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PHD THESIS

CARDIAC FUNCTION AND STRUCTURE CHANGES IN
ADVANCED AND END STAGE CHRONIC KIDNEY DISEASE

A B S T R A C T

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GENERAL PART

1.1 INTRODUCTION

Chronic kidney disease represents the progressive and irreversible kidney damage with increased mortality and morbidity. The outcome of this disease is represented by death in most of the cases and, the lucky patients can receive one renal replacement therapy (haemodialysis, peritoneal dialysis or kidney transplant). The epidemiology of chronic kidney disease show that the prevalence is rising in both pre-dialysis and dialysis states. Cardio-vascular disease represents the main comorbidity among the aforementioned disease.

Nowadays, cardio-vascular death represents the main cause of death worldwide and among patients with chronic kidney disease.

There is a link between cardiovascular disease (CVD) and chronic kidney disease (CKD). It is called in the literature CVD-CKD continuum. It was recognised back in 2003 by a pioneer of cardiology, Brenner. After the first description of this continuum, the published data acted like puzzle pieces and this concept become more and more clear. So far, there are clear evidence that chronic kidney disease is a major cardio-vascular disease risk factor and that cardio-vascular disease affects the kidney and hurries-up the progression of chronic kidney disease. It continue to influence mortality and morbidity even after the initiation of renal replacement therapy. Almost all patients on haemodialysis presents some form of cardio-vascular disease.

The exact links from this continuum are still under research and debate worldwide. It is difficult to describe them exactly due to many implicated factors. One could compare this continuum with the famous question: "Which was first, the egg or the hen?"

Besides the fact that cardio-vascular associated death is the leading cause of death among patients with chronic kidney disease, many other factors increase mortality. These factors are different among regions and even between countries. To assess accurately the risk of death of a patient, one should identify the specific risk factors from its area. In addition, with the detection of novel biomarkers, we should describe the exact link of them with CVD-CKD continuum.

In this thesis, we evaluated and identified specific mortality associated factors in patients with advanced chronic kidney disease on maintenance haemodialysis. In addition, we identified specific new biomarkers that influence cardiac function and are associated with increased mortality. The recognition of mortality risk factors is mandatory in order to intervene and increase survival. With this thesis, we wish to add some missing puzzle pieces from the CVD-CKD continuum.

1.2. EPIDEMIOLOGY

In 1971, Abdel R Omran (professor of epidemiology at the Chapel Hill university in North Carolina) published „The Epidemiologic Transition: A Theory of the epidemiology of Population", a milestone paper about the theory of global epidemiology transition. In the developed countries, at the end of the XIXth century, under the pressure of technology, socioeconomic changes and development of medicine and science, a receding of major epidemics, infectious diseases and famine decreased the overall mortality rate and

increased life span of the general population. Because of these changes, the number of the world population increased and the major causes of morbidity and mortality shifted from acute and infectious to chronic and degenerative diseases.

In the face of the epidemiologic transition, there is a need for a new approach towards chronic pathologies. These chronic diseases are conceived as a continuum between risk factors, chronic organ damage, progression to end stage disease and death. In this chain of events, there are multiple intervention possibilities: first: identifying and treating modifiable risk factors (as early as possible), second: early diagnosing and treatment of the chronic disease in order to reduce progression rate and prevent complications, having as a goal the reduction of mortality and increasing life span.

In 2002, The National Kidney Foundation released the first guidelines "The Kidney Disease Outcome Quality Initiative" (K/DOQI) introducing the concept of "Chronic Kidney Disease" (CKD), defined by abnormalities of kidney structure and/or function, lasting for more than 3 months, with implications for health. Currently, CKD is considered a worldwide public health problem with important implications on the morbidity and mortality, recognized as a major economic burden.

In a 2022 review of Kovesdy, he stated that CKD affects more than 10% of the general population worldwide, with an increasing mortality. It is estimated that until 2040, CKD will be the fifth cause of years of life lost globally. The concept and continuum of CKD was introduced in Romania in 2002 with a lot of scepticism. The first Romanian authors who addressed the concept were Covic A and Schiller A. In their paper published in 2008, they showed a high CKD prevalence in Romania (8.8% - 11.7%, stages 3 and up)) with one out of 10 elderly subjects with a severe reduction of renal function.

The natural evolution of CKD is towards end stage, but the prevalence of advanced stages of CKD in the general population is lower as compared to earlier stages due to high mortality rates. The major cause of high mortality in CKD patients is associated to cardio-vascular complications. The patient that survives long enough will become "the lucky loser" and will need renal replacement therapy (haemodialysis, peritoneal dialysis or renal transplantation).

In our days, the most common RRT is haemodialysis. In 2010, there were 2.6 million people worldwide receiving a form of RRT with the same number of patients that died because of the lack of access to dialysis or renal transplantation. The number of patients receiving RRT increased and in 2017, there were 3.9 million people performing a form of RRT. Hemodialysis accounts for 69% of all forms of RRT and 89% of dialyses. The latest data concerning this issue in Romania are form 2021, from the Romanian Renal Registry. In 2020, there were 13633 patients performing either hemodialysis or peritoneal dialysis.

1.3 CARDIOVASCULAR DISEASE AND CHRONIC KIDNEY DISEASE

Cardiovascular disease (CVD) represents a major public health care problem and a major economic burden with huge implications on morbidity, mortality and survival. Even though the mortality associated to CVD decreased in the past years, it remains the most important cause of death and morbidity worldwide. In 2016, according to the Global Burden of Disease Study, CVD was responsible for one-third of all deaths across the globe and was

the leading cause of morbidity and death. Cardiovascular disease addresses a family of pathologies that involves heart and blood vessels. It consists of heart failure, peripheral artery disease (PAD), coronary artery disease (CAD), cerebrovascular disease, hypertensive heart disease (HHD), rheumatic heart disease (RHD), and a number of other cardiac and vascular problems.

CKD proved to play an important role in CVD continuum. Lindner published the first article, which addressed atherosclerosis in HD patients in 1974. He observed that patients on maintenance HD had higher risk of death than the general population and that accelerated atherosclerosis plays an important role in the poor outcome. Linder suggested that renal failure could generate accelerated atherosclerosis. Sarnak and Levey were between the first to state that there could be a link between CVD and CKD. In the paper published in 2000, they pointed out that CVD and chronic renal disease (CRD- nowadays CKD) could be the outcomes of the same underlying conditions. In addition, the groups with end stage renal disease (ESRD) or chronic heart failure (CHF) are "the tip of the iceberg" with high morbidity and mortality. They observed the fact that patients with chronic renal disease are at high risk of developing CVD and many patients with established CVD had chronic renal disease.

Increased cardiovascular risk in CKD (i.e. morbidity, mortality) was attributed to major conventional cardiovascular risk factors as well as to novel risk factors emerging from chronic decrease of kidney function. Many of the traditional major CVD risk factors are also CKD risk factors but their prevalence is increasing in CKD with the decrease of the kidney function. On the other hand, the novel CVD risk factors are amplifying the progression of CKD.

The most important risk factors for CVD and CKD are represented by arterial hypertension, diabetes mellitus, albuminuria, hyperglycaemia, dyslipidaemia, obesity and smoking.

1.4 CARDIOVASCULAR PATHOLOGY IN CKD

Cardiovascular involvement in patients with CKD can be divided into two major categories: heart changes (remodelling) and vascular changes (atherosclerosis and vascular remodelling). It seems that endothelial dysfunction is involved from the beginning in all cardiovascular changes.

The vascular alterations are represented by endothelial dysfunction, arterial wall thickening and arterial stiffens and arterial calcifications. All of these changes in patients vessels lead to increased mortality and morbidity and progression of CVD and CKD.

The cardiac modifications are represented by left ventricular geometry changes, ultrastructural changes of the myocardium, left ventricular function changes, heart valve disease, and arrhythmias. All of these changes are closely linked to CKD progression and the presence of them has a direct impact on kidney function.

1.5 CKD IMPACT ON CVD

As Guyton stated, the kidney plays an important role in any form of HT. In kidney-HT relation, the kidney can be the aggressor (generating secondary HT) or the aggressed (developing HT related damage). The kidney is a major player in the blood pressure (BP) regulation. The kidney is capable to increase the BP through multiple mechanisms: salt and water retention, accumulation of endogenous vasopressors, activation of renin-angiotensin aldosterone system or sympathetic over activity. Afterwards, HT can damage the kidney and reduce the glomerular filtration rate (GFR), leading to a vicious cycle. There is evidence from kidney donors that a reduction in 10 ml/min/1.73sm of GFR generates an increase of 5 mmHg in systolic blood pressure (SBP).

There are several implications of the kidney in generating dyslipidaemia, especially after a reduction of GFR below 60ml/min/sm. The promoter is the accumulation of triglyceride-rich intermediate-density lipoprotein (IDL) and very low-density lipoprotein (VLDL).

There are several mechanisms that generates anemia in CKD like chronic inflammation, functional iron deficiency, erythropoietin deficiency, reduced red blood cells survival, deficiencies of vitamin B12 and folate and some mechanical factors related to HD. Many studies proved cardiac alteration and worst outcomes in patients with CKD and anaemia.

Another CKD complication that plays a major impact in CVD is mineral-bone disorder (CKD-MBD). Altered serum levels of calcitriol, parathyroid hormone, calcium and phosphorus influences cardio-vascular system. High levels of phosphate and fibroblastic growth factor 23 are associated with increased mortality. Several studies proved an increased risk of calcifications and myocardial fibrosis when parathyroid hormone is elevated.

The increased oxidative stress and elevation in asymmetric dimethyl arginine on CKD basis seems to be linked to an increased CVD prevalence.

SPECIAL PART

In the first chapters of the special part two studies on HD patients will be presented. The hard end-point of them is the mortality. The secondary end-points are represented by mortality risk factors, mainly cardio-vascular ones and possible interventions on them in order to decrease mortality. In the second part of the thesis, we present two pilot studies. First of them is an analysis of some specific biomarkers and their impact on cardiac parameters. The second pilot-study is performed on patients with diabetic kidney disease where we evaluate other novel biomarkers and their impact on cardiac changes.

2.1 CHANGES REGARDING COMORBIDITIES AND MORTALITY IN HAEMODIALYSIS PATIENTS

HD patients have an increased mortality and are at high risk of developing CVD. Over the last years, survival increased in ESKD after the improvement of CKD related risk factors (treatment and prevention of CKD complications, personalised HD therapy, reduction of the CKD progression and prevention and treatment of CVD). The future medical strategies are based on the analysis of the changes that appear in HD population (causes of death, mortality, comorbidities prevalence, etc.).

In our multicentre retrospective study, we compared baseline characteristics of two cohort of patients treated in the same eight HD centres from Romania. Our cohort from 2012 represented 9.4% (901 out of 9551) of the patients that performed HD in Romania, after analysing RRR data. The same registry reported, between 2012 and 2017, an average annual increase of HD treated patients with 7.8% compared to a 10.9% annual increase in our study. In 2017, our cohort represented 10.4% of the Romanian HD treated patients (1396 out of 13362). In 2020, the Romanian HD prevalence decreased with 1.4% because of Covid-19 pandemic.

Patients from the 2012 cohort were younger and with shorter history of dialysis treatment. In 2017, the average age of HD patients was 59.8 years, similar to ERA-EDTA report (60.7 years). The 566 survivors from the 2012 cohort influence the mortality, HD duration time and age in the 2017 one. European countries reported different mean age of HD patients (Portugal-67.9, Denmark-59, Spain-59.5, Scotland-56.8, Ukraine-50 and Albania-49.5 years). In our cohorts and as reported by ERA-EDTA 2020 registry, the mean age of HD patients is increasing. Increased history of HD treatment and age increase mortality among these patients. USRDS reported a reduction in mortality between 2010 and 2019, but the mortality increased in 2020, especially among older patients.

Average serum urea and creatinine before dialysis were lower in 2012 cohort (p=0.032 and p=0.004 respectively) with no difference in urea to creatinine ratio. The serum albumin was lower and C reactive protein higher in 2012 patients, suggesting a reduced inflammation and better nutritional status in 2017 cohort. Tanaka and Walther showed that higher urea to creatinine ratio and predialysis creatinine increases the risk of death through a possible correlation with malnutrition-inflammation complex. Our data suggest that the 2017 cohort had an improved diet compared to the 2012 one.

The 2017 cohort had higher ferritin levels with lower transferrin saturation and haemoglobin. The increased ferritin with almost normal C reactive protein is a result of higher IV iron therapy, as showed in the PIVOTAL study. The increased mortality risk in the presence of elevated ferritin levels is attenuated by a reduced prevalence inflammation and malnutrition. In 2017, the IV iron administration was lower compared to 2012, because of increased ferritin.

In the 2012 cohort, serum calcium and calcium x phosphate product was lower with no differences in parathyroid hormone and phosphate compared to 2017. This shows an increase need in use of phosphate binders and an excessive use of calcium based ones. Several studies show no differences in mortality regarding the type of phosphate binder's use, but other suggests better survival for non-calcium based one. The Cochrane meta-analysis published in 2018, found no benefits for Sevelamer use regarding stroke, coronary artery calcifications, myocardial infarction or cardiovascular death. The differences in our cohorts had no impact on survival.

There were no differences among HD time, the dialyzers type and target eKt/V of 1.4 between the two cohorts. One should mention that in 2012 cohort the dialysis average fluid flux was lower (580.9 ± 63.3 vs. 673.5 ± 86.5 ml/min) and the mean ultrafiltration volume was also lower (7ml/min vs. 8.3ml/min) compared to 2017 cohort. Though there is no cut-off value for optimal ultrafiltration volume, some studies suggest that fluid overload increases mortality. The increased ultrafiltration volume in 2017 cohort did not influenced mortality in our study.

As expected, the prevalence of some comorbidities was higher in 2017 cohort compared to 2012 one. DM, as a primary cause of ESKD, increased from 21% to 25.6%. This increase is in accordance with the trends seen worldwide. As a remark, the DM prevalence among HD patients is higher in USA compared with Europe, probably because of higher obesity incidence in America. The presence of DM in HD patients increase mortality and this raising prevalence will alter the HD patient's survival. Regarding hepatitis B and C, prevalence, there were no differences for hepatitis B, but the prevalence of hepatitis C was lower in 2017 as a result of national prevention program that started in 2000 and the national treatment program with novel antivirals that started in 2015. The presence of hepatitis did not influenced mortality in our cohorts.

The prevalence of history of myocardial infarction, PAD and CBVD remained the same, but the prevalence of valve calcifications and LVH increased in 2017 cohort. In addition, in 2017 the patients had a lower EF. This may be related to a higher prevalence of DM, longer time on dialysis and older patients in 2017 cohort. We identified 566 survivors from 2012 in the patients from 2017 (40.5% of the 2017 cohort). Our 5-years survival rate (62.8%) was higher compared with Japan, Europe and USA (60%, 41% and 39%). Several characteristics from 2012 cohort could explain this survival rate: higher haemoglobin levels, higher EF, lower prevalence on cardiovascular complications and DM, younger age and a low prevalence of catheters (6.7%). ANZDATA regarding the patients (non-indigenous) that started HD between 2009 and 2018 (age group 45-64) presented similar results. These characteristics influenced the increased 1-year mortality rate from 2017 cohort (14.1% in

2017 vs 6.6% in 2012). Nevertheless, USRDS reported an increasing mortality for the last two years.

Our statistical tests showed no differences regarding cause of death between the cohorts, even though there was a decreasing trend in cardiovascular mortality (50% in 2012 and 45.6% in 2017). Many regional and national registries reported a decreasing trend for cardiovascular mortality among HD patients, but it remains the leading cause of death (Europe-39%, USA-51.5%, New Zeeland-36.2% and Australia-30.6%). In RRR, in 2020, the cardiovascular death represented 53%. Europe, USA, Australia and New Zeeland reposted lower sepsis rates as cause of death (16.2%, 9.1%, 8.9% and 10.8%) compared to our sepsis mortality (20.8% in 2017). RRR had similar results (19%). Cancer related deaths in HD patients varies around the globe from 7.8% in Europe, 2.3% in USA, 1.4 % in New Zeeland and 4.4% in Australia. Our results were higher with an increased trend (8.3% in 2012 and 13.2% in 2017). RRR reported lower results regarding cancer mortality in HD patients (4%). There are many factors that influence the mortality differences among registries: RRT strategies and practice patterns, socioeconomic status, detection of CKD, access to treatment, etc. Kaiser Permanente integrated health care system from California, published in 2022 that there are differences regarding the coding and report of collected data and this influence also the results.

A meta-analysis of 23 studies from 2017 identified multiple risk factors that increase mortality of the HD patients: low levels of Apo-A3, ApoA2, total iron binding capacity, albumin, haemoglobin, body mass index and higher levels of brain natriuretic peptide, T troponin, glycosylated haemoglobin, ferritin, C reactive protein as well as increased age and prevalence of CVD and DM. The cardiovascular mortality risk is reduced in: higher levels of Apo-A2, total iron binding capacity and albumin. On the other hand, higher levels of glycosylated haemoglobin, HDL and ferritin, longer HD therapy history, presence of previous CVD and DM, female sex and higher age represents cardiovascular mortality risk factors. The mortality risk factors varies between geographic areas, HD centres and countries. There are differences even between Eastern and Western European countries. For instance, in Japan, in 2007, lower eKt/V and serum creatinine levels, presence of CBVD and high pulse pressure increased mortality. Hypoalbuminemia, prevalence on catheters, older age and rapid deterioration of CKD increased mortality in Spain. In addition, as we showed in a recent article, there are some methods to reduce mortality and improve cardiac function – the introduction of renin-angiotensin-aldosterone system inhibitors. In our cohorts, increased age and history of dialysis, high C reactive protein and phosphate were mortality risk factors. On the other hand, higher albumin and haemoglobin levels were protective.

2.2 THE CHANGES IN LEFT VENTRICULAR FUNCTION AND STRUCTURE AMONG HAEMODIALYSIS TREATED PATIENT

With the progression of CKD, the prevalence of common CVD risk factors increases with the emergence of new CKD related risk factors. The Joint National Committee 7, in 2003, one year after the Kidney Disease Outcome Initiative defined CKD, recognised CKD as a major CVD risk factor. The CVD related mortality and incidence is very high in ESKD. The cardiac function and structure anomalies appear even from the early stages of CKD.

Enlarged left atrial volume, left atrial late diastolic strain rate and altered systolic function are revealed by echocardiography and AD strain analysis from the stage 3 CKD. Left atrial decreased contractile function generates late diastolic strain rate of the left ventricle. Alterations of left ventricle like increased mass index, hypertrophy, low ejection fraction and diastolic dysfunction are common in ESKD patients with poor cardiovascular outcome. Increased cardiovascular risk is associated with impaired left ventricular myocardial function (evaluated by 2 and 3D speckle-tracking echocardiography) and normal left ventricular ejection fraction in CKD patients. Left ventricle dyssynchrony is associated with high mortality among ESKD patients. The CKD continuum related to fluid overload, uremic toxins, coronary artery disease, myocardial fibrosis, anaemia and hypertension progress along the myocardial function and structure anomalies.

With the HD start in ESKD, some of the risk factors are attenuated like elimination of uremic toxins, electrolyte and acid-base balance, fluid overload reduction and reduction of blood pressure. In the same time, other risk factors emerge: myocardial stunning, consequences of the arterio-venous fistula, hemodynamic stress due to HD and excess of endotoxins. Myocardial structure and function is influenced by the mentioned factors and lead to modification of echocardiographic parameters. The initiation of HD appears to reduce in patients with heart failure the eccentric or concentric remodelling of the left ventricle hypertrophy and left ventricular mass index. Apparently, patients with advanced CKD and preserved left ventricular ejection fraction have a worst left ventricular function (after 2D strain analysis) compared to HD ones. After this papers showed some benefit for HD patients, early HD initiation was proposed. A sub-study of the IDEAL trial (Echo) showed no echocardiographic benefits in the group of patients that started the HD earlier (at GFR of 10-14ml/min/1.73sm) compared to the late initiated group (at GFR 5-7ml/min/1.73sm). Susantitaphong published in 2012 a meta-analysis regarding the outcome of longer and more frequent HD sessions. There was an improvement in myocardial morphology and function in the intensive HD treated patients, but there was no exploration of cardiovascular survival.

In 2013, Bansal published the first study regarding echocardiographic changes of heart function and structure in patients with advanced CKD that started HD. The cohort represented a subgroup of the Chronic Renal Insufficiency Cohort (CRIC study). He showed no significant changes in left ventricular mass index and an important decrease of left ventricular ejection fraction one year before the initiation of HD evaluation compared to one year after.

According to our research, this is the first study (longitudinal and multicentre) that evaluate the changes of myocardial structure and function in stable HD patients. Our cohort was large (1034 patients) with an average HD history of 3.56 years at inclusion. During the 4 years follow-up, the prevalence of CVD comorbidities increased: cerebrovascular disease (from 20.4% to 30.8%, p<0.0001), peripheral vascular disease (from 29% to 40.9%, p<0.0001) and coronary artery disease (from 73.5% to 88.8%, p<0.0001). In addition, regarding the echocardiography parameters, the prevalence increased for valvular calcifications (from 65.6% to 89.3%, p<0.0001) and left ventricular hypertrophy (from 67.6% to 76.5%, p<0.0001). Compared to USRDS data (35-40%), our prevalence of valvular

calcification was higher. In the face of long-term HD therapy, the progression of cardiac structure anomalies and CVD is linked to the cumulative effect of novel CVD risk factors like: CKD-MBD, chronic inflammation, uremic toxins, anemia, oxidative stress, protein carbamylation, endothelial dysfunction, etc.

For our cohort, the mortality in 4 years was 25.3% (261 patients died). Survival rate decreased over the 4 years of follow-up being 90.5% in 1st year, 85.9% in 2nd, 77.3% in 3rd, 74.7% in fourth. The risk of death was higher in the presence of valvular calcification (1.77-times), cerebrovascular disease (1.59-times), peripheral artery disease (1.61-times) and coronary artery disease (1.59-times). Higher left ventricular ejection fraction, eKt/V and dry weight reduced mortality risk. Japanese published similar data in patients with more than 10 years of HD treatment.

15.08% of the patients had an EF<50% and overall average EF at inclusion was 57.7± 9.54 %. After we divided the patients in four group regarding heart failure (HF), the survival rate was higher among the group with EF>50% as compared to those with EF<50%. The estimated survival was 1270 days in no HF group, 1269 days in HF with preserved EF group, 1194 days in HF with mid-range EF group and 1009 days in HF with reduced EF one. In HF with mid-range EF group and HF with reduced EF one, the risk of death was higher by 1.5-times and 2.3-times respectively. A low EF at the initiation of HD is a strong predictor of cardiovascular death. According to 2016 ESC guidelines, 40.8% of the patients in our cohort had heart failure criteria. Our results showed higher risk of death only in the groups with EF<50% (representing 15.8% of the cohort), in contrast to some published papers, but similar with MAGGIC meta-analysis.

In the first-year survivors (936 patients), we discovered variations in EF at second echocardiography. We divided these patients into three groups: a group with an increase of EF of >5% (250 patients), a group with EF variation of ±5% (424 patients) and a group with a decrease of EF with >5% (262 patients). The group with an increase of EF with >5% had an increased risk of 1.43-times (adjusted for initial EF, gender and age) higher compared with the group with EF variation of ±5%. There was no statistically significant increased risk of death among the group with a decrease of EF with more than 5% (OR=0.83, CI: 0.55-1.24). In patients with heart failure, EF is a risk stratify for cardiovascular and all-cause mortality.

We have several hypotheses regarding our results in which an increase of the EF was associated with an increased risk of death: accelerated progression of coronary artery disease, uremic cardiomyopathy, valve calcification secondary to CKD-MBD, the changing pressure regimen on the valves and fluid overload that could lead to an increase of heart valve regurgitation level. The increase of the EF with more that 5% after one year of HD does not seem to generate reduction in mortality risk, but rather increase it. Another helpful determination would be global longitudinal strain (GLS). GLS alteration can be used as cardiovascular mortality risk factor, in addition to EF measurement.

2.3 SPECIFIC BIOMARKERS AND CARDIAC FUNCTION
IN PATIENTS ON HD OR WITH DKD

After more than 5 decades since haemodialysis become a reliable method of renal replacement therapy, the cardiovascular events remains the leading cause of death among

the HD patients. We showed in a previous article that even though the trend in fatal cardiovascular events among HD patients is declining, they remain the most important cause of death in this population.

In the face of CVD and CKD continuum, one should keep searching for better predictors and possible intervention means in order to reduce the mortality. In previous studies, we identified some specific markers that increase mortality and some that can reduce it. In this study, we searched even further for specific biomarkers and their correlation with specific cardiac alterations identified through echocardiography.

In the first part of this study, we conducted a pilot evaluation of 58 patients on maintenance HD with 1-year follow-up. The patients had a full echocardiographic evaluation between the 2nd and 3rd hour of dialysis. The mean age of the cohort was 60.4 years old (±11.73 years) with 70.2% males. Our cohort was younger compared to the ERA-EDTA annual report from 2020 (65.1 years) and similar to several European countries like Serbia (60.3 years), United Kingdom (61 years), Finland (61 years), etc. Mean age in Romania of the patients on HD in 2020 was 62.2 years. The mean age in our previously studies was lower compared to this cohort (58 years in 2012, 58.7 years in 2015, 59.8 years in 2017). As expected, the patients mean age in our cohorts follow the worldwide ageing trend. This ageing is accompanied by higher prevalence of comorbidities, especially CVD.

Mean history of dialysis years is rising as compared with our published data (6.2 years in this study from 2021, 3.46 years in 2015 and 4.7 years in 2012). The trend is rising worldwide and the increase vintage of dialysis appears to be correlated to higher mortality rates. In a study by Sumida, it seems that the history of HD increases the risk of infection-associated mortality and only modestly increases the risk of cardio-vascular death.

The patient's complications management was conducted in accordance to KDIGO Guidelines, with haemoglobin in therapeutic range (10.8g/dl), good nutritional status (albumin=4.06g/dl), low inflammation (CRP=0.42mg/dl) and a relatively low serum phosphorus (5.41mg/dl).

The mean EF was 49.29%. Compared to our previously studies, the mean EF was lower (58.2% in 2012, 57.8% in 2015 and 56.8% in 2017). This EF reduction trend may be associated to several cofounders. First, the patients are getting older and present a higher CVD prevalence. Secondly, as we showed in a previous article, the increase of EF after 1 year of HD was associated with higher mortality rate (OR=1.43, 95%CI: 1.01-2.04). In addition, the mortality rate is higher among patients with reduced EF compared to the ones with preserved EF (OR=2.375, 95%CI: 1.436-3.927).

The calcification rate was high with 65.5% having aortic calcifications, 77.6% mitral calcifications and 70.2% endomyocardial calcifications, higher than in previously years (65.6% in 2015 and 62.3% in 2012). In an interesting study by Kraus from 2015 on 243 HD patients, 100% of the patients had calcifications in aortic or mitral valve after echocardiographic evaluation.

Interestingly, all the patients had left ventricle hypertrophy, with a median interventricular septum value of 13mm and left ventricle mass of 250g. In our previously studies, LVH was present in 69.4% in 2012, 67.6% in 2015 and 85.1% in 2017. One should observe the increasing trend. LVH is a common cardiac target organ complication of arterial

hypertension. The prevalence of LVH is high in advanced CKD and HD patients, is different among countries and regions, and is influenced by the determination modality (echocardiographic or EKG). In a nice review by Di Lullo on LVH and CKD, it seems that the prevalence of LVH in dialysis patients is up to 90%. In addition, the presence and the severity of LVH is associated with higher mortality in dialysis patients.

Cardiovascular disease remains a major comorbidity in HD patients. Cardiac modifications are high even at the initiation of HD. Most easy way to asses these changes is by echocardiography. In previous published studies, we showed the necessity and importance of periodic cardiac ultrasound evaluation of HD patients and cardiac characteristic's changes impact on mortality (e.g. the increase in EF after one year is associated with higher mortality). With current study, we wanted to identify other specific biomarkers that could influence the cardiac outcome.

Interleukin 1B (IL-1B) is a pro-inflammatory cytokine involved in tissue repair, cell growth and inflammatory response. Increased levels of IL-1B are associated with worst cardiac outcome after myocardial infarction. HD patients have a pro-inflammatory state with higher IL-1B levels. In the face of these statements, we evaluated the correlations between IL-1B and cardiac ultrasound characteristics in this specific population.

The median values of IL-1B in our study was 44.43 pg/ml (95%CI: 43.84-45.19), being lower than the mean values in a cohort of 390 patients evaluated by Yu (84.82 ± 94.38 pg/ml). These differences are most likely due to a lower inflammation status in our cohort (CRP 0.42mg/dl vs 8.46mg/dl in Yu cohort). In another recently published study by Lisowska from 2022 on 67 HD patients, the mean IL-1B levels were 1.75 pg/ml, much lower than Yu's one and lower than the values from our cohort. These differences may be in the context of several factors: haemoglobin levels and anaemia management, inflammation and nutrition status, comorbidities, dialysis vintage and the number of patients.

We identified a good regression model where each increase in 1 unit of IL-1B generated a 0.434 mm increase in left ventricle tele systolic diameter. Also, each increase with 1 g in left ventricle mass generated a 0.02pg/ml increase in IL-1B. In a study by Ørn from 2022 on 42 patients that suffered myocardial infarction, IL-1B levels were associated with myocardial dysfunction and left ventricle mass 1 year after the acute episode. There could be a direct link between IL-1B and left ventricle mass and left ventricle hypertrophy respectively. Animal studies showed that IL-1B influences the myocardium through several factors: augmentation hypertrophy in cardiomyocytes, stimulation of fibroblast migration and growth and promotion of enhanced matrix metalloproteinase activity in myocardium. HD patients are in a chronic pro-inflammatory state with higher levels of IL-1B in most of the studies. IL-1B can contribute and can explain the increased prevalence of left ventricle hypertrophy in this specific population.

As expected, IL-1B was negatively correlated with EF in a regression model, where each unit increase in IL-1B generated a reduction of EF with 0.42%. We did not found any studies on HD patients that evaluated the possible connection between IL-1B and EF. Studies on animal models and on patients following myocardial infarction showed the same correlations between IL-1B and EF. In addition, patients with rheumatoid polyarthritis treated

with Anakinra (IL-1B receptor blocker) showed better cardiac function compared with the patients that received different treatments.

With these results, we can conclude that IL-1B plays an important role in cardiac dysfunction in patients on HD. In a recent randomized control trial in 40 HD patients that received Anakinra, it seems that the adverse events rate was even lower compared to placebo arm and there was a statistical significant reduction on inflammation. The results of this study are expected at the end of this year. This could be the beginning of target intervention on inflammatory cytokines in HD patients.

One last interesting result regarding IL-1B from our pilot study was the association between aortic valve calcifications and IL-1B levels higher than median value (OR=3.57). In a nice review by Shen from 2021 on atherosclerotic calcifications, Il-1B involvement is through several mechanisms: endothelial-to-mesenchymal transition, inhibition the mesodermal progenitor cells, activation of alkaline phosphatase and several factors that increase the levels IL-1B in this atherosclerotic state (hypercholesterolemia, capsase-1 upregulation, Rac2 stimulation of macrophages).

The super family of transforming growth factor has several members. One of them is represented by bone morphogenetic protein (BMP). The most important isoform is BMP2, which regulates cell growth and differentiation. Data about BMP-2 implications and effects in HD patients are scarce. There is a study by Costa from 2013 on 49 HD patients where BMP2 levels were negatively correlated with fistulas as vascular access type, erythrocytes, reticulocytes, haemoglobin, transferrin concentrations and age. Our median values of BMP-2 are much higher compared with the one from Costa's cohort (718.55pg/ml vs 46.41pg/ml). More than 75% of our cohort present at least one site of calcifications and could explain the increased BMP2 levels.

In our statistical analysis, BMP2 inversely correlates to FGF23 (FGF23 above its median value in a patient generated an odds ratio of 0.297 to have BMP2 levels above its median value). In addition, BMP2 levels above its median value generated an odds ratio of 4.08 in a patient of having aortic valve calcifications. In 2022, Ren wrote an updated review of vascular calcifications in CKD patients and presented the different mechanisms involved in vascular calcifications with the major role played by vascular smooth muscle cells (VSMC). BMP2 is upregulated by the inflammation, phosphorus levels and TLR4/NF-kB. Increased levels of BMP2 is associated with higher prevalence of vascular calcifications.

In a logistic regression model with BMP2 levels compared to its median value as dependant variable, it was positively correlated with calcium, E/A rapport, iPTh and iron vials/month and negatively with BMI. One should expect to have a positive correlation with phosphorus also. Our HD patients were already on phosphate binders, most of them being on calcium-based ones and this could explain our results. Also, phosphorus serum levels are influenced by the meal the patient had the prior the determination and its levels may not be that accurate. In Costa's cohort, the BMP2 was negatively correlated to erythrocytes and reticulocytes number, haemoglobin and transferrin concentrations. Our positively correlation with iron vials/month confirm her findings because the patients that need more iron doses are most likely with lower haemoglobin and lower transferrin concentrations. In a study by Ribeiro from 2017 on 38 overweight and 33 obese patients, BMP2 levels were positively

correlated with BMI and the authors concluded that BMP2 could influence the obesity status in these patients. Our mean BMI value was 28, so most of the patients were overweight or obese. Our findings are in opposition with Ribeiro's ones. In dialysis, there is a reversed epidemiology and increased BMI appears to increase survival. A study from 2013 by Zhang on 68 HD patients may confirm our results. Zhang evaluated the calcifications in radial artery and quantified the BMP2 expression in these vessels. He proved that malnourished patients had higher calcifications levels and higher BMP2 expression in the biopsy pieces of radial artery. Thus, in HD patients, it seems that BMP2 levels are correlated to vascular calcifications and in the present of malnourish, the calcification risk increases so does BMP2 expression.

FGF23 was not correlated with any of cardiac parameters. In a study by Xu from 2018 on 1179 people, FGF23 was directly correlated with visceral adiposity and had stronger correlation compared to BMI. In patients on HD, dry one represents the real weight. Thus, FGF23 levels could indicate a higher visceral adiposity in these patients.

Galectin-3 is a predictor of heart failure and it promotes myocardial fibrosis and inflammation. In our cohort, Galectin 3 was not correlated with cardiac parameters. We identified a negative correlation with dialysis vintage. A recent study by Liu suggest that Galectin-3 high levels increase the cardio-vascular death among HD patients. The mortality rate is higher in the first year of HD initiation. Our results can be explained by the fact that patients with lower HD vintage have higher Galectin-3 levels and are at a higher risk of death.

Suppression of tumorigenicity 2 (ST2) levels are correlated with left ventricular hypertrophy and with all-cause mortality rate in HD patients. In our cohort, we did not found any correlation with LVH or other cardiac parameters. We did found that males were more likely to have higher levels of ST2 compared to females, values confirmed by other studies. An interesting result in our study was the protection against aortic valve fibrosis with increased ST2 levels. The increase with one unit of ST2 levels reduced with 1% the probability in a patient of having aortic fibrosis. Also, the mean values of ST2 were higher in patients without aortic fibrosis compared with the ones that already developed it (p=0.02). Recent data from literature suggest that patients with aortic stenosis and EF<50% (and associated aortic fibrosis) have higher ST2 values compared to non-heart failure ones. Also, in a review by Gao from 2015 on IL33/ST2 effect on heart fibrosis, it seems that there are several mechanisms involved. ST2 is a decoy receptor for IL33. Increased ST2 levels are associated with increased risk of fibrosis. Several cells from the heart actively produce soluble ST2. In the face of fibrosis, the reducing in levels of soluble ST2 may be also a consequence of a reduced number of cells that produce it. Thus, patients with already developed fibrosis may have a reduction in soluble ST2 production, thus lower mean serum levels. This could explain our findings, but this should be confirmed by studies on a larger number of patients.

In the second part of this study, we evaluated several biomarkers and their impact on echocardiographic parameters in patients with diabetic kidney disease (DKD). The cohort consisted in 52 patients, mean age 64 years, mean eGFR of 56.5 ml/min/1.73sm and 53.8% males. The DM history was high (15.18 years) and uACR levels were 79mg/g. We measured

CTGF, FGF23, IL6, KIM-1, soluble Klotho and uMCP1/creatinine ratio. We also performed an echocardiography in all patients.

Kidney injury molecule-1 (KIM-1) is a type-1 transmembrane protein expressed in the kidney. It is a biomarker of proximal tubular injury but plays an important role in renal healing after an episode of kidney injury. Also, it is correlated with inflammation and fibrosis. Even though it is more of an acute kidney injury marker, it is a tubular damage marker also. The renal involvement in DM is complex and besides glomerular changes, there is tubular involvement too. More than 40% of patients with DKD do not have albuminuria, but biopsies showed the early tubular dysfunction in these patients. Also, diabetic patients with higher KIM-1 levels have a higher risk of kidney function decline.

In our study, we identified a strong correlation between KIM-1 and EF and each unit increase in KIM-1 generated a 0.017% increase in EF. Studies that evaluate cardiac function and KIM-1 were performed most on patients with acute kidney injury. In a study by Jungbauer on patients with chronic heart failure, KIM-1 levels were higher in patients with low ejection fraction. Our findings are opposite to ones of Jungbauer. An increased ejection fraction may be secondary to increased circulating volume. In patients with tubular injury, there could be a state of hypervolemia even at higher GFR levels. Thus, an increase in KIM-1 reflect the tubular injury that leads to over hydration and thus may increase EF. In addition, our cohort consisted of patients with no heart failure and it is possible that the relationship between KIM-1 and EF to be different in normal cardiac status. There is a need for prospective studies in order to evaluate if KIM-1 levels can predict future cardiac outcomes.

Connective tissue growth factor (CTGF) is a regulatory and signalling protein that is involved in many biological processes, like tumour development, fibrosis, angiogenesis and cell proliferation. KIM-1 is mostly a marker of tubular damage. In our study, we found a strong positive correlation between these two biomarkers. Secondly, in a regression model, each unit increase in CTGF generated an increase with 43.5 units in KIM-1. This strong correlation seems to be logical and may represent an already associated important tubular damage with an important degree of kidney fibrosis. Ito proved since 1998 that in kidney biopsies with glomerulonephritis and tubulointerstitial fibrosis, there an increase in CTGF expression. In a recent study by Bauer on zebrafish larvae exposed to gentamicin, there was an upregulation in KIM-1 and CTGF genes after proximal tubular injury.

Even though the correlation between KIM-1 and uACR in our study was not strong enough (p=0.056), we identified a good regression model with KIM-1 as dependant variable. Therefore, each unit increase in uACR generated 0.1 units increase in KIM-1. Albuminuria is a marker of tubular injury, besides glomerular one. In a recent study by Gohda on 600 DM patients, the authors identified a strong correlation between KIM-1 levels and uACR.

KIM-1 had a strong correlation with Klotho levels (p=0.0058) and in a regression model with Klotho as dependant variable, each unit increase in KIM-1 level generated 1.25 units increase in Klotho. It seems that proximal tubular injury is associated with elevated s-Klotho circulating levels, as we showed in a previously published article.

We found strong negative correlations between s-Klotho and initial eGFR and respectively the eGFR after 1-year follow-up. Thus, higher s-Klotho levels were correlated with lower eGFR values. Most of the authors described increase in s-Klotho levels in CKD

and AKI patients, while others describe a reduction of it. In our cohort, each 1-unit increase in s-Klotho levels generated 0.057 unit reduction in eGFR. The excretion route of s-Klotho is unknown. We can assume that elevated s-Klotho in patients with reduced eGFR values because of reduced excretion. Hu MC identified Klotho in urine of the mice after intravenously injection, but not in the Bowman's space. Klotho is a heavy protein and is not passing the glomerular membrane; its urine excretion is most likely through tubular mechanisms. This excretion route could explain elevated s-Klotho levels in patients with reduced GFR and tubular damage, as seen in our cohort. S-Klotho was positively correlated with uACR and each unit increase in uACR generated 0.17 units increase in s-Klotho levels. Albuminuria could be a marker of tubular injury. The "retrieval pathway" of albumin suggests that the excess albumin is retrieved from urine at proximal tubular level through a transcytosis mechanism. Thus, increased albuminuria could mean proximal tubular injury. In our cohort, uACR had a direct correlation with s-Klotho and KIM-1, in concordance with the "retrieval pathway" theory.

Monocyte chemoattractant protein-1 (MCP-1) is a member from the chemotactic cytokines. It is a small, signalling protein with important roles in inflammation. MCP-1 is correlated with reduced GFR and rapid decline in eGFR, increased albuminuria and atheroma plaque instability. Augmentation index is an accepted measurement of arterial stiffness and reflects endothelial function. Higher values of arterial augmentation index are associated with higher arterial stiffness. Several studies showed positive correlations with hypercholesterolemia, systolic blood pressure and heart rate. We identified a negative correlation between uMCP-1 and aortic augmentation index (AAX). Also, in a regression model with AAX as dependant variable, each unit increase in uMCP1 generated 0.08 units decrease in AAX. In a study by Zinehh on 98 children with type-1 DM there was no correlation between augmentation index and serum MCP-1 levels. In a article by Sveen on 27 type-1 DM patients, the authors found a positive correlation between serum MCP-1 and AAX. Finally, in a study by Alpay on 15 renal transplant recipients, there was no correlation between AAX and uMCP-1. As we can see, the published data about AAX and uMCP-1 are scarce and different. One should expect that increased levels of uMCP-1 reflect an inflammatory and fibrotic status, thus leading to endothelial dysfunction translated in increased AAX. There an increase need in studies with larger cohorts to better assess the relationship between augmentation index and uMCP-1 levels.

As we showed in these two pilot studies, many biomarkers permit clinicians to better assess cardiac function in patients with DKD or on HD. The possible intervention on these markers could increase survival. These studies could become a starting point in large-scale evaluation on intervention in specific biomarkers with hard end points like survival or cardiac function.