogenetic and genetic factors in the development of diabetic nephropathy in patients with type 2 diabetes mellitus" and to award Dr. Stanislava Lyubomirova Ilieva the educational and scientific degree "PhD" in the field of higher education 7. Healthcare and sport, professional direction 7.1. Medicine, doctoral programme "Internal Medicine"**

06/01/2025

Sofia

Prepared the opinion:

Prof. Dr. Milena Staneva, PhD