

**To the Chairman of the Scientific Jury  
appointed by Order №-112-109/11.03.2022  
of the Rector of Prof. Dr. Paraskev  
Stoyanov Medical University, Varna**

## **REFERENCE**

**Prepared by:** Assoc. Prof. Marianka Genova Petrova-Yanachkova, MD, PhD, Member of the Scientific Jury

**Scientific specialty:** Clinical Laboratory, Faculty of Medicine, Medical University, Sofia

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Appointed a member of the Scientific jury according to the decision of the Faculty Council of Medical University, Varna and protocol №61 / 01.03.2022

**Regarding:**

A thesis on the topic of the PREDICTIVE ROLE OF NGAL AS AN EARLY MARKER FOR KIDNEY INJURY IN PATIENTS WITH DIABETIS MELLITUS TYPE I AND DIABETIS MELLITUS TYPE II for acquiring the educational and scientific degree of "Doctor".

PhD Candidate: Dr. Sevim Ahmed Shefket, full-time doctoral student, Department of Clinical Laboratory at Medical University, Varna, Assoc. Prof. Dr. Yana Bocheva, Ph.D. as a scientific supervisor.

**Biographical data and professional development of the doctoral student**

Dr. Shefket was born in Shumen where she graduated from high school. In 2010 she graduated from Medical University, Varna with a Master's degree in medicine. From 2014 through 2018 she specialized in Clinical Laboratory at the University. In 2019, she acquired a specialty in Clinical Laboratory. In 2016, she started working as a resident doctor at the Clinical Laboratory of St. Marina University Hospital. From 2019 until now, Dr. Shefket is an assistant professor and full-time doctoral student in the Department of Clinical Laboratory at Medical University, Varna. As an assistant professor, Dr. Shefket participates in the academic work of the department, conducts practical classes of students. In 2019, by order № P-100-35/28.01.2019, she started to work on the current doctoral program with Assoc. Prof. Dr. Yana Bocheva, Ph.D. as a research supervisor. In 2022, by order of the Rector № P-109-112/11.03.2022, Dr. Shefket was expelled within the set term of the doctoral program.

Dr. Shefket is a member of the Bulgarian Society of Clinical Laboratory and the Bulgarian Medical Union.

### **General data on Dr. Shefket's thesis**

There is no doubt that diabetes mellitus (DM) is a socially significant disease with an increasing incidence that is reaching epidemic proportions. Diabetes mellitus seriously affects a number of organs in the human body and one of the serious complications is diabetic kidney disease (DKD), which is associated with increased cardiovascular disease (CVD) and risk of developing end-stage renal disease (TKF). Early detection, good follow-up and treatment of DKD can delay progression to TKF. Currently used laboratory indices for diagnosis, classification and risk stratification of DKD show a number of limitations and shortcomings, which necessitate the search and validation of new markers for reliable assessment and diagnosis of DKD.

The above mentioned determines the relevance of the submitted thesis. Dr. Sevim Shefket aims to define the diagnostic reliability of neutrophil gelatinase-associated lipocalin (NGAL) as a marker of DBP in patients with T2DM and T1DM. The topic of this thesis is essential and does not reproduce the NGAL study as a marker of acute kidney injury which significantly reflects on its relevance. Early diagnosis of DKD, followed by an adequate therapeutic approach, correlates with successful treatment by improving the prognosis for T1DM and T2DM patients.

The thesis is written in clear and accurate language, in a good scientific style, with a logical consistency, which makes the material easy to read and understand. The thesis contains a total of 150 pages, illustrated with 36 figures and 47 tables. The bibliography consists of 221 references. Of these, 211 are in Latin and 10 in Cyrillic - the works of Bulgarian researchers are also taken into account and it makes a good impression. The bibliography is structured as per the requirements. The thesis is constructed according to the usual order (introduction, literature review, aim and objectives, material and methods, results of own research, conclusions, contributions, bibliography).

The introduction is short, accurate and points to the research aim and the topic of the thesis. The literature review is well structured and demonstrates the doctoral student's ability to handle literature sources. It is presented in 38 standard pages. Dr. Sevim Shefket has a good knowledge of the core issue, which is obvious from how the analyzed literature is presented. The thesis describes current trends in the English-language writings regarding risk factors for DKD development, precisely exposing the pathogenetic and pathomorphological changes in the tubulointerstitial in DN and the role of tubular structures in the DKD development. Two markers (GFR and ACR/AER), applied in up-to-date diagnosis of CKD and DKD, are also characterized, providing a good basis for comparison with the advantages and disadvantages of the NGAL laboratory indicator studied in the thesis. The overall presentation of NGAL with its molecular characteristics, its role in the diagnosis of CKD and DMD and in particular as an early marker for DMD development and progression, and its connection with CV risk is well presented. The advantages and disadvantages of the marker are well described and also its role in differential diagnosis as a marker differentiating DKD from Non DKD, as an indicator of urinary tract infections in DM patients. The number of laboratory markers to assess renal function is increasing, but the benefit availability of analysis performed in urine samples is insufficient. On the other hand, there is a need for new markers for early DM diagnosis. This is an additional strength of the thesis, considered also in the context of

patients with T1DM and T2DM, including children with T1DM. The literature review ends with a conclusion that logically leads to the aim and objectives of the thesis.

The material is presented in five main chapters: chapter one includes nine subheadings, chapter two is presented in six subheadings, chapter four-in seven, chapter five-three subheadings. The literature sources are predominantly from the last 10 years and authors from the last 2-3 years are also cited.

The aim is clear and well defined-the diagnostic reliability of the laboratory indicator NGAL to be determined as a marker of DKD in patients with T1DM and T2DM. For the set goal, the presented seven tasks logically follow and are exposed accurately and consistently. The material used in the study is adequate for obtaining statistically reliable results. Depending on the objectives, the number of participants, inclusion and exclusion criteria for the study varied.

To determine the NGAL reference limits in plasma and urine for the Bulgarian population, 127 healthy volunteers divided into two groups-85 healthy adults and 42 healthy children-were included in the study. To assess the diagnostic reliability of pNGAL, uNGAL and UNC as markers of DKD, 167 DM patients divided into two main groups were included in the study: 92 adult patients with T2DM and 75 children with T1DM. Methods for taking, testing and keeping of research materials are well described. The study was conducted at St. Marina University Hospital, Varna from 2019 through 2021. The study protocol was approved by the Research Ethics Committee of Medical University, Varna. All study participants received and completed an informed consent protocol.

Up-to-date laboratory methods and equipment were used for the accomplishment of the set tasks. The doctoral student shows good knowledge, proficiency and interpretation of the obtained results. The validation of the latex enhanced immunoturbidimetric analysis method for the NGAL determination on the ADVIA 1800 biochemical system was performed accurately and in detail according to accepted rules. The formulas for the calculation of eGFR in adults and in children are described in detail. The statistical methods used in the study are described in detail. The SPSS Statistics 27 was used for the statistical processing of data.

The obtained research results are clearly shown in tables and figures, including histograms, graphical distribution of results and ROC curves of very good quality, which illustrates the position of the thesis. Each result is followed by a logical, scientific consideration, which helps to make the material easier to grasp and comprehend.

The doctoral student makes skillful use of the literature materials in order to flesh out data from other studies as per the need to compare with the results from this study. After summarizing and considering the results, the 13 conclusions logically follow. The drawn conclusions and the contributions are in accordance with the set tasks and obtained results. The contributions are divided into theoretical and practical and applied ones.

**Theoretical contributions:** It is confirmed the high analytical reliability of the method for determining NGAL concentration in plasma and urine; Showing the uNGAL results as a proportion of uCreatinine allows better comparability of results and obtaining more reliable results when using spot urine; In

T2DM and T1DM patients, the use of UNC alone is an effective marker for the diagnosis of DBP, and the simultaneous use of pNGAL and UNC - for the prognosis of DBP; Concentration of uNGAL depends on the availability of UTI and leukocyturia.

**Practical and applied contributions:** For the first time in Bulgaria, an analytical verification of an immunoturbidimetric assay for NGAL determination has been performed; For the first time in Bulgaria, sex-differentiated reference limits of pNGAL, uNGAL and UNC in adults as well as in children have been determined; For the first time in a Bulgarian cohort of patients with T2DM and T1DM, the prognostic role of NGAL as a marker for the diagnosis of DMD and for the prognosis of DMD has been evaluated.

In relation to the topic of her dissertation, Dr. Sevim Shefket has two publications in scientific journals and two participations in scientific forums where she was the first author.

**Abstract:** It meets the requirements, written in 80 pages, includes all the main parts of the thesis and also tables, figures and ROC curves. The contents of the abstract give a detailed notion of the overall thesis.

**In conclusion:** in my opinion, Dr. Sevim Shefket's thesis is complete, up-to-date, thorough, well-structured, with clear and precisely formulated and completed tasks, with important conclusions and contributions the to contemporary science and of great practical orientation. The thesis shows profound theoretical knowledge and good professional preparation of the doctoral student. All the above gives me a reason to recommend to the esteemed members of the Scientific jury to award Dr. Sevim Ahmed Shefket degree of Doctor of Education and Science in the scientific specialty Clinical Laboratory in the professional field 7.1 Medicine in Higher Education 7. Health and Sports.

April 18, 2022

Sofia

Reference prepared by:

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