

## Opinion

from Professor Dr. Violeta Mihova Iotova, MD, PhD, DSc  
Head of the Department of Pediatrics  
MU "Prof. Dr. Paraskev Stoyanov", Varna

**Subject:** dissertation of Dr. Sevim Ahmed Shefket, PhD student in full-time education at the Department of Clinical Laboratory, MU-Varna, for obtaining the scientific-educational degree "Doctor" in the field of higher education 7. Health and Sports, professional field 7.1. Medicine, specialty "Clinical Laboratory".

Based on Order № P-109-112/11.03.2022 of the Rector of MU - Varna and by decision of the Scientific Jury (Minutes №1/23.03.2022) I am appointed to prepare an opinion for a dissertation on "**PREDICTIVE ROLE OF NGAL AS AN EARLY MARKER FOR KIDNEY INJURY IN PATIENTS WITH DM TYPE I AND DM TYPE II**" for acquiring a scientific-educational degree "Doctor" in a *professional* field 7.1. Medicine, scientific specialty "Clinical Laboratory".

The opinion has been prepared in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation at the Medical University - Varna.

### **Biographical data.**

Dr. Sevim Shefket was born in 1986. After graduating from high school in Shumen, she studied medicine from 2004 to 2010. She graduated from the Medical University in Varna with honors. She began her career as a doctor at the Central Medical Center - Varna, and between 2010 and 2014 she worked as a doctor at the Medical Center "South". Since 2014 she has been specializing in clinical laboratory, successfully acquiring a specialty in 2019. Since 2016 she has been working in the Clinical Laboratory of the University Hospital "St. Marina", and since 2019 she has been enrolled after a competition as an assistant in clinical laboratory at the department of the same name. Dr. Shefket is a member of the Bulgarian Clinical Laboratory Society and the Bulgarian Medical Union. In 2018, after a competition, she was enrolled as a full-time doctoral student on the topic of this dissertation, with supervisor Assoc. Prof. Dr. Yana Bocheva, PhD, Head of the Department of Clinical Laboratory.

### **1. Assessment of the topicality of the dissertation:**

The present work is the first in our country to study and draw sound conclusions about the reliability, diagnostic value and use of the renal tubular biomarker *neutrophil gelatinase-associated lipocalin (NGAL)* in patients with type 1 and type 2 diabetes mellitus in the assessment of chronic renal damage.

### **2. Structure of the scientific work.**

The dissertation is well structured, with all the main parts presented:



- *Review of the literature (39 pages)*. The review is contemporary, logically setting out the current views on the diagnosis of chronic kidney disease (CKD) and in particular, diabetic kidney disease (DKD) and the role of NGAL as a biomarker. The role of the renal tubular and tubular-interstitial involvement, as well as NGAL as a modern early laboratory marker, is particularly well elucidated. Throughout the review, the in-depth interpretation of the literature data by the doctoral student is clear and the formulation of the need for additional research and their set-up is clear.
- *Purpose and objectives (1 page)*. Seven main tasks are clearly formulated, fully meeting the set goal.
- *Materials and methods (13 pages)*. Dr. Shefket's dissertation combines several separate studies - a study of healthy people to define reference limits for the Bulgarian population; study of patients with type 1 and type 2 diabetes mellitus (DM) to assess the diagnostic thresholds for DKD. The design of the studies is suitable for multifaceted elucidation not only of the potential of NGAL as a marker of renal damage, but also of the specific entities in which the best performance can be expected, as well as the methods for its study. The organization of the surveys, the participants and controls involved are presented competently and understandably. The rules of good scientific practice have been worked on with the permission of the Commission for Ethics of Research at the Medical University of Varna, and all the necessary documents are presented in the appendices. Every next step of the research is explained in detail, as the PhD student has prepared the analyzes independently or under guidance.
- *Results (37 pages)*. In over 37 pages, 47 tables and 36 graphs, accompanied by understandable text and showing the competence of the author, the dissertation presents its results. The sequence of the set tasks is observed and the idea of the scientific development is followed. For the first time in Bulgaria, the significance (specificity and sensitivity) of plasma and urinary NGAL, its diagnostic role in chronic kidney disease and especially in the diagnosis of such in type 2 diabetes have been studied. The applied analyzes are selected and performed accurately and correctly, as well as presented in an understandable way. The exact meaning of the studied protein was found in different ratios (with glomerular filtration, with the ratio of albumin-creatinine in the urine, with urinary creatinine). Reference norms have been developed in order to improve its diagnostic role and the most stable prognostic indicator - the NGAL/creatinine ratio in urine - has been specified. The gender difference with higher values in women was studied. From a pediatric point of view, two of the study results are particularly valuable. First, the relative share of patients with existing DKD in children and adolescents is insignificantly small, despite the relatively long duration and poor control in the study group. This is a direct reflection of the improvement in the treatment and prognosis of type 1 diabetes mellitus, which began in childhood in recent decades in the world and in our country. Second, the NGAL/creatinine ratio in a single portion of urine (the so-called spot urine) appears to be a reliable early marker of the onset of renal



damage in type 1 diabetes. Against the background of the desire for more and more easy follow-up for the patient, this is a very important result.

- *Discussion (33 pages)*. Very consistently, in clear literary language, showing the excellent preparedness of the PhD student, Dr. Shefket presents the evaluation of her scientific results and their comparison with the knowledge in literature at the moment. Throughout the text, its clinical and analytical depth, insight into the pathogenesis of renal impairment and especially the modern concepts of diabetic renal impairment are evident. The obligatory for a young scientist openness and in-depth analysis of the limitations in the present work is found throughout the text.
- *Conclusions, contributions and publications on the topic (5 pages)*. A total of 13 conclusions have been consistently, clearly and concisely formulated in accordance with the set tasks. All of them are original for the national scientific space, as the studies available so far do not cover such groups of patients. Based on the conclusions and with a high degree of analytical work, 14 contributions are formulated, 7 of which are of a practical-applied nature. Four publications were presented (2 articles in Bulgarian and 2 participations in international conferences in English, published as abstracts).
- *Appendices (4 pages)*. This part of the scientific work is valuable for future scientific developments as an example of good scientific practice.
- *References (22 pages)* - 221 sources, 10 of which are in Cyrillic. The dissertation shows skillful handling of published resources and extremely correct citations. A total of 162 (73.3%) publications are from the last 10 years, which makes the bibliography extremely contemporary. No significant repetitions were found in the citations.

### **3. Evaluation of the results:**

The results of this dissertation are in-depth, scientifically sound, fully applicable in practice and available for use in future research (e.g. to track the natural evolution of diabetic kidney disease). The competent assessment of the PhD student and the visible own opinion on the presented results are impressive.

### **4. Evaluation of contributions:**

The contributions are original, with clear scientific and practical significance, especially for the national science and practice. Theoretical contributions outline the exact meaning and conventions of NGAL use, especially in type 1 and type 2 diabetes, but also potential interferences with its concentrations (from urinary tract infections and leukocyturia) and the importance of gender. The development of reference values for children and adults, diagnostic limits and the choice of the most accurate presentation of the results as a ratio of creatinine in a single portion of urine are of great practical importance and determine the value of the dissertation beyond purely scientific contributions.

### **5. Critical remarks:**

This dissertation is an honor for our science and an example that with a dignified attitude to the problems and in-depth work truly valuable scientific results can be achieved. It is an excellent certificate for both the supervisor and the entire department. I have no critical remarks and I hope that Dr. Shefket will continue her research work as a postdoctoral fellow.

## 6. Conclusion:

The dissertation of Dr. Sevim Shefket is a well-planned, excellently performed and significant contemporary study of the role of a relatively new laboratory marker for chronic kidney disease in the specific populations of adult patients with type 2 diabetes and children and adolescents with type 1 diabetes compared with healthy individuals and in the development of reference standards and diagnostic limits. The design of the study is well thought out and modern, and the participants are properly selected and properly researched. The selected methods for processing the results are described and clearly explained, with the main contribution of the candidate. Of great importance is Dr. Shefket's perfectionism in quoting literature and interpreting her own results. The basis of the success of this dissertation is the development of clinical laboratory diagnostics in the hospital, the persistence in maintaining high standards at all stages of developing a reliable laboratory result, as well as working with the hospital clinics. In this sense, Dr. Shefket's dissertation is a complex reflection of the modern way of working in a clinical laboratory and in a hospital, and its main conclusions are the basis for further building on the success of the diagnostic and treatment process.

All this gives me a reason to express **positive opinion** and to recommend to the esteemed Scientific Jury to award Dr. Sevim Ahmed Shefket the scientific and educational degree "Doctor".

April 26, 2022  
Varna



/Prof. Dr. V. Iotova, PhD, DSc /