“Victor Babeș” University of Medicine and Pharmacy

Timișoara, Romania

HABILITATION THESIS
- SUMMARY -

An innovative, trans-disciplinary research approach in the study of Diabetes, its complications and co-morbidities

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The Habilitation Thesis contains the most important professional achievements, obtained after receiving the title of *Philosophiae Doctor* in 2012 with the thesis entitled "The comprehensive management of Type 1 Diabetes Mellitus in Romanian Children and Adolescents", awarded with the *Magna Cum Laudae* distinction after its public defense.

This Habilitation Thesis is divided in three major sections: in the first section, the main academic, professional and scientific achievements are presented; The second section briefly describes the future academic and scientific perspectives of the candidate; the last section of the thesis contains the references used for the research and documentation work presented in the first two sections.

The post-doctoral research and the professional path of the candidate are in a close relation to the two positions appointed: specialist physician in Diabetes Mellitus, Nutrition and Metabolic Diseases respectively Associate Professor at the Discipline of Biostatistics and Medical Informatics. The candidate was responsible with the research methodology, study design and data analysis inside an innovative, multidisciplinary research nucleus developed inside our University which included clinicians (diabetologists, nephrologists, pneumologists, cardiologists and neurologists), as well as fundamental science specialists (histologists and pathology specialists) and specialists in informatics or medical informatics. This research team focused its research on Diabetes Mellitus, its complications, co-morbidities and state of the art high-tech interventions for the treatment of Type 1 Diabetes. The importance of this research is emphasized in the context of the global public health issue generated by diabetes, being a disease affecting approximately 425 million persons, having a worrying increasing trend of in-time incidence, having a major impact on both the individual's health as well as on the public health systems, being estimated that diabetes yields a direct and indirect cost of about 730 billion USD.

At the moment of writing this thesis, the author had disseminated his research work in 69 papers indexed in Clarivate's Web of Science (42 in extenso papers, 8 proceeding papers and 19 meeting abstracts), which received a total of 200 citations from 163 different citing articles indexed in the Web of Science. In April 2018 the candidate had a Hirsch index of 8 in Web of Science respectively 11 in Google Academic. In the Google Academic, at the moment of writing this thesis the author has a total of 129 articles indexed, summing a number of 343 citations.

The research was published in scientific journals with high impact on the research community. This statement is emphasized by the fact that the author's cumulated impact factor of the published papers is 81.521 (the cumulated impact factor of the published papers in which the candidate is principal author being 41.465).

To summarize, the candidate's research was disseminated in:

- 17 *in extenso* articles indexed in Clarivate's Web of Science as principal author
- 25 *in extenso* articles indexed in Clarivate's Web of Science as co-author
- 14 *in extenso* articles indexed in international scientific databases as principal author
- 17 *in extenso* articles indexed in international scientific databases as co-author
- 19 abstracts or proceedings after conferences in supplements of journals indexed in Clarivate's Web of Science, principal author
- 21 abstracts or proceedings after conferences in supplements of journals indexed in Clarivate's Web of Science, co-author
- 77 abstracts in supplements of recognized scientific journals
- 22 posters presented at international scientific meetings
- 33 posters presented at national scientific meetings

A part of the research done was funded by five international, national or internal scientific grants which were won in a competitive process. In these grants the candidate was project director, local project director or member. In the same time, the candidate is currently involved in global projects of state of the art medical research, being an active participant in major clinical trials from the area of diabetes holding positions of principal or co-investigator in 11 clinical trials. Of a special interest is the *Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results* a study which demonstrated first that intervention with GLP1-RA may decrease the cardiovascular risk and the rate of death, improving thus the global outcome and prognosis of patients with T2DM and changing in this way the treatment paradigm in T2DM. The results of this trial were published in *The New England Journal of Medicine* in an article in which the candidate is acknowledged as contributor in the trial. The clinical importance of the results obtained here is emphasized by the fact

that this article was cited 799 times in just two years after is publication, at the moment of writing this thesis in the Clarivate's Web of Science.

A of the knowledge obtained during his medical experience and biostatistics training were published in the pages of 7 books in which the candidate was author, co-author or co-author in chapters.

In this Thesis, the author's main scientific achievements are underlined by presenting the results of 14 of the most important studies in which the candidate participated.

1. **The development of a prognosis tool for pregnancies and fetal risk in women with Type 1 Diabetes Mellitus.** The study aimed to analyze the pregnancies outcomes in the Romanian women population with Type 1 Diabetes Mellitus and to evaluate which clinical and biological factors from the first trimester of pregnancy are significantly influencing the prognosis of these pregnancies. Based on the findings, it was concluded that the presence of Type 1 Diabetes Mellitus may be considered only a fetal and not a maternal risk factor. In addition, there were no any major maternal complications or deaths in the studied cohort. After analyzing the results of the study and evaluating them in the context of other published findings, we concluded that by achieving optimal glycemic control and thus avoiding the occurrence of hypoglycemic or ketosis events, as well as by eliminating the habitual risk factors, the aim of the St. Vincent declaration of 1989 may be realized. Moreover, the results suggested that, by implementing these measures, the fetal risk in women with Type 1 Diabetes may be decreased to a value that is similar to that in women without diabetes.
2. **The epidemiology of Type 1 Diabetes in Romanian children.** The epidemiology of type 1 diabetes mellitus may provide insights into the pathogenesis of the disease. The aim of this work was to characterize the trend of the incidence of type 1 diabetes mellitus in Romanian children aged from 0 to 17 years over a 10-year interval. Data regarding new cases were obtained from two sources: The Romanian Childhood Diabetes Registry and Records of the Medical Center "Cristian Serban", Buziaş. The demographic data were retrieved from the National Institute

for Statistics. The incidence was calculated for the age groups 0-4, 5-9, 10-14, and 15-17 years. The results pointed to the conclusions that Romania is a country with an intermediate incidence of Type 1 Diabetes Mellitus in children, which is slightly higher in boys than in girls. The incidence of Type 1 Diabetes Mellitus increased continuously during the 10-year survey, with the exception of the oldest teens, the age and gender adjusted incidence increasing from 6.2/100,000/year in 2002 up to 9.3/100,000/year in 2011.

3. **Metabolic syndrome in Type 1 Diabetes.** The study aimed to assess the prevalence of metabolic syndrome and evaluate proinflammatory status in patients with type 1 diabetes, and to analyze the relationship between inflammation, metabolic control and insulin resistance in these patients. For this, patients with Type 1 Diabetes were stratified according to the presence or absence of metabolic syndrome. Serum adiponectin, leptin, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and high-sensitivity C-reactive protein (hsCRP) were quantified. Patients with metabolic syndrome had lower adiponectin concentrations and higher leptin, TNF-α, IL-6 and hsCRP concentrations compared with patients without metabolic syndrome. The proinflammatory state associated with metabolic syndrome in patients with Type 1 Diabetes leads to deterioration of glycemic control and an increase in the required daily dose of insulin. Early and proactive diagnosis of metabolic syndrome in these patients will allow medication and lifestyle optimization, in order to prevent the occurrence of diabetes complications and improve health-related quality-of-life.
4. **Artificial intelligence in the prediction of glucose dynamics in patients with Type 1 Diabetes.** In patients with Type 1 Diabetes Mellitus, glucose dynamics are influenced by insulin reactions, diet, lifestyle or other factors and are being characterized by instability and nonlinearity. With the objective of a dependable decision support system for Type 1 Diabetes self-management, we aimed to model glucose dynamics using their nonlinear chaotic properties. A group of patients was monitored via continuous glucose monitoring sensors for several days under free-living conditions. We assessed the glycemic variability

and chaotic properties of each time series. Time series were subsequently transformed into the phase-space and individual autoregressive models were applied to predict glucose values over 30-minute and 60-minute prediction horizons. The logistic smooth transition model provided the best prediction accuracy for patients with high glycemic variability. Without the burden of measuring exogenous information, nonlinear regime-switching auto-regressive models provided fast and accurate results for glucose prediction.

5. **A study regarding patient's attitude towards mobile health involvement in the management of Type 1 Diabetes.** Nowadays, mobile technologies are part of everyday life, but the lack of instruments to assess their acceptability for the management of chronic diseases makes their actual adoption for this purpose slow. The objective of this study was to develop a survey instrument for assessing patients' attitude toward and intention to use mobile technology for diabetes mellitus (DM) self-management, as well as to identify sociodemographic characteristics and quality of life factors that affect them. The instrument showed good reliability and internal consistency, making it suitable for measuring the acceptability of mobile technology for DM self-management. Additionally, we found that even if most of the patients showed positive attitude toward mobile applications, only a moderate level of intention to indeed use them was observed. Moreover, the study indicated that barriers were truthfulness and easiness to use.
6. **A novel approach in neuropathy's diagnosis: quantifying its severity by assessing the density of intra-epidermal nerve fibers density.** Distal symmetric polyneuropathy (DSPN) is one of the most common complications of Diabetes and the most common form of peripheral neuropathy. The presence of this complication increases the risk of foot ulceration up to seven-fold and is a significant risk factor in more than 60 % of the amputations of the lower limbs in patients with Diabetes. The aims of our study were to evaluate the difference in the density of intraepidermal nerve fibers (IENF) in patients with respectively without DSPN, to evaluate the strength of the relationship between the symptomatology of the DSPN and IENF density and to define a cutoff

value of the IENF density for the diagnosis of DSPN. The presence of neuropathy was significantly associated with a decreased density of IENF for both the proximal and the distal biopsies. The optimal threshold value of IENF density (the point with the maximum sum of specificity and sensitivity), according to our model, was 10.1 fibers/mm. Skin biopsy followed by IENF density quantification proved to be a valid, reliable tool for the diagnosis of DSPN.

7. **Balance impairment in patients with Diabetes: an unforeseen complication.** Diabetic neuropathy is a prevalent complication of Type 2 Diabetes Mellitus with a major impact on the health of the affected patient. We hypothesized that mediated by the dysfunctionalities associated with diabetic neuropathy's three major components: sensitive (lack of motion associated sensory), motor (impairments in movement coordination) and autonomic (the presence of postural hypotension), the presence of neuropathy may impair the balance in the affected patients. Our study's main aim is to evaluate the possible association between the presence and severity of diabetic neuropathy and both the balance impairment and the risk of falls in patients with Type 2 Diabetes. The study's results pointed to the conclusion that the presence of neuropathy in patients with Type 2 Diabetes is associated with impaired balance and a consecutive increase in the risk of falls.
8. **A closed loop feedback in diabetes: neuropathy and adherence to self-care activities.** The purpose of this study was to evaluate the impact of the presence and severity of neuropathy and depression on the patient's adherence to diabetes-related self-care activities (DRSCA) in a cohort of patients with Type 2 Diabetes Mellitus (T2DM). The presence of neuropathy is associated with decreases in the quality of adherence to DRSCA in patients with T2DM and with increases in the symptomatology of depression. The significant, negative association between the severity of neuropathy and the quality of disease self-management points to a possible loop-type relationship between these two components, being possible a reciprocal augmentation with negative consequences on the global management of the disease.

9. **Diabetic neuropathy insights: its development in relation to aging.** Population aging is unprecedented, without parallel in the history of humanity. As T2DM is predominantly more prevalent in aging populations, this creates a major public health burden. Older adults with diabetes have the highest rates of major lower-extremity amputation, myocardial infarction, visual impairment, and end-stage renal disease of any age group. The aims of the study were to assess whether age is an independent factor for the occurrence of diabetic neuropathy (DN), and to evaluate the relationship between the presence and the severity of DN and the diabetes duration and blood glucose level. The conclusions were that age influences the occurrence of DN, independent in regard to other risk factors. This influence persists even after adjusting for other, very important risk factors, like blood glucose level or diabetes duration.
10. **A predictable inter-relationship: the prevalence of neuropathy in patients with diabetic chronic kidney disease.** The study aimed to investigate the prevalence and severity of neuropathy in hospitalized, insulin treated, diabetic patients, in relation to the severity of chronic kidney disease. We analyzed the two-way relationship between the scores in the Michigan Neuropathy Instrument and the severity of kidney disease according to KDIGO 2012 classification. In patients with diabetes mellitus, chronic kidney disease and neuropathy are two of the most prevalent chronic complications, both having a major impact on the quality of life and overall health. Furthermore is hypothesized that the presence of neuropathy is associated with a consecutive worsening in the prognosis of other diabetes complications, indirectly mediated by the decrease of the quality of the diabetes self-management, thus, studying the association between neuropathy and chronic kidney disease is of a paramount importance for clinicians - being a guidance by providing the odds of having one complication if other is already diagnosed and for researchers - by providing a regional overview of the situation.
11. **Novel biomarkers used in the assessment of diabetic nephropathy.** Diabetic nephropathy (DN) is a frequent and severe complication of diabetes mellitus (DM). Its diagnosis in incipient stages may allow prompt interventions and an improved prognosis. Towards this aim,

biomarkers for detecting early DN can be used. Microalbuminuria has been proven a remarkably useful biomarker, being used for diagnosis of DN, for assessing its associated condition-mainly cardiovascular ones-and for monitoring its progression. New researches are pointing that some of these biomarkers (i.e., glomerular, tubular, inflammation markers, and biomarkers of oxidative stress) precede albuminuria in some patients. However, their usefulness is widely debated in the literature and has not yet led to the validation of a new "gold standard" biomarker for the early diagnosis of DN. Currently, microalbuminuria is an important biomarker for both glomerular and tubular injury. Other glomerular biomarkers (transferrin and ceruloplasmin) are under evaluation. Tubular biomarkers in DN seem to be of a paramount importance in the early diagnosis of DN since tubular lesions occur early. Additionally, biomarkers of inflammation, oxidative stress, podocyte biomarkers, and vascular biomarkers have been employed for assessing early DN. The purpose of this review is to provide an overview of the current biomarkers used for the diagnosis of early DN.

12. **The predictive role of Vitamin D in patients with Diabetes and end-stage renal disease.** End stage renal disease (ESRD) patients on renal replacement therapy (RRT) with diabetes mellitus (DM) have a higher mortality rate and an increase prevalence of vitamin D deficiency compared to those without DM. It is still debated if vitamin D deficiency is a risk factor or a prognostic marker for mortality in these patients. This study investigated the prevalence of vitamin D deficiency and its impact on all-cause mortality in HD patients with DM. This prospective, non-interventional cohort study included 600 patients on hemodialysis therapy (HD) recruited from 7 HD centers, from all main geographical regions of Romania. The prevalence of DM was 15.3%. They were then followed regarding: dialysis duration, dialysis efficiency, renal anemia, CKD-MB, inflammatory status and comorbidities: coronary artery disease, peripheral vascular disease and stroke. The study concluded that ESRD patients with DM treated with HD have a higher overall mortality than non-DM patients. Vitamin D deficiency is significantly

more prevalent in HD patients with DM. Low 25-OH vitamin D levels were associated with increased all-cause mortality in these patients.

13. **Renal function in patients with Diabetes and solitary kidney.** Due to the shortage of living kidney donors and the current diabetes mellitus (DM) pandemic, studying the association of solitary kidney (SK) with DM is of paramount importance. The aim of the study was to assess the significance of the association between SK and DM. A higher proteinuria was found in patients with Diabetes as well as significant metabolic abnormalities, a higher prevalence of hypertension and coronary artery disease. However, no differences with respect to GFR were found, which could have significant implications for transplantation.
14. **The prevalence of urinary tract infections in patients with Diabetes.** Patients with diabetes mellitus (DM) have an increased risk of infections, especially urinary tract infections (UTIs). The aim of this study was to assess the prevalence and etiology of UTIs and identify the risk factors for their development in patients with DM. In this retrospective, noninterventional study, the medical records of 2,465 adult patients with DM who were hospitalized in a Diabetes Clinic of Timisoara were reviewed. The prevalence of UTIs in patients with DM was 12.0% (297 cases), being higher in females than in males and higher in patients with type 2 DM compared with patients with type 1 DM. In univariate logistic regression analysis, risk factors associated with UTIs were female gender, age, type 2 DM, longer duration of DM, and the presence of chronic kidney disease and coronary artery disease. The gram-negative bacilli from the Enterobacteriaceae family were predominant, with Escherichia coli being the most frequent of them (70.4%).

The second part of the Habilitation Thesis focuses on the candidate's future perspectives, from an academic as well as scientific perspectives. From an academic point of view, the candidate would like to develop the current existing teaching and knowledge base regarding biostatistics, research methodology and medical informatics, emphasizing on the existing link of these sciences with clinical medicine. The author aims to develop new lectures for both medical students, post-graduates and PhD candidates broadening the spectrum of the existing topics and integrating

them in the context of state-of-the art medical research areas. Besides the pre-existing lectures, an immense step was made with the major re-designed biostatistics for PhD students course held by the candidate in the Doctoral School of “Victor Babes” University of Medicine and Pharmacy. The author plans to develop tailored biostatistics and medical research methodology courses for medical doctors practicing in different specialties and organized by our University in different scenarios.

From the researcher’s point of view, the candidate aims to continue the research topics already started, a work in which his PhD students will have a central place being pro-actively involved in applied and theoretical state-of-the-art medical research. Part of the approached areas of research will be continuing the already started ones: insights of the epidemiology and pathogenesis of Diabetes, particularities of the clinical manifestations in the loco-regional populations, the pro-active search for unforeseen complications or co-morbidities and cutting edge technological interventions in Type 1 Diabetes. As further areas of interest for the nucleus of research the candidate intends to develop by co-opting his PhD students as well as other researchers are the involvement of the “closing the loop” for the artificial pancreas - the hallmark of biological cybernetics. From the hardware’s point of view this device is nowadays feasible to be built, being mainly a connected system composed from a dual insulin-glucagon pump and a real-time glycemic sensor; all these components being available. However, to be completely independent the system lacks a safety-critical predictive algorithm for the dual-hormone delivery. The major burden is represented by the individualized, highly variable response to both hormone delivery and glucose input as well as the important lags regarding the responses in the cybernetics of the system: the time elapsed from action to response, both regarding the action-biological response of insulin or glucagon as well as glucose input. The foundation stone for this research was already laid when the candidate was involved in a scientific research project regarding the cybernetics of this system by the most influential industry player in the field of insulin pumps and glycemic sensors: Medtronic Inc. Another innovative area of research which the author aims to address is the mathematical modelling of several biological phenomena. This activity was also studied during his PhD, when the candidate aimed to develop complex mathematical models which included in analysis emerging modeling theories, like the theory of the information entropy or the Akaike’s criterion for the selection of the predictor. During the PhD, the model addressed the rate of auto-immune mediated destruction of β-

pancreatic cells in patients with Type 1 Diabetes. These mathematical models may lead to the discovery of innovative and unforeseen insights regarding the biological phenomena. Moreover, their importance is emphasized by the fact that these models may be used in the development of virtual, theoretical, close to reality experiments in a harmless scenario.

Of course, this short list of further perspectives doesn't intend to be an exhaustive but just an exemplificative one, the candidate being acknowledged that research is in fact an experience full of unpredictable results and events and thus many new perspectives will arise during the journey.

Finally, having the premises of his previous training and experience and considering his future plans, the candidate hopes that by achieving the previously mentioned goals he will be a member of the academic staff who will reach the expectations of the University which formed him, his Alma Mater, the "Victor Babes" University of Medicine and Pharmacy from Timisoara.