”VICTOR BABEŞ” UNIVERSITY OF
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MEDICINE

A TRANSLATIONAL APPROACH TO PERSONALIZED MEDICINE IN NEPHROLOGY – FROM A BIOMARKER, MOLECULAR, AND BIOINFORMATICS PERSPECTIVE TO CLINICAL APPLICABILITY.

ABSTRACT

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ABSTRACT

My name is Florica Nicoleta Gădălean and I was born in 1980, in Salonta, Bihor County. I decided to study at the Faculty of General Medicine of the University of Medicine and Pharmacy “Victor Babes” Timișoara, from which I graduated in 2004.

The thesis “A translational approach to personalized medicine in nephrology - from a biomarker, molecular, and bioinformatics perspective to clinical applicability”, is elaborated according to the recommendations of the Ministry of Education and Research (Order of the Minister of Education and Research no. 3121/27.01.2015), as well as the Guide for the Preparation and Editing of the Habilitation Thesis of the University of Medicine and Pharmacy "Victor Babeș" Timișoara, developed in accordance with the recommendations of the National Council for Attestation of University Degrees, Diplomas and Certificates (CNATDCU) (H.S. Nr.201/18470/18.12.2020) .

The habilitation thesis presents the results of the scientific, academic, and medical activity since 2011, when I finished the PhD programme, becoming PhD (Ord. MS nr.4387/2011).

The work is organized in four parts: the 1st part is dedicated to scientific activity; the 2nd part is dedicated to academic activity and academic achievements; the 3rd part is dedicated to professional activity; the 4th part includes the development plan of the academic career.

After completing my PhD, I continued the research in the field of solitary kidney (SK). Furthermore, I studied the outcome and particular risk-factor for mortality in a large cohort of Romanian hemodialysed patients. Another field of research was the acute kidney injury among patients from non-nephrology departments and its associated-risk factors, namely the impact of acute kidney injury on short-term mortality burden. The latest scientific research direction, with ongoing studies, is represented by the investigation of the neuropsychiatric dysfunction associated to chronic kidney disease, with a focus on subclinical cerebrovascular changes, such as the cognitive impairment in patients with chronic kidney disease. Currently these topics are considered the new and innovative avenues of research at international level.

In parallel to the research activity, I started to work in different projects and I participated as co-investigator in clinical trials. Also, I participated as a member in three internal research grants funded by the "Victor Babes" University of Medicine and Pharmacy, Timisoara. Currently, I am a full-member of the Centre for Molecular Research in Nephrology and Vascular Pathology, which represents one of the most important advanced research centres, funded by the "Victor Babes" University of Medicine and Pharmacy, Timisoara.

To date, I have published in ISI indexed journals, in full and in abstracts: 85 works, 48 ISI in full articles (21 main author, 27 co-author), 37 works published in ISI abstracts (9 first author). The research results were published in journals with a high impact on the scientific community, accumulating a Hirsch index of 12 (438 citations) and the cumulative impact factor of the works published as the main author FCIAP= 57.671 (21 articles).

The research activity was carried out in interdisciplinary teams, in which we collaborated with specialists in biochemistry, neurology, gastroenterology, diabetology, and cardiology.

The purpose of my research activity was to improve the diagnosis and subsequently the therapies in several fields, such as the single kidney, end-stage chronic kidney disease treated by hemodialysis, acute kidney injury, and cerebrovascular pathology associated with chronic kidney disease, with the description of complications, risk factors, and impact on mortality rate.

In the first stage of my postdoctoral research activity, I continued the research in the field of solitary kidney by highlighting the value of urinary biomarkers as diagnostic indicators of early kidney damage in the course of congenital and acquired solitary kidney. The results of these researches were published in 2013, in two articles, “Is the urinary biomarkers assessment a non-invasive approach to tubular lesions of the solitary kidney?” (Renal Fail, IF: 0.775), and “Biomarkers in assessing tubular lesions of the solitary kidney. The solitary kidney in special conditions” (Rom J Intern Med, BDI, PubMed).

In the next stage, I aimed to assess tubular dysfunction due to drug-induced nephrotoxicity through urinary biomarkers in solitary kidney patients with urinary tract infections treated by aminoglycosides or fluoroquinolones. We demonstrated that proximal tubular urinary biomarkers can early detect gentamicin- and ciprofloxacin-induced kidney dysfunction in patients with solitary kidney, even in its subclinical

stage, before eGFR decline. The results were published in 2 articles, in 2014, and 2016, in ISI journals, being the first literature reports concerning this topic: „Urinary biomarkers in assessing the nephrotoxic potential of gentamicin in solitary kidney patients after 7 days of therapy" (Renal Fail, IF: 0.944), and „Is ciprofloxacin safe in patients with solitary kidney and upper urinary tract infection?" (Biomed Pharmacother, IF: 2.759)

Further on, I deepened the research on the single kidney through studies that assessed the impact of comorbidities on kidney function to the single kidney status. The importance of an added comorbidity, the so-called "second hit", is revealed by its potential negative consequences on kidney function, being a subject with profound ethical valences when deciding to remove one kidney from a living donor. Therefore, I aimed to investigate the differences between kidney function among single kidney patients having a similar duration of solitary kidney existence, with diabetes mellitus versus with no diabetes mellitus. Our results highlight that there is no difference in the eGFR between solitary kidney patients with and with no diabetes mellitus. The results were published in 2017 in an ISI article "Renal function is similar in solitary kidneys from patients with and without diabetes", (Nephrologia, IF: 1.167). This topic has also been the subject of two extensive review studies, which were published in 2013 "The solitary kidney- a nephrological perspective" (Rom J Intern Med, BDI, PubMed), and in 2021 „The Inter-Relation between Solitary Kidney and Diabetes Mellitus-What Patterns are Known" (BDI). In another line of research, I observed that there is a strong association between lower potassium levels and rapid decline in renal function in patients with both congenital and surgical single kidney.

Another direction of research was the study of haemodialysis patients in Romania. Currently, end-stage chronic kidney disease treated by renal replacement techniques is associated with an extremely high mortality rate, although significant progress has been made in these therapeutic methods. Epidemiological aspects of risk factors for mortality and prognosis of dialysis patients in Eastern European countries, although presenting certain characteristic features as compared to the rest of Europe, have been scarcely reported in the literature due to the specific socio-economic environment. Therefore, I aimed to assess the prevalence of vitamin D deficiency and its relationship with risk of all-cause mortality in hemodialysed patients with diabetes mellitus, in a prospective non-interventional cohort study including 600 patients on hemodialysis therapy. To the best of my knowledge, this paper was the

first Eastern European study which demonstrated the fact that hemodialysis patients with diabetes mellitus had a higher prevalence of vitamin D deficiency as compared to those with no diabetes, which was associated with an important burden of mortality. The results were published in 2015, in the article entitled “Vitamin D deficiency--prognostic marker or mortality risk factor in end stage renal disease patients with diabetes mellitus treated with hemodialysis - a prospective multicenter study”, (Plos One, IF: 3.057). A particular aspect of chronic kidney disease treated by haemodialysis is that of populations considered “special”, i.e., the black race, as well as certain ethnicities. In Romania, Roma ethnicity represents 8.32% of the general population, being the second most prevalent minority in the country, that is why I studied Roma subjects with end-stage chronic kidney disease treated by hemodialysis. For the first time, these results showed that Roma people are initiating chronic hemodialysis at a younger age than Caucasians and they are at a higher risk of dying at a younger age. Furthermore, in the long-term survival analysis, Roma ethnicity represents per se, a significant, independent risk factor for mortality in hemodialysis patients. My interest for this theme of research was materialized in the following article, published in a high IF journal “Survival of Roma Minority Patients on Chronic Hemodialysis Therapy - A Romanian Multicenter Survey”, (Plos One, IF: 2.806). A third line of research including haemodialysis patients was the study of the risk factors for loss of arteriovenous fistula patency. I found that C-reactive protein represented an independent predictor for loss of arteriovenous fistula functionality. The results were published in 2019 in the article "C-reactive protein as a prognostic risk factor for loss of arteriovenous fistula patency in hemodialyzed patients", (J Vasc Surg, IF: 3.405).

Another line of research was the assessment of the risk of acute kidney injury and the impact on in-hospital mortality in patients at high risk through cumulative mechanisms, i.e., the underlying disease added to the risk of therapeutic interventions. In a first paper, we assessed the risk of acute kidney injury in stroke patients treated by intravenous thrombolysis with alteplase. The observations of our study demonstrated that intravenous thrombolysis per se does not increase the risk of acute kidney injury in stroke patients. Moreover, acute kidney injury was an independent predictor of mortality, this study being the first international report which is addressed to this issue. The results were published in 2017 in the paper "The impact of acute kidney injury on in-hospital mortality in acute ischemic stroke patients undergoing intravenous thrombolysis", (Plos One, IF: 2.766). In another study we evaluated the

impact of acute kidney injury in patients undergoing endoscopic retrograde cholangio-pancreatography, a complex and invasive method of digestive endoscopy recognized as the endoscopic technique with the most associated complications. The independent factors associated with acute kidney injury were baseline eGFR, non-renal Charlson score, choledocholithiasis, and serum bilirubin level upon admission. Patients who developed acute kidney injury after endoscopic retrograde cholangio-pancreatography had a significantly longer period of in-hospital stay. Also, in-hospital mortality rate gradually increased with the rising of post-ERCP AKI severity. Furthermore, in multivariate analysis, moderate to severe AKI (stages 2 and 3) was a strong independent predictor for in hospital mortality. The interest for this topic of research was materialized recently, in 2023, in the following high-IF ISI paper "„Acute Kidney Injury after Endoscopic Retrograde Cholangiopancreatography-A Hospital-Based Prospective Observational Study", (Biomedicines, IF: 4.757).

In the 4th sub-chapter, we presented the studies conducted on the kidney ↔ brain axis. Chronic kidney disease is now recognised as a major risk factor for neurological diseases, whereas patients with stroke have an increased risk for acute kidney injury. Furthermore, silent brain infarction is demonstrated to be an independent prognostic factor for progression of kidney disease in subjects with chronic kidney disease. In the first neuroimaging study I showed that among young and midlife adults, even mild reduction in eGFR may play a crucial role in reduction of hippocampal volume. Also, there was a strong association between decreased eGFR and an increased medial temporal atrophy score, calculated by Schelten scale. These results were published in 2022, as an abstract in the paper "Mildly impaired kidney function may be associated with risk of hippocampal atrophy in young and midlife adults", (NDT, IF: 7.186).

To date, the most common cause of end-stage kidney disease across the world is diabetes mellitus and the lesions of small cerebral vascular disease spectrum are more common in people with diabetes mellitus. Therefore, we evaluated aspects of the molecular mechanisms of cerebrovascular disease associated with diabetic kidney disease, based on the hypothesis of multiple structural and functional similarities between the microcirculation of the two organs. The study of epigenetic mechanisms involved in the occurrence of cerebrovascular disease associated with diabetic kidney disease has been an important topic. Thus, starting from the epigenetic alterations that characterize the early stage of diabetic kidney disease, we extended our research

by assessing the impact of these epigenetic dysregulations on cerebral vascular dysfunction.

I observed a specific pattern of miRNA expression related to cerebral endothelial dysfunction. Plasma miRNA-21 and 192 promote cerebral vessels remodelling, while plasma miRNA-124, 125a, 126, and 146a exert neuroprotective effects. However, cerebrovascular changes occurred even in normoalbuminuric patients, with 'high-to-normal' levels of podocyte damage and proximal tubular dysfunction biomarkers, thus pointing to significant cerebral vessels remodelling in early diabetic kidney disease. Variability of miRNA expression within the kidney and the brain may explain the dissociated time frame of cerebral vessels remodelling and renal involvement in early diabetic kidney disease. I have summarized the main observations on epigenetic mechanisms of cerebrovascular disease associated to diabetic kidney disease in the following articles: "Urinary podocyte-associated mRNA levels correlate with proximal tubule dysfunction in early diabetic nephropathy of type 2 diabetes mellitus" (2017, Diabetol Metab Sydrome, IF: 2.413); „Deregulated profiles of urinary microRNAs may explain podocyte injury and proximal tubule dysfunction in normoalbuminuric patients with type 2 diabetes mellitus" (2018, J Investi Med, IF: 1.994); „MiRNA Expression is Associated with Clinical Variables Related to Vascular Remodeling in the Kidney and the Brain in Type 2 Diabetes Mellitus Patients" (2020, Endocr Res, IF: 1.553), respective „Long noncoding RNAs may impact podocytes and proximal tubule function through modulating miRNAs expression in Early Diabetic Kidney Disease of Type 2 Diabetes Mellitus patients" (2021, Int J Med Sci, IF: 3.642). Inflammation plays a crucial role in the pathophysiology of small cerebrovascular disease. Also in diabetic kidney disease, inflammation is considered a main phenomenon for the initiation and progression of diabetic kidney disease. The study of inflammatory molecular mechanisms involved in the genesis of cerebrovascular and glomerular lesions represented another concern. I demonstrated that in early diabetic kidney disease there is a panel of pro-inflammatory cytokines, such as IL-1α, IL-8, and IL-18, a specific pattern of miRNAs that controls the expression of pro-inflammatory effectors (miR-21, miR-124, miR125a, miR-192; miR-126 and miR-146a). Furthermore, in my study advanced glycation end-products, which are important players of chronic vascular inflammation, were associated with impairment of cerebrovascular reactivity. The interest for this topic of research was materialized in the following high-IF ISI papers, some of them having a large number of citation in Clarivate analysis, „ Glycated

peptides are associated with the variability of endothelial dysfunction in the cerebral vessels and the kidney in type 2 diabetes mellitus patients: a cross-sectional study" (2015, J Diabetes Complications, IF: 2.955, 12 citations); „Interleukins and miRNAs intervene in the early stages of diabetic kidney disease in Type 2 diabetes mellitus patients" (2019, Biomark Med, IF: 2.479, 8 citations), and „Pro-inflammatory cytokines are associated with podocyte damage and proximal tubular dysfunction in the early stage of diabetic kidney disease in type 2 diabetes mellitus patients." (2020, J Diab Complic, IF: 2.852, 29 citations).

The second chapter includes academic achievements, which I had throughout my career from assistant professor to associate professor. I have mentioned and detailed the activity of teaching, from practical work and courses for students, graduate thesis to other educational activities, such as participation with active involvement in lectures at scientific events organized by the Timisoara Society of Medical Students. I have developed study materials for students and residents, focusing on Nephrology.

My involvement in the academic activity is also evidenced by my status as reviewer for 7 journals indexed in Clarivate's Web of Science. Parallel to my academic activity, I am a senior consultant in Nephrology and consultant in Internal Medicine, with a certificate in General Ultrasonography (2010), in the Nephrology Department of "Pius Brînzeu" County Clinical Emergency Hospital Timisoara.

In the last chapter, are presented the projects of academic and scientific development. My main objectives are to develop Nephrology, through interdisciplinary collaboration with Neurology, Biochemistry, Radiology, and Psychology experts, in order to create an exceptional School of Nephrology in Timișoara. An interdisciplinary approach to research and the involvement of young researchers are essential for academic development. With the aid of students and young resident doctors of Nephrology, I have built up a team which went on to receive very good results, presented through research papers at national and international congresses. Some of them will be co-opted in a future team of PhD students that I intend to coordinate in the field of Nephrology.

In the long term, I intend to continue the academic activity and the scientific research in the field of Nephrology, through development of the study of neuropsychiatric dysfunction associated with chronic kidney disease. I will focus on international collaborations, in order to establish a Centre of Excellence on the study

of the kidney ↔ brain axis within the University of Medicine and Pharmacy "Victor Babeș" Timișoara, thus raising Timisoara nephrological medicine to a European level.

The bibliography and list of ten representative scientific papers conclude this habilitation thesis.