REVIEW

by Assoc. Prof. Atanas Kundurdjiev, MD

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By order №68 / 28.02.2020 of the Executive Director of MHAT "NHH" EAD I have been appointed a member of the scientific jury for conducting a procedure for defense of the dissertation of Dr. Iliyana Hristova Petrova - Stoyanova in scientific specialty "cardiology" (Minutes of the Scientific Council of the NCB №11 of 22.02.12020).

Dissertation topic: Study of renal function in patients undergoing invasive angiographic examination with a new biomarker – Neutrophil Gelatinase Associated Lipocaline (NGAL)

Scientific advisers: Prof. Dr. Nina Gocheva, MD

Prof. Dr. Boris Bogov, MD

The review was prepared in accordance with the law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for its application in MU-Sofia.

Biographical data:

Dr. Petrova graduated in medicine in 2005 with honors. Since 2006 she has been an assistant in the cardiology clinic of the National Heart Hospital. In 2013 she acquired a degree in cardiology and a degree in invasive cardiology. Since 2016 she has been a doctoral student in self-study. She has over 40 publications in national and international publications and 6 chapters in monographs. She has been a lecturer in many national and international publications. She is a member of the Bulgarian Society of Cardiology and the Society of Interventional Cardiology.

The dissertation was discussed and approved for public defense at meetings of the Primary Scientific Unit. The presented dissertation is 282 standard pages and is structured in the classical way. It includes 95 figures and 82 tables, and an additional 28 tables and 13 figures are included in the annexes. The figures and tables are illustrated very well graphically and this makes the work extremely well illustrated. The dissertation is written in literary Bulgarian. The literature review covers 82 pages, contains 12 chapters and ends with an analysis of - 3 pages; Purpose and tasks - 2 pages; Material and methods - 15 pages; Results, analysis of results and discussion - 126 pages; Summary and conclusions - 12 pages; Contributions - 2 pages; Appendix - 17 pages and Bibliography - 18 pages. 407 literary sources are cited, of which 4 in Cyrillic and 403 in Latin.

Assessment of the significance of the topic:

With the increasing development of invasive hightechnology diagnostic and therapeutic procedures using contrast agents in practical medicine, along with the improvement of clinical results and quality of life, the possibility of kidney damage increases and this requires increased attention and preliminary assessment of the risk of complications. The complex pathophysiological mechanisms by which contrast-induced nephropathy occurs make it difficult to predict and safe ways to prevent it have not yet been developed. Along with the search for new therapeutic strategies and preventive measures, new renal biomarkers are being introduced for early diagnosis. Serum creatinine is a widely studied biomarker, but is unsuitable for early diagnosis due to its late positivity in developing of kidney damage. The introduction of new structural biomarkers can significantly improve the diagnostic process and distinguish some conditions such as "subclinical AKI" and "hemodynamic AKI", as well as to distinguish the different phases of acute kidney injury. These aspects of the problem are still underdeveloped and this makes the topic extremely relevant.

Evaluation of the literature review:

The literature review is more extensive than necessary and there are many details that could be saved. It sets out in detail the definition of the basic concepts on the subject and emphasizes that the terms 'post-contrast acute kidney injury' and 'contrast-induced nephropathy' are not interchangeable, as the latter is a subset of the new, more general condition. The type and manner of application of the contrast agent are commented, as well as the specialiy of the standard biomarkers for assessment of renal function in CIN. The evidence for risk factors and the risk assessment scales for CIN are logically cited. Particular attention is paid to the Mehran`s score and scale developed by Brown. The debates on the factors and mechanisms for the occurrence and development of renal damage and the causal relationship between them are followed. Attention is also paid to the methods of prevention, describing in detail the mechanisms of action, the collected evidence and a critical analysis is made of each of them. Special attention is focused to modern innovative approaches in invasive cardiology and especially to the use of IVUS images in order to reduce the amount of contrast agent. The review points out the short comings of the creatinine-based definition of AKI and logically considers the strengths and weaknesses of the new biomarkers. In a separate chapter, special attention is paid to Neutrophil Gelatinase Associated Lipocaline (NGAL) as a new biomarker of renal damage, which is also the basis of the dissertation. The long literature review ends with an analysis on three pages with very useful summaries and a logical transition to the purpose and tasks of the dissertation, by marking the still unsolved and debatable problems. I believe that the literature review provides very rich information for anyone who is interested in the topic.

Evaluation of the goal, tasks and methodology:

The goal is correctly formulated: To study renal function in a group of patients who underwent routine invasive angiographic examination using a new biomarker - Neutrophil Gelatinase Associated Lipocaline (NGAL) and its comparison to serum creatinine and GFR.

To achieve this goal, 8 tasks are set: In addition to monitoring renal function through these biomarkers, determining their independent diagnostic abilities, their role in determining the clinical profile of the patient, sensitivity and specificity, the last task deserves attention, namely developing their own risk scale. This is an extremely ambitious task and its correct solution makes the dissertation especially valuable.

Object of the study: The study was performed in patients with known or suspected coronary artery disease and scheduled for invasive angiography and/or percutaneous coronary intervention (PCI). The possibilities of the new structural biomarker NGAL to detect the occurrence of acute kidney injury are compared to the diagnostic capabilities of the functional biomarker serum creatinine in a cohort of patients with different baseline levels of kidney function. The study is prospective and with an appropriate design in order to achieve the goal. 135 patients were included and were carefully selected according to baseline kidney function in patients with preserved renal function (GF $\geq$ 60 ml / min / 1.73 m^2) (n = 87) - Group A and patients with moderate renal impairment (moderate form of CKD) (GF 30-59 ml/min/1.73 m^2) (n = 48) - Group B. Based on the registered dynamics of biomarkers, patients from the two main groups are divided into the following groups: 1). Patient Control group; 2) group named "CIN without CKD"; 3). "NGAL +" group; 4). group "baseline normal NGAL and small variations of biomarkers"; 5). group "CIN with CKD"; and 6). Group CKD without CIN. The application of the generally accepted classification and the criteria of KDIGO for determining the degree of chronic kidney disease allows secondary distribution of the main group "CKD without CIN" as follows: Group "CKD 3a stage" and Group "CKD stage 3b". The detailed examination of the whole group "CKD without CIN" compared to the registered changes only of plasma NGAL allows to form two additional subgroups: 1). Group "CKD with subclinical AKI" and 2). Group "CKD without subclinical AKI". Based on baseline plasma NGAL levels, the classification is supplemented by the following groups: 1). General group of "baseline normal NGAL" with preserved renal function and 2). General group of "baseline high NGAL" in preserved kidney. They include respectively 1). Group with "baseline ↑ NGAL with a small variation of BM" and 2). Group with "baseline ↑ NGAL with dynamics of BM". Separately are considered 1). Group "CKD with baseline normal NGAL" (CKD with baseline nNGAL) and Group "CKD with baseline high NGAL".

This seemingly complex distribution of groups and subgroups is in fact a very original and innovative approach that overcomes some of the weaknesses of other studies, namely dealing with inhomogeneous groups and making it difficult to classify some intermediate cases.

Clinical and statistical research methods are comprehensively described.

Evaluation of the results and conclusions:

Appropriately designed graphs and figures show the main characteristics of the groups and the intragroup distribution in relation to the initial level of creatinine, NGAL and the dynamics in NGAL. The age-sex distribution is also presented in detail. On the basis of gender there is no significant difference between the groups, but on the basis of age the greatest age is in the group "CKD 3a stage". Regarding the risk factors - arterial hypertension, diabetes mellitus, dyslipidemia and body mass index, there is no significant difference between the groups. Regarding coronary artery disease, there is no significant difference between the

groups, but with a history of coronary artery bypass surgery. There is a similar significant difference with regard to heart failure. No significant difference in echocardiographic ejection fraction was observed. The intake of basic classes of drugs has also been studied and no significant difference has been established. An analysis of the features of the invasive study - type and degree of vascular changes, the amount of contrast agent and the ratio of the amount and glomerular filtration rate. The dynamic changes in serum creatinine, glomerular filtration rate and plasma NGAL, both in each group and between groups, are appropriately presented. A ROC analysis of the diagnostic capabilities of plasma NGAL was performed in all patients with CIN. All studied indicators in all time intervals of all groups are presented compared to the control. A correlation was also sought between the studied indicators in all groups. The results of the groups "baseline normal NGAL with small variations of BM" and "baseline high NGAL with small variations of BM" from all patients who do not develop CIN shed light on a kind of "gray area". The groups of patients with CKD were treated in a similar way. This allows to separate the groups "CKD without subclinical AKI" and "CKD with subclinical AKI".

Regarding the relationship between the level of NGAL with coronary artery disease and heart failure, it has been shown that it is not related to coronary artery disease, but is related to heart failure. All groups were assigned the risk scale of Serious Renal Dysfunction (SRD) developed by Brown and the risk of CIN on the Mehran`s score. Thus, it is clear that the use of NGAL for risk assessment leads to the calculation of a higher risk as a whole and to a detailed delineation of more groups of patients. This shows that the tested hypothesis to implement plasma NGAL as part of a risk calculator is extremely innovative. There is no such description in the literature and therefore it deserves its attention. Of particular interest is the differentiation of the group of "baseline ↑ NGAL and dynamics of BM", where the established laboratory finding differs radically from all described. A significant contribution to the enrichment of knowledge has the formed group "NGAL +", which is considered as very similar with the group CIN. This group shows that these patients have structural changes in the kidney without having functional ones. Applied this approach in the group with CKD shows that patients with subclinical AKI can be found in it as well. The consideration of patients with such a laboratory constellation in relation to the general sample gives a clear example of the size of the persons who would be unrecognised when applying the classical approach to diagnosing AKI. In general, there are few studies in the literature examining patients with CKD and they do not describe the state of subclinical AKI. This study marks the beginning of an important problem that will need further research.

Regarding the results, it can be said that the rich material has been processed in the best possible way and dependencies have been purposefully sought and established, enabling the achievement of the set goal.

An important conclusion is that the simultaneous comparison between these two approaches - static values of baseline and subsequent dynamic changes, leads to the creation of a number of possible combinations between the two biomarkers, which respectively reflect different clinical conditions. The detailed analysis that is done on these conditions is very innovative and brings clarity to a still poorly studied area.

We can say that in patients without CIN, using NGAL, 7 more groups of patients are identified. In one part there is a subclinical AKI, in others transient prerenal damage or an silent

process of renal involvement. The identification of these groups of patients undergoing interventional procedures makes a significant contribution to day-to-day clinical practice.

The role of ***plasma NGAL*** in patients undergoing contrast testing is summarized and originally presented, namely:

1. NGAL outlines a "gray area" of silent structural damage of kidney and possibly increased risk;

2. Recognised subclinically AKI and hemodynamically AKI;

3. Completed the diagnostic process of functional kidney damage with early detection of structural demage.

Contributions: I fully accept the above contributions, namely:

Theoretical and methodological contributions:

1. A detailed approach is presented, according to which the assessment of kidney function in patients undergoing contrast angiography is performed simultaneously on the basis of the registered baseline levels and the reported dynamic changes in two types of biomarkers.

2. For the first time, the incorporation of functional and structural biomarker data into the new conceptual framework for acute renal injury proposed by the Working Group on Acute Dialysis Quality Initiative (ADQI) is described in the field of invasive cardiology.

3. The scientific relevance of the classification of a standard sample to the new NGAL biomarker is determined and an own scale is proposed to assess the degree of renal impairment.

4. The possibilities of a new approach for integration of plasma NGAL in the risk stratification of patients are demonstrated, by applying it in a risk scale for assessment of serious renal dysfunction.

5. A comparative model on the independent role of each of the two types of biomarkers (functional and structural) in the assessment of renal function and the level of risk among patients undergoing contrast angiographic examination is presented.

Scientific and applied contributions:

1. For the first time in Bulgaria the use of NGAL in contrast angiographic examinations is demonstrated.

2. The role of NGAL as an early biomarker for the diagnosis of CIN has been demonstrated, both in patients with preserved renal function and in the conditions of chronic kidney disease.

3. The presence of groups with subclinical acute kidney injury, regardless of the degree of baseline renal function, has been demonstrated in patients undergoing contrast angiography.

4. Different reference limits of plasma NGAL are established according to the stage of chronic kidney disease.

5. The influence of the accompanying diseases and risk factors on the reported levels of NGAL is demonstrated.

6. The classical scale for risk assessment of development of contrast-induced nephropathy is supplemented by comparing it with the structural biomarker.

Abstract

The presented abstract has a volume of 87 pages and reflects the content of the dissertation.

Publications: On the topic Dr. Petrova has 8 publications in our foreign journals, 2 book chapters and 7 participations in Bulgarian and international forums.

Conclusion: In her dissertation Dr. Petrova has studied and summarized a rich clinical material in an interclinical field, which is important not only for cardiologists and nephrologists, but also for all specialists who are involved in contrast imaging. I am convinced that the dissertation provides new additional opportunities for more precise selection of patients undergoing contrast studies in order to prevent possible complications.

This gives me reason to convincingly vote **"yes"** for awarding the scientific and educational degree **"Philosophy doctor" to Dr. Iliyana Hristova Petrova - Stoyanova.**

16.06.2020 **Assoc. Prof. Atanas Kundurdjiev, MD** .....