

REVIEW

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Member of the Scientific Jury for the defense of the dissertation for acquisition of Educational and Scientific Degree "Doctor" in scientific specialty "Clinical Laboratory" on the basis of order № P-109-112 from 11.03.2022 of the Rector of the Medical University Varna.

Topic: „Predictive role of NGAL-neutrophilgelatinase-associated lipocalin as an early marker of renal impairment in patients with type 1 and type 2 diabetes”.

Author: Dr. Sevim Shefket, Clinical Laboratory, UMHAT “St. Marina”, Varna.

Scientific adviser: assoc. prof. Jana Bocheva, MD, PhD

Brief presentation of the candidate:

Dr. Sevim Shefket graduated from the Medical University of Varna in 2010. She began her career as a doctor in 2010 at the Varna Medical Center. Until 2014 she worked at the South Medical Center. Since 2016 and until now she has been working consecutively as a doctor and a specialist in the Clinical Laboratory of the University Hospital "St. Marina". Since 2019 she has been appointed as an assistant in the Department of Clinical Laboratory of MU "Prof. Dr. P. Stoyanov ", Varna. and was immediately enrolled as a full-time doctoral student in the scientific specialty "Clinical Laboratory". That same year, Dr. Shefket successfully passed an exam and acquired a degree in Clinical laboratory. She is a member of the Bulgarian Society of Clinical Laboratory.

Relevance of the topic of the dissertation:

In 2021, the International Diabetes Federation (IDF) reports a steady global increase in the prevalence of diabetes mellitus (DM), reaffirming the resulting significant global challenge to the health and well-being of individuals, families and societies. IDF data for 2021 show that: approximately 537 million adults (20-79 years) live with diabetes; about 36% of elderly people living with diabetes are not diagnosed; more than 1.2 million children and adolescents (0-19 years) live with type I diabetes; diabetes is responsible for 6.7 million deaths. That is why the prevention of diabetes and its complications is essential. It has been found that the incidence of chronic kidney disease (CKD) among patients with diabetes varies from 20 to 40% in different countries. The ratio of albumin to urine creatinine and the formulas for estimating the calculated glomerular filtration rate are currently used parameters to assess the presence and progression of DKD. However, they are inaccurate and relatively insensitive to small changes in renal function. The gold standard for diagnosing DKD - kidney biopsy - is an invasive and time consuming method. The standard non-invasive diagnostic test currently used in clinical practice to predict the onset of monitoring the progression of diabetic nephropathy is the microalbuminuria study. However, this test has considerable limitations in determining disease progression due to the observation that some patients with type I diabetes return to normoalbuminuria without treatment. It has also been suggested that tubulointerstitial damage may precede glomerulopathy in diabetic nephropathy.

It is therefore imperative to look for more sensitive and non-invasive markers in this area, to identify and validate new biomarkers for early diagnosis of renal impairment to help predict nephropathy and monitor its progression. All this determines the

exceptional relevance of the problem developed in the dissertation of Dr. Sevim Shefket.

Structure and content of the dissertation:

The dissertation is presented on 150 pages with an optimal ratio between the individual parts: introduction, literature review, purpose and tasks, material and methods, results, discussion, conclusions and contributions. It contains 47 tables and 36 figures. The bibliography includes 221 titles, 10 of them in Cyrillic.

The literature review is detailed and comprehensive, well structured and presented in a volume of 40 pages. In a logical sequence are presented modern scientific data about: diabetes mellitus and diabetic kidney disease; structure, biological role, mechanisms of induction and elimination of NGAL (Neutrophil Gelatinase Associated Lipocaline); on the basis of 15 cited authors, existing reference limits are presented, differing depending on the method, platform, selection and distribution of the reference group. A significant part of the review focuses on the current understanding of the role of NGAL as an early marker for the diagnosis of DKD and as a marker for disease progression. The literature review ends with a brief summary analysis, aimed at formulating the purpose of scientific research.

The aim of the dissertation is briefly and clearly defined - "To determine the diagnostic reliability of NGAL as a marker for DKD in patients with diabetes I and diabetes II". The set goal is a logical consequence of the problems discussed in the literature review and is achievable through well-formulated 7 tasks related to the analytical and diagnostic reliability and clinical applicability of the NGAL marker.

In the section "**Material and methods**" with extreme precision are presented data on: the introduction and verification of latex enhanced immunoturbidimetric analysis of NGAL through the use of 7 criteria for analytical reliability; the establishment of NGAL

reference limits in plasma and urine for the Bulgarian population, involving 127 healthy volunteers with indication of inclusion and exclusion criteria; assessment of the diagnostic reliability of NGAL in plasma and urine and uNGAL / uCreatinine (UNC) as markers of DKD in patients with diabetes, which included 167 patients with diabetes, including 92 adult patients with diabetes and 75 children with diabetes. In the statistical data processing the possibilities of the statistical package SPSS 27 were used, and 10 statistical methods were applied as appropriate.

In connection with the complex solution of the set goal, the doctoral student presents the **results** of her work in 6 sections, which can be summarized as follows:

1. Verification of immunoturbidimetric method for determination of NGAL: Bioporto test was used, analyzes were performed on biochemical analyzer ADVIA 1800. Reproducibility over time and in series, unreliability and inaccuracy, proportional system error by % recovery and % carryover are valued. The results show very good analytical characteristics that meet national and international standards.
2. Building NGAL reference limits for adults and children: Due to the proven lack of Gaussian distribution for all three indicators - pNGAL, uNGAL and UNC in adults, as well as for uNGAL and UNC in children, the derived reference limits include values between 2.5 and 97.5 percentile, with 95% confidence interval.
3. Diagnostic reliability of NGAL: The included persons with DM II are divided into subgroups according to ACR, eGFR and the presence of DKD. According to the guidelines of KDIGO 2020 in the dissertation criteria for diagnosing DKD are adopted: ACR > 3 g/mol and/or eGFR <60 mL/min/1.73 m², against which two groups have been formed: DM II without DKD and MD II with DKD. ROC analysis of the data demonstrated that pNGAL in adults has good diagnostic efficacy in differentiating patients with diabetes mellitus II with decreased eGFR from those

with preserved eGFR: AUC-ROC - 0.753. However, pNGAL demonstrated poor diagnostic efficacy in distinguishing patients with type II diabetes with albuminuria from those with normoalbuminuria: AUC-ROC - 0.580. The author accepts that because in the natural course of renal impairment in diabetes mellitus II, the increase in ACR is most often the first clinical indicator of the presence of DKD, biomarkers that correlate with albuminuria are suitable for the detection of DKD. The results of Dr. Sheffett's dissertation show that uNGAL and UNC have the necessary diagnostic reliability in distinguishing DKD patients from those with type II diabetes, with UNC showing better diagnostic efficacy than uNGAL.

In assessing the diagnostic reliability and clinical applicability of pNGAL, uNGAL and UNC as markers of DKD progression in patients with type II diabetes, the PhD student found that the values of all three indicators increased progressively with the degree of renal impairment, but this difference reached significance only in the results of uNGAL and UNC.

Regarding the diagnostic reliability and clinical applicability of pNGAL, uNGAL and UNC in patients with DM I, the results of ROC analysis demonstrate very good diagnostic efficacy of uNGAL and UNC in differentiating patients with DM I with elevated ACR (A1 vs A2/A3) with AUC-ROC, respectively: 0.836 and 0.873.

As a result of the research and analysis in the dissertation, 13 important **conclusions** of great practical value have been made. Some of them are related to the established pNGAL reference limits in the Bulgarian adult population, where age differences correlate with age changes in eGFR, as well as statistically significant gender differences in UNC values. Gender differences also exist in patients with diabetes mellitus without DKD in terms of UNC values. Women have higher scores for UNC, which is in parallel with lower urine creatinine.

The second part of the findings refers to patients with diabetes mellitus I and II, in whom the concentration of pNGAL correlates with a decrease in eGFR, while changes in uNGAL and UNC values - with an increase in ACR. Of the three evaluated indicators - pNGAL, uNGAL and UNC, with the highest prognostic value for the diagnosis of DKD are the changes in the values of UNC.

Based on the conclusions of the dissertation, the doctoral student has formulated **7 contributions of theoretical and 7 contributions of practical-applied nature**. They are semantically and factually determined by the results obtained and their analysis, confirming the scientific and practical value of this work.

The presented **Abstract** is written on 80 pages. It contains 36 figures and 38 tables, is prepared according to generally accepted rules and reflects the results presented in the dissertation.

In connection with the dissertation, the doctoral student presented **2 publications** in scientific journals: "Nephrology, Dialysis and Transplantation" and "Current Nephrology", both published in 2021 as first author. There are also 2 participations in scientific forums.

The **minimum national requirements** for obtaining the Educational and Scientific Degree of "Doctor" have been met.

In conclusion:

I firmly believe that with this work, Dr. Sevim Shefket proves that she has the necessary qualities to obtain the educational and scientific degree "doctor". This gives me reason to give a **POSITIVE REVIEW** for the dissertation on "Predictive role of NGAL-neutrophilgelatinase-associated lipocalin as an early marker of renal impairment in patients with type I and type II diabetes" for the award of educational and scientific degree "Doctor" in the scientific specialty "Clinical Laboratory" of Dr. Sevim Shefket.

April 18, 2022

Prof. Dr. A. Ruseva: