Statement

by Prof. Borislav Georgiev Georgiev, PhD Head of the Cardiology Clinics, MHAT "National Heart Hospital" Sofia, Member of the Scientific Jury for awarding the educational and scientific degree "Philosophy doctor" on the basis of order № 68/28.2.20202 by the Executive director of the National heart hospital

Subject: dissertation of Iliana Hristova Petrova-Stoyanova, MD, PhD student in independent training in the Doctoral Program "Cardiology" on "Study of renal function in patients undergoing invasive angiographic examination with a new biomarker – neutrophil gelatinase-associated lipocalin (NGAL)" Scientific advisers - Prof. Dr. Nina Gotcheva and Prof. Dr. Boris Bogov

For the competition Iliana Hristova Petrova-Stoyanova, MD presented all necessary documents - dissertation, abstract and additional documents in accordance with the requirements of the Academic Staff Development Act in the Republic of Bulgaria and the Regulations of the Ministry of education and MHAT "National Heart Hospital" for its implementation. I do not find any gaps in the submitted documentation.

I declare that I have no conflict of interest with the candidate.

All presented materials are precisely arranged and described.

No evidence of plagiary.

Significance of the topic

The topic of the dissertation is contemporary and relevant. Today, contrast-induced nephropathy (CIN) and new biomarkers for renal impairment in contrast invasive procedures have been the subject of many studies and publications. The advantages and disadvantages of serum creatinine as a marker of renal function have long been known in the literature, and despite its widespread use in clinical practice, ways to overcome its weaknesses are increasingly being sought. The discovery, study and collection of data on new biomarkers in recent years has led to a change in the conceptual framework for acute renal impairment. The adoption of CIN as a form of acute tubular injury has led research in last years to prove the application of new structural biomarkers. However, not all of the biomarkers studied in nephrology prove their diagnostic ability in CIN. Many of them could not differentiate the occurrence of the complication in subjects undergoing contrast testing compared to the group "without CIN" or do not demonstrate serious advantages over serum creatinine. Neutrophil gelatinase-associated lipocalin (NGAL) manages to overcome these shortcomings with sufficient evidence that its molecules are subject to rapid overregulation after the application of a contrast agent and it is possible to register elevated levels within the first 4-6 hours. The applicability of NGAL as a measure of renal status, reflecting the interaction between comorbidities and contrast agent administration, is an area of scientific knowledge that has been poorly studied and raises many questions. However, there is evidence that elevated NGAL levels may correlate with an unfavorable prognosis in the future and increased mortality and/or morbidity, as well as with accelerated progression to CKD.

At the start of the research work, the topic was almost unknown to the world scientific community.

Structure of the dissertation:

The scientific work of Iliana Petrova, MD is formed on 282 pages according to the requirements and contains a literature review, goals, tasks, materials and methods, results and discussion (in one section), conclusions, contributions, bibliography. Before to the bibliography, Iliana Petrova applied 16 pages of appendices. The dissertation material is illustrated with 82 tables and 95 figures, and 28 additional tables and 13 figures are included in the appendices.

The literature review presented on 89 pages shows a very good awareness of the author regarding contrast-induced nephropathy (CIN), contrast-induced acute renal impairment and post-contrast acute renal impairment, markers for assessment of renal function in CIN, risk assessment of CIN, pathophysiology, clinical course, methods of prevention and current problems in the diagnosis of acute renal impairment and the need for new biomarkers. Neutrophil-gelatinase-associated lipocalin (NGAL) has been extensively analyzed as a new biomarker of renal impairment, the role of NGAL in contrast-induced nephropathy, and in cardiovascular disease. The bibliography contains 407 cited titles, 4 of which are in Cyrillic and the rest in Latin.

Iliana Petrova, MD **aims** to study renal function in a group of patients who underwent routine invasive angiography through the application of the new biomarker NGAL and its comparison with serum creatinine and GFR.

To achieve this goal the author sets itself the following tasks:

- 1. To monitor renal function in patients undergoing elective invasive angiography by determining serum creatinine and plasma NGAL levels
- 2. To investigate the diagnostic ability of the structural biomarker NGAL to identify individual clinical conditions or to help distinguish them from a general group defined according to the criteria of serum creatinine
- 3. To establish the role of NGAL in studying the influence and impact of risk factors, and/or clinical profile of patients on the level of kidney function
- 4. To investigate the correlations between standardized scales for risk assessment in patients undergoing invasive angiographic examination and the applicability of the new structural biomarker
- 5. To determine the theoretical justification and clinical applicability in the classification of the sample according to the levels of the new biomarker NGAL
- 6. To introduce a combined approach for analyzing the results against the simultaneous reporting of baseline levels and registered dynamic changes in biomarkers
- 7. To determine the role of NGAL, its sensitivity and specificity as an early biomarker for the diagnosis of contrast-induced nephropathy, both in patients with preserved renal function and with baseline chronic kidney disease.
- 8. To develop its own risk scale, which will incorporate the data from the measurement of NGAL.

Methodical approach: The data of 145 patients were analyzed, of which a total of 135 patients were included in the final analysis. They are divided according to baseline renal function at admission in hospital. Based on the dynamics of biomarkers, patients from the two main groups are further divided into 6 groups, one of which is control.

In addition to the control group, patients were divided into those with CIN without CKD, «NGAL +», «baseline normal NGAL and small variations of biomarkers», CIN with CKD, CKD

without CIN. The application of the generally accepted classification and KDIGO criteria for determining the degree of chronic kidney disease allows a secondary distribution of the main group of CKD without CIN as follows: CKD stage 3a and CKD stage 3b. The whole group "CKD without CIN" compared to the registered changes only of plasma NGAL allows to form two additional subgroups: CKD with subclinical AKI and CKD without subclinical AKI. Based on baseline plasma NGAL levels, the classification is supplemented by the following groups: baseline normal NGAL, baseline high NGAL, CKD with baseline normal NGAL, CKD with baseline NGAL.

Statistical analysis includes various analyses that are consistent with the hypothesis and objectives.

Results: The obtained results of Iliana Petrova are diligently presented on 126 pages of the dissertation. The results are well illustrated.

In separate sections the main characteristics of the patient groups, dynamic changes of serum creatinine, GFR and plasma NGAL in generally; changes in creatinine, GFR and NGAL in each group separately and intergroup comparisons; ROC analysis; relative changes in serum creatinine, GFR and NGAL are traced by groups; correlation analysis, and in the last section the discussion of the results was carried out.

Conclusions: Dr. Iliana Petrova offers 11 conclusions. They stem from the study. In the dissertation the conclusions are presented on 12 pages in different directions according to the directions of the scientific search. They are presented briefly and clearly in the abstract.

Contributions: The contributions are 11, divided into two groups - theoretical and methodological (5) and scientifically applied (6).

Publications: In connection with the dissertation the author presents 8 full-text publications (7 as the first author), 2 chapters of books and 7 participations in scientific forums at home and abroad (6 of them are the first author).

The abstract contains 87 pages and reflects what was written in the dissertation. It is precise as required.

Recommendations to the doctoral student:

> To continue the research work on the topic and to publish some of the results of the research in foreign scientific periodicals, if possible with an impact factor.

According to the minimum requirements of NACID for awarding educational and scientific degree "Philosophy doctor" Iliana Petrova meets the requirements as follows: with the required 80 points, she scored 135.5 points.

Conclusion: I evaluate the work of Iliana Hristova Petrova-Stoyanova, MD on "*Study of renal function in patients undergoing invasive angiographic examination with a new biomarker – neutrophil gelatinase-associated lipocalin (NGAL)*" as interesting in scientific terms and important for clinical practice. I believe that this dissertation meets the requirements for awarding the educational and scientific degree "Philosophy doctor" laid down in the Law for the development of the academic staff in the Republic of Bulgaria and the Regulations for the development of the academic staff of MHAT "National Heart Hospital".

Based on the above merits of the dissertation of Iliana Petrova, I recommend the members of the esteemed Scientific Jury to vote positively and to award Iliana Hristova Petrova-Stoyanova the educational and scientific degree "Philosophy doctor" in the scientific specialty "Cardiology", professional field 7.1 Medicine, field of higher education 7 Health and sports.

June 8, 2020

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(prof. Borislav Georgiev, MD. Ph.D.)