



**Fund “Nauka” Project № 24007 Resume – Special Competition-based Session
2024:**

“Biomarkers for assessment of inflammatory response, oxidative stress and dyslipidemia in patients with acute ischemic stroke - effect of recovery therapy with a nutritional supplement containing citicoline and plant extracts”

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The aim of the project is to study the role of molecular biomarkers for oxidative stress, dyslipidemia and inflammation in patients with acute ischemic stroke and the effect of restorative therapy with the food supplement Cytodeox, containing citicoline and extracts of chokeberry, green tea and rosehip.

The object of the study will be previously collected and reliably stored (at -80°C) blood serum samples and isolated RNA from lymphocytes of patients with acute ischemic stroke under an active scientific project (No. 21015), financed by the "Science" Fund, for which approval received from Research Ethics Committee (Protocol No 119/21.07.2022). The informed consent signed by each participant explicitly states that the samples will be preserved under appropriate conditions for future research. The collected and stored samples of 100 patients with acute ischemic stroke before and after supplementation with Cytodeox will be analyzed.

An innovative and original combination of **methods** will be applied:

1. Clinical activities – assessment of the disease course and severity of the disorders, as well as subgrouping by vascular territory of the stroke, concomitant diseases, medication intake;
2. Immunochemical method – determination of 8-iPGF2-alpha;
3. Chromatography – determination of vitamin D status;
4. Spectrophotometry – assessment of indicators of oxidative stress;
5. Molecular-biological methods – expression of specific cytokines and enzymes.

Expected results:

1. New data will be obtained on insufficiently studied markers of oxidative stress, assessing the process of lipid peroxidation in membranes;
2. Data will be obtained on vitamin D status, pro-inflammatory cytokines and enzymes, which are insufficiently studied in relation to ischemic stroke worldwide and in Bulgaria;
3. Data will be obtained on the expression levels of pro-inflammatory cytokines and enzymes related to the inflammatory response and oxidative stress in patients with acute ischemic stroke;

4. The changes of the studied biomarkers after recovery therapy with a nutritional supplement containing citicoline and plant extracts will be analyzed and evaluated;
5. Correlations between the serum levels of the investigated biomarkers and those of classical and specific biochemical markers will be investigated, which will allow the assessment of their reliability in relation to the course of the disease and the effect of citicoline supplementation.

This project is a thematic and methodological continuation of a previous project, also funded by the Science Fund at the Medical University of Varna. The present study builds on earlier findings on the effects of the Cytodeox food supplement in patients with ischaemic stroke and in healthy volunteers following a six-month intervention. Several biomarkers investigated in the previous project, including sirtuin 1 (*SIRT1*), underwent additional stratified analysis by stroke aetiology, comorbidities and concomitant medication. At the same time, the biomarker panel was expanded to include 8-*iso*-prostaglandin F₂α (8-*iso*-PGF₂α) as a marker of lipid peroxidation, brain-derived neurotrophic factor (*BDNF*) as an indicator of neurotrophic and neuroplastic mechanisms, Kelch-like ECH-associated protein 1 (*Keap1*) as a component of the redox-regulatory system, and methylmalonic acid as an additional metabolic marker.

The integrated analysis covered lipid metabolism, lipid peroxidation, redox regulation, cellular adaptation, mitochondria-related metabolism and neuroplasticity. Following the intervention, patients showed favorable changes in selected lipid-profile parameters and a reduction in the total cholesterol/HDL-cholesterol ratio. Serum methylmalonic acid decreased significantly in patients, whereas no substantial change was observed in healthy controls. The results for arachidonic acid and 8-*iso*-PGF₂α indicated that lipid-peroxidation markers were strongly influenced by baseline metabolic status. Both markers decreased in healthy volunteers, whereas oxidative stress remained elevated in patients with pronounced comorbidity, particularly diabetes mellitus.

Molecular analyses showed increased expression of *BDNF* and *Keap1* in both healthy volunteers and patients, whereas *SIRT1* expression increased predominantly in the patient group. Taken together, these changes indicate concurrent modulation of neurotrophic mechanisms, redox regulation and adaptive cellular stress responses. Stratified analysis identified differences by stroke subtype and comorbidity, highlighting the importance of clinical heterogeneity when evaluating therapeutic response.

The scientific contribution of the project lies in extending the findings of the preceding study, deepening the analysis of previously investigated biomarkers and broadening the panel with new molecular and metabolic indicators. The integration of clinical, biochemical and gene expression data provides a basis for more precise monitoring of metabolic and neurorecovery-related adaptation, and for future controlled studies aimed at stratified and personalized supportive approaches in ischaemic stroke.