# REVIEW

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According to Order No. R-109-160/ 14.03.2025 of the Rector of the Medical University of Varna and Protocol No. 1/ 25.03.2025, I have been selected to prepare a review of the dissertation work of Dr. Zhaneta Atanasova Yaneva.

Topic of the dissertation:

# "Glycemia in acute ischemic stroke – prognostic significance and association with metabolic and inflammatory markers"

for awarding of educational and scientific degree "PhD" in professional field 7.1. Medicine, doctoral program "Endocrinology"

with scientific supervisor: Assoc. Prof. Mila Bogdanova Boyadzhieva, MD, PhD

## 1. General presentation of the procedure

I was provided with all the necessary materials for preparing a review. I confirm that the procedure for disclosure, development and admission to defense was carried out in accordance with the requirements of the Academic Staff Development Act in Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Development of the Academic Staff of MU-Varna for obtaining the educational and scientific degree "PhD"

## 2. Brief biographical data of the applicant

Dr. Zhaneta Yaneva was born in 1988 in Varna. In 2007 she graduated from the First Language School in Varna, studying German and English. In 2013, she graduated in medicine at the Medical University of Varna with honors, and in 2016, she obtained a Master's degree in health management. Since February 2014 Dr. Yaneva has been part of the team of the Clinic of Endocrinology and Metabolic Diseases, University Hospital "St. Marina", Varna. In October 2017 she was appointed as an assistant at the Department of Endocrinology, Second Department of Internal Medicine, MU-Varna, and since July 2018 she has become a full-time doctoral student. In January 2022 Dr. Yaneva obtained a specialty in endocrinology and metabolic diseases. In 2023 she conducted an internship in rare endocrine diseases at the University Hospital in Munich, Germany. Her main research interests are in the field of diabetes mellitus and stress hyperglycemia.

## **3.** Relevance of the topic

Ischemic stroke is a leading cause of mortality and long-term disability worldwide, and glycemic disorders often accompany it. On the one hand, diabetes mellitus (DM) is a well-known risk factor for acute cerebrovascular accident, and on the other hand, acute stress reaction often provokes the manifestation of stress hyperglycemia (SH). It is associated with adverse consequences in various categories of acutely ill patients, especially in those unadapted to chronically high blood glucose (BG) values. It can potentially affect the course of the stroke, as well as the clinical and functional outcome of it. In this sense, the concept of SH is a current problem in everyday clinical practice, including its prognostic significance in individuals with acute ischemic stroke (AIS). This is the subject of scientific research of the dissertation of Dr. Zhaneta Yaneva, along with the search for an association between glycemia and metabolic and inflammatory markers in these patients. The identification of laboratory parameters associated with BG, as well as biomarkers with predictive value in AIS, will contribute to a more accurate assessment of the prognosis of these patients and would help determine the therapeutic behavior.

#### 4. Structure of the dissertation

The dissertation contains 143 standard pages, is illustrated with 38 tables, 28 figures and 4 schemes and is well-structured in the following main sections: Title page (1 page); Content (3 pages); Abbreviations (2 pages); Introduction (2 pages); Literature review (33 pages); Aim, tasks, materials and methods (6 pages); Results (57 pages); Discussion (19 pages); Conclusions, contributions and conclusion (4 pages); Publicity and scientific projects related to the dissertation (2 pages); References (13 pages). The bibliography contains 285 titles, of which 9 in Cyrillic and 276 in Latin. The style is extremely good, clear, with a correct discussion of the data and conclusions.

#### 5. Nature of the dissertation

The **literature review** is very well structured and based mainly on current literature sources from the last 5 to 10 years. The spectrum of glycemic disorders in AIS is reviewed, with a focus mainly on SH. Detailed information is included on the epidemiology, clinical significance, pathogenesis and damaging mechanisms of SH in general, and in AIS in particular. Glycated hemoglobin (HbA1c)-based glycemic variables are described, considered to be more reliable indicators for identifying SH, given their ability to account for the usual levels of BG (so-called background glycemia) in the individual patient. The behavior of various inflammatory biomarkers and some neuroactive steroids in AIS is reviewed. The marker progranulin and its role in the pathogenesis of ischemic stroke are presented, as well as the view on it in recent years as an adipokine involved in the regulation of glucose metabolism.

The **aim** of the dissertation is precisely and clearly formulated: "To look for a relationship between glycemia level and outcome in patients with AIS, as well as an association with metabolic and inflammatory markers".

To achieve this goal 5 specific tasks have been set:

- 1. To assess the level of glycemia in hospitalized patients with AIS with and without type 2 DM using indicators of absolute and relative glycemia and glycemic variability.
- 2. To conduct a comparative characteristic of some clinical, glycemic, metabolic and inflammatory parameters between groups with different glycemic status, different severity of AIS and outcome from hospitalization.

- 3. To investigate the association of AIS severity and hospitalization outcome with some clinical, glycemic, metabolic and inflammatory parameters.
- 4. To examine the relationship between glycemic parameters and some metabolic and inflammatory markers.
- 5. To assess whether HbA1c-based glycemic variables are better determinants of stress response compared to the absolute value of BG at hospitalization and to evaluate their prognostic value.

#### Materials and methods:

The study, the subject of the dissertation, was conducted in two stages. The first stage is a retrospective study of a cohort of 555 patients hospitalized for AIS for the period of May 2016 to April 2017, divided into groups with SH, with type 2 DM and with normoglycemia. The second stage is a cross-sectional study, covering 114 patients for the period of June 2021 to May 2023, selected in the first 24 hours of hospitalization for AIS. They were divided into four groups, and one with newly diagnosed DM was additionally formed based on the HbA1c value. In both stages, the inclusion and exclusion criteria were well selected and adequate to the purpose and design of the study. Clinical and laboratory research methods are well described and optimally selected. Appropriate statistical methods were used to process the data in relation to the studied population and the studied variables - descriptive statistics, hypothesis testing methods, correlation analysis, multiple regression analysis, ROC analysis.

#### **Results and discussion:**

The **results** of the study are presented in detail and are organized precisely and clearly following the tasks set. They are well illustrated in tables and graphical versions and are accompanied by adequate and objective commentary.

The **discussion** is well structured, presented in 6 sections, and the results obtained are analyzed very logically and thoroughly in the context of the data available in the literature on the problem. The self-criticism of the doctoral student, pointing out some limitations of the dissertation work, is striking.

The **first section** examines the prevalence of SH among patients with AIS. A similar distribution of SH in AIS was found, which is in accordance to the published data, namely 20.9% in stage I and 13.16% in stage II of the study. The significant proportion of patients with abnormal glycemic status (over <sup>3</sup>/<sub>4</sub> of the patients) is noteworthy, indicating how widespread glycemic disorders are in patients during the course of AIS.

The **second section** compares HbA1c-based glycemic variables and BG on admission for assessment of SH, with the parameters analyzed according to the severity of AIS at admission, and serum cortisol levels were used to more accurately determine the stress response. A significantly greater severity of AIS was observed in the SH group compared to those with normoglycemia and newly diagnosed type 2 DM. In contrast to BG on admission, glycemic variables demonstrated the highest values in the SH group, in which both serum cortisol and stroke severity were significantly higher compared to the normoglycemia and DM groups. Additionally, there was a significant difference in cortisol levels and HbA1c-based glycemic variables between the different degrees of AIS severity, as well as a tendency for their progressive increase with increasing stroke severity. The same trend was observed in the admission BG, but without statistical significance. Both cortisol and glycemic variables, but not admission BG, were positively and independently correlated with stroke severity. These results suggest that HbA1c-based glycemic variables are better determinants of stress response

than admission BG, and serum cortisol levels can be used to adequately assess the severity of AIS.

The **third section** is dedicated to the factors associated with the severity of AIS. In addition to the mentioned dependencies with serum cortisol and HbA1c-based glycemic variables, an association with inflammatory markers – leukocytes, neutrophils, Neutro/Lympho ratio and CRP was also observed. Of particular interest is the relationship of the marker progranulin with the severity of AIS. A significant difference in its levels was observed between groups with different severity of AIS, a positive correlation with the severity of the stroke, as well as participation in the final reduced regression model for predicting the severity of AIS. These results indicate that progranulin has the potential to find application in the assessment of the severity of AIS.

The **fourth section** describes factors associated with glycemic status in patients with AIS. It was found that patients with SH demonstrate elevated levels of inflammatory and stress markers, including HbA1c-based glycemic variables. These observations were not typical for patients with DM, in which changes in lipid parameters were reported, as well as elevated levels of all glycemic parameters, except glycemic variables.

The **fifth section** focuses on the prognostic significance of glycemia in AIS. Indirect evidence of increased mortality risk was observed in patients with SH. SH was found to be associated with poor functional outcome of AIS in patients without DM, i.e. those who are not accustomed to chronically elevated glucose levels. On the other hand, persistent fasting hyperglycemia was associated with both poor functional outcome of AIS and mortality, regardless of the diabetic status of the patients.

The **sixth section** presents other factors with prognostic significance in AIS. As such, the severity of AIS on admission, cortisol, as well as the Neutro/Lymph ratio, neutrophil count and CRP were distinguished. The severity of AIS stands out as a leading factor that influences the outcome of stroke. It was positively associated not only with fatal outcome, but also with poor functional outcome, as well as with the severity of AIS at discharge. Of interest is the observation that high cortisol levels were independently associated, including the severity of AIS, with poor functional outcome and with fatal outcome. This indicates that cortisol not only reflects the severity of AIS, but is probably also directly related to adverse consequences. High values of the Neutro/Lymph ratio also demonstrated an independent association with poor functional outcome and fatal outcome from AIS.

In accordance with the obtained results, nine **conclusions** have been drawn, which are clearly formulated and follow the tasks set in the dissertation.

The **contributions** of the dissertation are divided into two categories – of original and confirmatory character.

Of original character:

- 1. For the first time in the country, the prevalence of SH in AIS has been studied and HbA1c-based glycemic variables have been used for its assessment.
- 2. This is the first time that HbA1c-based glycemic variables have been compared with cortisol levels in assessing the stress response.
- 3. For the first time, the marker progranulin was analyzed simultaneously in patients with AIS and different glycemic status.

Of **confirmatory** character:

- 1. HbA1c-based glycemic variables have been found to provide a better estimate of SH compared to the absolute value of admission BG, which is consistent with most observations worldwide.
- 2. It has been confirmed that SH occurs in patients with more severe AIS, and a higher mortality rate has been observed in the SH group, but no direct relationship of SH with mortality has been established.
- 3. The few published observations that progranulin can be used to assess the severity of AIS have been confirmed, but no association with prognosis has been observed, except for the severity of AIS at discharge.

## 6. Abstract

The abstract is 76 pages long and is completely sufficient in content to present the main results achieved in the dissertation.

## 7. Publications related to the dissertation

A list of 4 full-text publications related to the dissertation (2 reviews and 2 with results) is attached, which meets the requirements for obtaining the educational and scientific degree "PhD". Parts of the work have been presented at three national scientific forums and one international congress.

## 8. Critical remarks and recommendations

I do not have critical remarks or recommendations to the conducted research and the materials provided to me.

## Conclusion

The dissertation work of Dr. Zhaneta Yaneva reflects her in-depth work – precisely planned and conducted, correctly and critically analyzed – on a current and socially significant problem. Given the volume of work performed, the precise statistical processing, the numerous results, their in-depth comparison with the data published to date and the indicated contributions, I believe that the scientific work fully meets the requirements of the Academic Staff Development Act in Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Development of the Academic Staff of MU-Varna for obtaining the educational and scientific degree "PhD".

I give my positive assessment of the dissertation work of Dr. Zhaneta Atanasova Yaneva and appeal to the esteemed members of the Scientific Jury to do the same.

17.04.2025г. Varna Prof. Kiril Hristov Hristozov, MD, PhD

Заличено на основание чл. 5, §1, б. "В" от Регламент (ЕС) 2016/679