

**VICTOR BABEȘ UNIVERSITY OF MEDICINE
AND PHARMACY TIMIȘOARA
FACULTY OF MEDICINE
DEPARTMENT VII OF INTERNAL MEDICINE II**

ALBULESCU NICOLAE



PhD THESIS

**THE PARTICULARITIES OF CARDIOVASCULAR
DISEASE IN HEMODIALYSIS PATIENTS IN ROMANIA:
FROM SCREENING TO THERAPY**

A B S T R A C T

Scientific Coordinator

PROF. UNIV. DR. HABIL ADALBERT SCHILLER

**Timișoara
2021**

CONTENT

List of published papers	VII
List of abbreviations	VIII
Index of figures.....	X
Index of tables.....	XII
INTRODUCTION – MOTIVATION FOR THE RESEARCH	XIII
GENERAL PART	
1. Epidemiology of cardiovascular disease and prevalence of cardiovascular mortality in patients with chronic end-stage kidney disease	1
2. Classic and specific risk factors for cardiovascular disease in the population with chronic end-stage kidney disease	3
2.1 Age and gender	3
2.2 Hypertension.....	3
2.3 Dyslipidemia	4
2.4 Diabetes mellitus	5
2.5 Smoking	5
2.6 Ischemic coronary artery disease	6
2.7 Sedentarism	7
2.8 Oxidative stress	7
2.9 Uremic cardiomyopathy.....	8
2.10 Anemia.....	8
2.11 Proteinuria	9
2.12 Mineral and bone disorder and cardiovascular calcifications	10
2.13 Inflammation and the role of CRP	11
2.14 Hemodynamic instability during hemodialysis	11
3. Cardiovascular disorders in the hemodialysis population	12
3.1 Left ventricular hypertrophy.....	12
3.2 Myocardial ischemia and hemodialysis.....	14
3.3 Hemodialysis-induced myocardial stunning.....	14
3.4 Hibernating myocardium and cardiac fibrosis	15
3.5 Ischemic preconditioning.....	16
3.6 Cardiac troponins in hemodialysis patients.....	16
3.7 Ventricular arrhythmias and hemodialysis	17
3.8 Atrial fibrillation in hemodialysis.....	18
3.9 Intradialytic hypotension and ultrafiltration rate	19
3.10 Left ventricular systolic and diastolic dysfunction	20
3.11 Calcifications and valvular heart disease in hemodialysis	22
3.12 Heart failure	23
3.13 Cardiac implications of vascular access in hemodialysis	25
4. Sudden cardiac death in patients with chronic end-stage kidney disease	26
4.1 Definition, etiology and epidemiology of SCD in patients with ESRD	26
4.2 Ischemic coronary artery disease and SCD	28
4.3 Factors promoting myocardial fibrosis	28
4.4 Autonomic dysfunction	29
4.5 Hydroelectrolytic imbalance and hyperkalemia	29
4.6 Uremic cardiomyopathy and SCD	29
5. Electrocardiographic and echo screening of the hemodialysis patient.....	31
5.1 Standard 12 lead resting electrocardiogram.....	31
5.1.1 Heart rate variability	31
5.1.2 Dispersion of the QT segment.....	32
5.1.3 Micro-voltage T-wave alternation.....	32
5.2 Transthoracic echocardiography.....	33
6. The particularities of therapy in the hemodialysis patient	37
6.1 Beta-blockers.....	37

6.2 RAAS blockers	37
6.3 Statins	38
6.4 Antiplatelet agents	38
6.5 Anticoagulants	38
6.6 Implantable devices	39
6.7 Diuretics	39
SPECIAL PART - PERSONAL CONTRIBUTIONS	
1. Furosemide Pharmacodynamics and Cardiovascular Effects in Hemodialysis Patients	41
1.1 Introduction and study objectives	41
1.2 Materials and methods	44
1.3 Results	47
1.4. Discussions	50
1.5 Conclusions	53
2. Predictive Value of Several Echo Parameters for Cardiovascular Events in Hemodialysis Patients with Mid-range and Preserved Ejection Fraction Heart Failure	54
2.1. Introduction and study objectives	54
2.2. Materials and methods	56
2.3. Results and discussions	62
2.4 Conclusions	65
3. Effects of chronic hemodialysis on echocardiographic parameters	67
3.1 Prevalence and prognostic implications of valvulopathies in dialysis patients	
3.1.1 Introduction and study objectives.....	67
3.1.2 Materials and methods.....	67
3.1.3 Results	69
3.1.4 Discussions	72
3.1.5 Conclusions.....	74
3.2 Prognostic implications and prevalence of significant mitral-aortic calcifications in dialysis patients	74
3.2.1 Introduction and study objectives.....	74
3.2.2 Materials and methods.....	75
3.2.3 Results.....	77
3.2.4 Discussions	79
3.2.5 Conclusions.....	80
4. RAAS blockade in hemodialysis population. Impact on mortality and echocardiographic parameters.	81
4.1 Introduction and study objectives	81
4.2 Materials and methods	84
4.3 Results	85
4.4 Discussions	90
4.4 Conclusions	92
5. The effects of ferric carboxymaltose in dialysis patients with heart failure	94
5.1 Introduction and study objectives	94
5.2 Materials and methods.....	95
5.3 Results	98
5.4 Discussions	101
5.4 Conclusions.....	103
6. Pharmacokinetics of apixaban treatment in dialysis patients	104
6.1 Introduction and study objectives	104
6.2 Materials and methods	105
6.3 Results	106
6.4 Discussions.....	107
6.4 Conclusions	110
FINAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS	111
REFERENCES	120
ANNEXES	I

INTRODUCTION

Chronic kidney disease is known (CKD) to be an important public health problem worldwide. The estimated global prevalence of CKD is 13.5% (11-15%) and the number of ESRD patients requiring renal replacement therapy is increasing. Mortality is very high among dialysis patients and almost half of all deaths were due to cardiovascular causes. About half of these were due to sudden cardiac death or cardiovascular death within one hour of the onset of symptoms. This high incidence reflects the frequency of structural and functional cardiac abnormalities in this group of patients and early detection of these changes may be possible with echocardiography and electrocardiography performed periodically.

The largely unexplained reverse epidemiology of cardiovascular disease among end-stage renal disease (ESRD) patients is an indicator that, despite the ongoing advances in understanding and managing cardiovascular disease and chronic kidney disease, we still do not understand the intersection of these comorbid associations. In addition, the impact of renal replacement therapy on cardiovascular function and its alterations is not well understood and may inadvertently contribute to the accelerated development of cardio-renal syndrome. This thesis aims to provide an overview of cardiovascular changes in ESRD, a description of the mechanisms reported for HD-induced myocardial injury, a comparison of various treatment modalities in the context of cardiovascular disease, and possible future management strategies.

GENERAL PART

Chronic kidney disease is a concept based on a continuum starting with progressive chronic kidney damage and ending with loss of kidney function. It is a disease with a significant global epidemiological increase, and studies of recent years have increasingly associated this pathology with an increased risk of cardiovascular morbidity and mortality.

With a prevalence between 10-15% in Western countries, CKD is a public health problem, and more than half of all deaths among patients with ESRD are due to cardiovascular disease. Interestingly, patients with CKD show reverse associations of traditional CVD risk factors compared to the general population. Obesity, hypercholesterolemia and hypertension seemed paradoxically to be protective pathologies in contrast to the general population. In fact, in patients on renal replacement therapy, the risk of prevalence of coronary heart disease and left ventricular hypertrophy was found to be 40% and 70%, respectively. The prevalence of hypertension, a major risk factor for coronary artery disease and left ventricular hypertrophy (LVH), is high in patients with chronic kidney disease by up to 90%. At least one third of patients with CKD have evidence of an ischemic event (myocardial infarction or angina pectoris) at the time of presentation to the nephrologist. The prevalence of LVH increases with each stage of CKD, reaching more than 70% at the time of dialysis initiation, and favorable/modifiable risk factors for LVH include anemia and systolic blood pressure, which also progress with each stage of CKD. A better understanding of the impact of these many factors on CVD would be an important step towards prevention and treatment.

There is evidence that uremic factors play a major role in the pathogenesis of CV disease in hemodialysis patients, as CV survival improves after renal transplantation even in high-risk patients. At the moment, other factors are under investigation in patients with ESRD, such as cardiotonic steroids, a new class of hormones with the ability to bind and inhibit the enzymatic activity of the ubiquitous Na/K pump. Serum levels of ouabain, telocinobufagin and marinobufagenin are substantially elevated in ESRD patients. There is evidence that chronic exposure to cardiotonic steroids may contribute to the development of cardiovascular disease characterized by the presence of LVH, fibrosis, diastolic dysfunction, arrhythmias and a reduction in ejection fraction.

In patients with chronic kidney disease, FGF23 (fibroblast growth factor 23) levels increase from the onset of CKD (even in stage 2), earlier than phosphate and PTH increases. Klotho and FGF23 could be new targets for early diagnosis of renal dysfunction and prediction of chronic complications, including CVD in ESRD. Thus, a high plasma FGF23 concentration is independently associated with an increased risk of CKD progression, CVD complications and increased mortality in these patients.

Although HD is the most effective method of correcting metabolic acidosis in patients with ESRD, the changes in serum bicarbonate levels produced by a dialysis session are sometimes too abrupt, producing adverse effects. There is an association between dialysis bicarbonate, serum calcium and phosphorus levels, and PWV values with the frequency of vascular calcifications. Large fluctuations in serum bicarbonate levels in HD patients should be avoided, as these fluctuations may increase vascular stiffness, vascular calcifications and overall CV risk. Blood urea levels increase with progressive decline in renal function. Increased urea levels cause disturbances in various target organs. Protein carbamylation is linked to increased CV risk in patients with ESRD. HD intensification may help control plasmatic urea levels and reduce carbamylated protein concentrations, reducing CV risk. HD itself may induce intestinal ischemia during intradialytic hypotension with alteration of intestinal wall integrity. Changes in gut microbiota composition produce excessive amounts of uremic toxins such as p-cresol sulfate, trimethylamine-N-oxide, and these contribute to the progression of chronic kidney disease as well as cardiovascular disease, accompanied by high CV mortality.

Premature cardiovascular death is the most common cause of death in patients with end-stage renal disease, including those receiving or scheduled to receive renal replacement therapy. Myocardial structural changes associated with uremic cardiomyopathy, have been associated with sudden cardiac death. In patients with CKD, deaths from cardiovascular causes are most commonly due to malignant arrhythmia and SCD, 10-20 times higher compared to the general population, where ischemia and acute myocardial infarction predominate. The development of fatal cardiac arrhythmia requires an abnormal "substrate" (LVH, fibrosis, cardiomyopathy) interacting with a "triggered mechanism" (ischemia, hyperkalemia). Both substrate and triggers are extremely common in patients with CKD. On the other hand, the huge cardiovascular risk is enhanced by the accumulation of classical (hypertension, diabetes, dyslipidemia, obesity, smoking) and "new" CKD risk-related factors (oxidative stress, hyperhomocysteinemia, endothelial dysfunction, inflammation, proteinuria, anemia, mineral-bone disorder), as well as due to arrhythmic vulnerability secondary to pathophysiological changes due to LVH, myocardial ischemia/fibrosis and ischemic coronary artery disease. Myocardial ischemia can be precipitated by hemodialysis, and this concept, of subclinical dialysis-induced ischemia (occurring without acute atherosclerotic plaque rupture), has received remarkably little attention, despite its theoretical plausibility. Thus, transient myocardial ischemia in HD can lead to left ventricular dysfunction, which may persist even after restoration of normal coronary flow. Therefore, if myocardial ischemia and myocardial "stunning" are induced by hemodialysis, then the hemodialysis process itself, repeated three times a week, may contribute to chronic cardiac damage in this group of patients. Thus, myocardial "stunning" is being increasingly considered as an underappreciated causal mechanism of heart failure in the hemodialysis population. Identification of high-risk patients is desirable in order to implement prophylactic measures and reduce the rate of SCD in ESRD patients. However, this remains very difficult due to the multifactorial nature of SCD in this population. Echocardiography and electrocardiography remain indispensable tools in the management and prognostic prediction of dialysis patients. However, the interpretation of electro- and echocardiographic changes in this context should be differentiated from that of the general population.

Heart failure is very common among patients with CKD and it reflects a particularly severe prognosis. Pressure and volume overload, as well as non-hemodynamic factors associated with CKD, will induce LVH, lowering coronary microcirculation density and increasing myocardial fibrosis, ultimately resulting in diastolic and systolic LV dysfunction. Moreover, the direct effects of uremia, hemodialysis-induced myocardial ischemia, and the

hemodialysis process itself may well contribute to the development of congestive heart failure. High output heart failure has been associated with the presence of arteriovenous fistula. All of these pathophysiological mechanisms and almost irreversible structural abnormalities in uremic cardiomyopathy are common in patients with ESRD, and have been incriminated as determinants of increased morbidity and mortality.

Patients with ESRD are also at increased risk of developing valvular heart disease, partly due to structural/hemodynamic changes and partly due to complex metabolic alterations that predispose to valvular calcification, dilated cardiomyopathy and endocarditis. Valvular calcification may be a good marker of negative prognosis, as it is also a marker of vascular calcification. Calcium-phosphate product value is an independent predictor of heart valve calcifications, and it may be possible that optimal treatment of CKD-MBD might ameliorate or prevent advanced valvular calcification.

Atrial fibrillation is the most common arrhythmia detected in hemodialysis patients and its development is due to structural and electrical remodeling of the atrial myocardium. In dialysis patients with AF, the metabolic and hemodynamic imbalance associated with hemodialysis seems to modulate the AF substrate, thus contributing to its high incidence. Echocardiography allows us a comprehensive assessment of LA structural and functional changes that may precede the onset of AF. However, the association between these echocardiographic parameters and AF in ESRD, remains unexplored. Special attention should be dedicated to this atrial arrhythmia, and in particular to the practical measures that derive from echocardiographic assessments that can predict the timing of onset, as well as to the treatment challenges, in particular anticoagulation therapy, but also to whether Xa factor inhibitors are appropriate for the hemodialysis population.

In a large number of dialysis patients, cardioprotective drugs are not prescribed. The reason is simple, namely that there are no relevant data for patients with ESRD, due to their exclusion from interventional studies and the absence of post hoc studies on the analyzed population subgroups. Given the high cardiovascular mortality in this at-risk population, greater attention to cardiovascular risk reduction by all available therapeutic means, is warranted.

The aim of the present paper was to investigate and objectify the biunivocal character of these two resounding pathologies: advanced chronic kidney disease and cardiovascular disease, this continuous intricacy between the cardio-renal/reno-cardiac syndrome and the permanent "switch" between the aggressed organ - aggressor organ, but also the cardiovascular/renal pathophysiological continuum, approached by modern methods, adapted treatment and careful screening for a better achievement of the surrogate end-points.

SPECIAL PART - PERSONAL CONTRIBUTIONS

1. FUROSEMIDE PHARMACODYNAMICS AND CARDIOVASCULAR EFFECTS IN HEMODIALYSIS PATIENTS

Furosemide is a drug that has not only a renal but also a vascular effect, resulting in decreased atrial and left ventricular filling pressures accompanied by an increase in venous compliance, all with significant effects on cardiac hemodynamics. In dialysis patients, the efficacy of furosemide, this inhibitor of the Na⁺, K⁺, 2Cl co-transporter in the tubulo-renal system, is still controversial. In addition, furosemide needs to be used in much higher doses because of pharmacokinetic changes in the context of impaired renal clearance. The aim of our study was to investigate whether furosemide induces changes in cardiovascular hemodynamics in dialysis patients using standard echocardiography and Tissue Doppler Imaging.

We included 101 patients with ESRD who were treated by hemodialysis, three times a week, for more than 6 months, in whom there was some residual diuresis (between 250 - 650 ml/day). Patients were divided into 2 groups according to the presence of furosemide treatment (furosemide group-n=47 and control group-n= 54). We did not establish significant

morbidity-mortality related objectives and thus not being a study with primary/secondary hard end-points, we simply evaluated the changes in echocardiographic parameters and the influence of furosemide on the cardiac structural and functional changes. In order to calculate statistical significance, we performed comparisons between the two groups using the Student's t-test and Fisher's exact test. Correlation was assessed with Pearson's test. Comparisons between baseline and follow-up measurements were performed using ANOVA (Turkey post-hoc).

The conclusions were as shown below:

- There were no significant differences between group characteristics (gender, age, etiology, dialysis duration, vascular access, BMI, BSA and presence of other risk factors).
- There was a significantly better urine volume in the furosemide group ($p < 0.0001$), proportionally correlated with a higher furosemide dose.
- None of the patients had normal diastolic function and almost all had LVH/Cardiopathy with normal EF.
- There were no differences in blood pressure values throughout follow-up in either group.
- All patients were examined immediately before dialysis, which was demonstrated by elevated E/e' values, especially in the non-furosemide group, indicating elevated capillary-pulmonary pressure > 12 mmHg and E/e' ratio > 15 .
- Standard echo parameters such as E wave, A wave, E/A ratio, EDT, LA parameters (diameter, volume) and PSAP were significantly higher in the control group and they worsened over time, reaching statistical significance ($p < 0.05$).
- TDI parameters (e' wave, a' wave, E/e' ratio) are directly proportional to capillary-pulmonary pressure and increased LA-LV filling pressures.

The issue we often face is whether or not, diuretic treatment should be continued on dialysis, especially if patients have been/are on dialysis for a period of time. For the hemodialyzed population with remaining diuresis, an adequate dose of furosemide, controls interdialytic weight gain, may lead to better control of symptoms and may reduce specific HF risk. Moreover, there may be a favorable impact on cardiac remodeling and cardiovascular morbidity and mortality.

The loop diuretic can also increase potassium excretion, thereby reducing the risk of hyperkalaemia and its dangerous complications. The administration of loop diuretic must take into account the heterogeneity of patients, the volume of remaining diuresis, the interdialytic weight variation, but also aspects related to alterations in vascular elastance and vasomotricity, all of which, together with echocardiographic assessment, are essential for establishing the best therapeutic strategy.

To summarize, our data suggest that HD patients who are using long-term furosemide, in proper doses, have a higher residual diuresis volume than patients who are not using this drug. Corroborated with favorable changes in tissue and spectral Doppler, we suggest that a prospective analysis could be performed in a larger number of patients with more parameters, both sonographic and biological/clinical, in order to identify possible mechanisms of long-term furosemide use, and its role in preserving residual renal function as well as improving symptomatology and cardiovascular status.

We found correlations between furosemide use and improvement in clinical and cardiovascular parameters, assessed by multiple echocardiographic variables, thus furosemide has complementary effects in dialysis patients with residual diuresis. In our study group we noticed a significant difference in the final echocardiographic report. All furosemide patients had a significant hemodynamic impact, thus atrial and ventricular filling parameters quantifiable by tissue Doppler were improved, and also there was a balance of interdialytic weight gain and diuresis, providing valuable data on volume overload and allowing treatment individualization. Therefore, our results indicate that the use of furosemide after initiation of dialysis could be an effective and inexpensive strategy to both individualize patient care and improve renal and cardiovascular endpoints.

2. PREDICTIVE VALUE OF SEVERAL ECHO PARAMETERS FOR CARDIOVASCULAR EVENTS IN HEMODIALYSIS PATIENTS WITH MID-RANGE AND PRESERVED EJECTION FRACTION HEART FAILURE

Cardiac structure and function are frequently altered in ESRD patients treated with hemodialysis. Most of the cardiac parameters assessed by echo are predictive of poor prognosis. The development of heart failure, which can occur in up to 50% of hemodialysis patients, reflects poor prognosis. The main challenge in this population is to identify the patient at risk and adjust treatment for better clinical outcome and longer survival. Specific ESRD alterations at the cardiovascular level leads to morphological and functional alterations of the heart that can be detected echocardiographically and can have a significant impact on long-term prognosis.

Two-dimensional transthoracic echocardiography is a reliable method as well as a rapid and widely used tool in clinical practice for assessing any underlying abnormality. However, it is not perfect and has limitations, with some challenges specific to the hemodialysis population. This chapter highlights some of the difficulties in obtaining accurate results and sensitive markers of cardiac dysfunction using transthoracic echocardiography in hemodialysis patients, including an explanation for the need of new echocardiographic techniques.

The prevalence of diastolic dysfunction is reported to be between 45% and 75% in dialysis patients. Diastolic dysfunction, independently but also in combination with other clinical factors and echocardiographic parameters, has been shown to be a predictor of adverse cardiovascular events and mortality. Diastolic dysfunction with elevated filling pressures in dialysis patients often exists without the presence of systolic heart failure and can be assessed by Doppler ultrasonography, especially Tissue Doppler Imaging (TDI), a technique that has proven its prognostic value for CV events in the general population.

In this retrospective analysis, 61 patients undergoing chronic HD (>3 months) with preserved and mid-range EF, were selected. Thus, patients were classified into the 2 types of HF:

1. Heart failure with preserved EF: presence of signs and symptoms of HF, EF>50%;
2. Heart failure with mid-range EF: presence of signs and symptoms of HF, EF between 40 and 49%;

During the study we monitored echocardiographic, clinical and paraclinical parameters and looked for those changes that might be predictive of cardiovascular events or mortality. To calculate statistical significance, we performed between-group comparisons using Student's t-test and Chi-Square test. Hazard ratios (HRs) and 95% confidence intervals (CIs) for morbidity and mortality were calculated using Cox proportional hazards models. Survival curves were projected using the Kaplan-Meier method and the log rank test was used to compare survival curves in univariate analysis. Statistical significance was considered if $p < 0.05$.

Therefore, the following conclusions resulted:

- The CV event group had an increased prevalence of diabetes, previous CV disease, higher LVEDD and lower EF/SF with systolic and diastolic dysfunction.
- The most common echocardiographic changes were as follows: LV diastolic dysfunction (85%), LV systolic dysfunction(27%), LA dilatation (48%), LVH (84%) and LV dilatation (21%).
- Each echocardiographic parameter was included in the univariate risk model as a continuous variable, while some were included as a fixed variable. Statistical significance was obtained for EF, SF, LVEDD, LVEDV, kinetic abnormalities, s' , e' and E/e' ratio.
- In the final regression model, history of cardiovascular disease and severe diastolic dysfunction were found to be independent predictors for fatal and non-fatal cardiovascular events.

- Only diastolic dysfunction, E/e' ratio and previous history of cardiovascular disease were parameters that reached statistical significance associated with cardiovascular mortality in univariate analysis.
- Increased E/e' ratio was correlated with an increased risk of cardiovascular events. Thus, the E/e' ratio, representative of diastolic LV dysfunction, was the strongest predictor of all cardiovascular events.

All these results pave the way for important considerations when it comes to future studies involving transthoracic echocardiography, such as the importance of performing echocardiography on a non-dialysis day when patients are dry weight in order for echo parameters to be measured accurately. Secondly, it outlines the main rationale for the aims and objectives of this thesis, namely to explore novel echocardiographic techniques in providing prognostic information. Future parameters to explore include speckle tracking technique and mitral annulus tissue motion, as well as three-dimensional transthoracic echocardiography with more accurate measurements of left ventricular mass, volume and contractility.

Thus, this study highlights that diastolic dysfunction and E/e' ratio are the most significantly predictive parameters for cardiovascular events and mortality. Diastolic dysfunction (grade II and III), assessed by Echo and tissue Doppler, was an independent predictor for cardiovascular events and should be added in the standard evaluation of dialysis patients. The present study may help us for better guidance and surveillance of dialysis patients with abnormal echo parameters. Therefore, by enabling early detection of these high-risk patients, we can take appropriate measures to reduce morbidity and mortality.

3. EFFECTS OF CHRONIC HEMODIALYSIS ON ECHOCARDIOGRAPHIC PARAMETERS

3.1. Prevalence and prognostic implications of valvulopathies in dialysis patients

Patients with ESRD on hemodialysis are at increased risk of developing significant valvulopathies due to altered hemodynamics and metabolic status which promote calcification and secondary valve dysfunction. However, the prevalence of these valvulopathies in dialysis patients remains partially known. There are a variety of proposed contributing factors such as aging, hyperphosphatemia, elevated calcium-phosphorus product values, and inflammation. Besides maximal drug therapy, surgical/transcatheter valve replacement/repair are the only curative or supportive therapies for these valvular heart diseases. The prognostic implications of severe, untreated valvulopathies in the general population are known, but they have not been extensively studied in hemodialysis patients.

Therefore, the objectives of the study were to assess the prevalence of valvular heart disease (VHD) in a cohort of dialysis patients and its prognostic implications, including the frequency of need for surgery or transcatheter interventions and all-cause mortality.

For this retrospective study, patients were selected from an annual echocardiographic registry of dialysis centers. All patients on chronic (>1 year), periodic (3/week) dialysis who had transthoracic echocardiography were included (n=279). Patients were divided into two groups according to the existence of one or more significant valvulopathies, defined as moderate or severe mitral regurgitation (MR), mitral stenosis (MS), tricuspid regurgitation (TR), aortic regurgitation (AR) and/or aortic stenosis (AS). Differences between the two groups were analyzed using Student's t-test or Mann-Whitney U test for continuous data and chi-square test or Fisher's test for categorical data, as needed. Kaplan-Meier curves were used to calculate cumulative overall mortality-free survival rates. To compare cumulative event-free survival rates between the two groups, the log-rank test was used. To determine the independent association between significant valve disease and all-cause mortality, univariable and multivariable Cox regression analyses were performed.

Out of 279 HD patients (55% male, mean age 61 ± 13 years), 73 (25%) had significant valvular heart disease. To be more specific, 37 (38%) patients had significant MR (25 patients with moderate MR, 12 patients with severe MR), 24 (25%) patients had significant TR (19 patients with moderate TR, 5 patients with severe TR), 16 (15%) patients had significant AR (15 patients with moderate AR and 1 patient with severe AR) and 24 (23%) patients had significant AS (21 patients with moderate AS, 3 patients with severe AS). There were no patients with significant MS. Of the 37 patients with significant MR, 24 patients had secondary MR and 13 patients had primary MR. Patients with significant VHD were older, had a higher heart rate, and more frequently had symptoms of NYHA class III-IV heart failure, peripheral arterial disease, and atrial fibrillation compared to patients without significant valvular disease. In addition, patients with significant valvulopathy were using significantly more oral anticoagulants and diuretics compared to patients without. They also had larger diameters and volumes (LVEDD, LVESD, LVEDV, LVESDV) lower LV ejection fraction, larger LA volume index and a higher LV mass index compared to patients without. LV filling pressures (E, A, E' waves, E/e' ratio) were higher in patients with significant valvulopathy compared to the others.

During an average follow-up of 36 months, 15% of patients with significant VHD had an indication for surgery/transcatheter, and 32% of patients later died. Cumulative mortality rates at 12, 24, and 36 months were 28%, 39%, and 44% for patients with significant valvular heart disease versus 9%, 11%, and 19% for patients without ($P < 0.01$). On univariate Cox analysis, heart rate, NYHA class, presence of peripheral arterial disease, atrial fibrillation, SV mass index, SV ejection fraction, AS volume index, E/e' ratio, and presence of significant valvular disease were associated with all-cause mortality. In multivariate Cox analysis, significant valve disease was independently associated with increased risk of cardiovascular mortality after adjusting for age, NYHA class, LV mass index, ejection fraction, lateral E/e' ratio, and LA volume index.

Therefore, the prevalence of significant valvular heart disease in hemodialysis patients is around 25%. Surgical or transcatheter treatment of the valve was avoided, both by the patient and the multidisciplinary team, given the comorbid cumulus. In this population, patients with significant valvulopathy had a significantly higher risk of overall mortality compared to other patients. The high mortality risk in patients with ESRD and significant valvular heart disease indicates the importance of an appropriate protocol and multidisciplinary team for proper management. Prospective studies are needed in the future, to investigate the role of valve surgery and transcatheter interventions in patients with ESRD and severe valve disease.

3.2. Prognostic implications and prevalence of significant mitral-aortic calcifications in dialysis patients

In patients with ESRD and dialysis, valvular calcium is an important mechanism underlying valve dysfunction. Valvular calcifications are associated with an increased risk of all-cause mortality in patients with end-stage chronic kidney disease. However, little is known about the prognostic implications of left side valvular calcifications in dialysis patients. Calcification of the valve and mitral annulus is common in hemodialysis patients, with prevalence ranging from 19% - 84%. Knowledge of these valvular diseases in the general population is not always applicable to dialysis patients, as the pathophysiology may be different and patients with ESRD may have a high prevalence of comorbid conditions as well as a high risk of periprocedural complications and mortality. Therefore, the aim of the present study was to investigate the prevalence of mitral and aortic valve calcifications using transthoracic echocardiography and to assess their association with all-cause mortality.

As it is a sub-analysis of the above study, we used the same methods and materials, retrospectively selecting dialysis patients from an annual echocardiographic registry of dialysis centrum. Valvular calcification can be observed as echocardiographic hyperechogenicity. We used as a cut-off, hyperechogenic >1 mm at the valve level as an indicator of its presence, according to some international recommendations.

Continuous variables were compared between groups using Student's t-test or Mann-Whitney U test as appropriate. Categorical variables were compared between groups using

the chi-square test. The Kaplan-Meier method was used to calculate cumulative event-free survival rates for all-cause mortality. The log-rank test was used to compare cumulative event-free survival rates between groups. Univariable and multivariable hazard models and Cox regression were used to assess the independent association of mitral or aortic valve calcification with all-cause mortality.

Out of 206 dialysis patients (55% male, mean age 60 ± 13 years), 67 (33%) patients had significant mitral-aortic calcifications. During a mean follow-up of 36 months, 28 (13%) of the patients died. Specifically, 16 (23%) patients had both mitral and aortic calcifications, 41 (63%) patients had only aortic calcifications, and 10 (14%) patients had only mitral valve calcification. Patients with mitral and/or aortic valve calcification were older, had a higher body mass index and more frequent ischemic coronary artery disease compared to patients without calcifications. Patients who had significant mitral-aortic calcifications had significantly higher mortality rates compared to patients without them ($p=0.010$). On univariable analysis, age, dialysis age, body mass index, atrial fibrillation, previous myocardial infarction and mitral and aortic valve calcium were associated with secondary cause mortality. On multivariable analysis, mitral valve calcification was independently associated with an increased risk of all-cause mortality after correction for age, sex, anterior myocardial infarction and atrial fibrillation, whereas aortic valve calcification was not similarly associated.

In the present study, one third of dialysis patients had mitral and/or aortic calcifications. The presence of mitral and/or aortic valve calcium was associated with poor survival in this population, but only mitral valve calcium was independently associated with an increased risk of all-cause mortality, whereas aortic valve calcification was not. The prevalence of left valve calcification in ESRD patients treated by hemodialysis is high and was associated with poorer survival. Future studies are needed to investigate the association between mitral valve calcium and CKD, and further potential therapeutic targets to prevent these significant calcifications.

4. RAAS BLOCKADE IN HEMODIALYSIS POPULATION. IMPACT ON MORTALITY AND ECHOCARDIOGRAPHIC PARAMETERS

The last part of the thesis was dedicated to the therapeutic particularities in dialysis and thus we analyzed by echocardiographic quantification patients with/without RAAS blocker therapy and the role of EF and some specific echo parameters in their chronic evolution. Echocardiographic assessment of these patients is extremely important in risk stratification and therapeutic strategy in terms of improving prognosis.

Our analysis in dialysis patients seeks to answer the eternal question: is RAAS therapy useful in dialysis patients and can it influence morbidity and mortality? So, how far can we go with the administration of RAAS? Should we stop after dialysis initiation when there is a vulnerable window and a high mortality? Should we re-start RAAS in the chronic phase of the dialysis when the patient is more stable?

The initiation of dialysis is accompanied by very high mortality in the first 3 months, and thus one may wonder, whether the risk is even higher if we stop cardioprotective medication in ESRD. On the other hand, if nephroprotection disappears in the advanced stages of ESRD, it is possible that by keeping ACEI/ARB we may achieve a faster progression towards dialysis. The aim of our multicenter, longitudinal observational study was to further explore changes in left ventricular function and structure and their impact on mortality in chronically ESRD patients treated with HD, especially associated with RAAS medication. We also revealed predictors of adverse cardiovascular events in this study. Further, we outline the mortality according to baseline treatment and we also describe the impact of changes in baseline treatment and EF value on mortality.

Patients ($n=1104$) with ESRD on chronic HD treatment (>3 months), as of December 2014, from 9 HD centers in Romania, were included in this study (mean age at inclusion 57.8 years (670 males), mean duration of HD treatment at inclusion 4.6 years, 25% with DZ). Thus, patients were divided into several groups. The effects of ACEI/BRA therapy, as well as the impact of heart rate in this population were assessed on the patient groups studied.

Thus, we monitored in all these groups, data on comparative trends of the survival curves over 36 months.

Differences between the groups were analyzed using the Student's t-test or Mann-Whitney U test for continuous data and the chi-square test or Fisher's exact test for categorical data as appropriate. Kaplan-Meier curves were used to calculate cumulative overall mortality-free survival rates from the time of echocardiography. To compare cumulative event-free survival rates between groups, the log-rank test was used.

The results are as follows:

- 11% of patients had ultrasound within age range; LVH was present in 68% and LV dilatation in 43% of cases.
- Valvular calcifications(VC) were found in 61%, some of them on ischemic and/or functional substrate (18%), and some of the calcifications were found at endomyocardial level (19%); 51% - a single valvular calcification, 10% - mitral-aortic calcification. Of patients without VC at inclusion, 15% died during follow-up and 50% developed progression of one or more valve calcifications. In patients with VC, mortality during follow-up was significantly higher - 24% ($p=0.001$).
- Patients were in evidence for ischemic coronary artery disease(CAD) in 71% of cases, peripheral arterial disease (PAD) in 28% and cerebrovascular disease(CD) in 20%.
- At inclusion, 68% of patients had echocardiographic LVH. Of patients without LVH, 20% died and 20% developed LVH. In patients with LVH at inclusion, mortality was 25%.
- Patients with FEVS > 50% had significantly higher estimated survival compared to those with FEVS < 50%. EF <50% prior to inclusion was found in 15% of patients. Follow-up mortality was significantly higher in these patients, as expected ($p=0.001$).
- Mortality at 3 years was 23% (being 9% in the first year, 8% in the second year, 7% in the third year). Mortality was correlated with cardiovascular disease (CAD, PAD and CD, all $p<0.0001$) and was negatively correlated with weight ($p=0.001$) and EF at inclusion ($p=0.049$).
- Contrary to our expectations, during the 3-year follow-up, mortality was higher in the group with a slight increase in EF and significantly lower in the group with a decrease in EF (24%).
- We compared all parameters at baseline and during the 3 years of dialysis for all patients. There were significant differences between groups ($p=0.003$) in echocardiographic parameters and also in EF values.
- The surviving patients had more normal LV dimensions, with EF ranging within normal limits and annual variations within normal limits, with values above 50%. On the other hand, deceased patients have significantly larger end-systolic and end-diastolic diameters, a hypertrophic heart with increased parietal thickness (IVS= 15 ± 2.8 and reduced EF = $41\pm 3.7\%$).
- Blood pressure control with RAAS blockers had the best prognosis; the difference is significant after 3 years of treatment in patients with and without RAAS inhibitors, considering that patients with the same characteristics (etiology, age, comorbidities, duration of dialysis treatment) and in whom optimal blood pressure control was achieved, were compared.
- Survival curves showed that the best prognosis was achieved in patients treated with RAAS inhibitors with near normal morphological parameters and a heart rate below 70 b/min.
- Patients in the NHF and HFpEF groups had a similar estimated survival up to 36 months, while in the HFmrEF and HFrEF groups, the estimated survival was lower.
- Lower EF was associated with increased risk of death, and the HFmrEF group had a 1.5-fold increased risk, while the HFrEF group had a 2.5-fold increased risk of death compared to those without HF.

All of these findings suggest the need for randomized controlled trials in this vulnerable population, both to confirm our findings and for additional therapies that may alter long-term prognosis. Thus, all this supports the idea of repeated echocardiographic evaluation, to highlight as early as possible morphological changes, that may have hemodynamic consequences with significant prognostic impact.

5. THE EFFECTS OF FERRIC CARBOXYMALTOSE IN DIALYSIS PATIENTS WITH HEART FAILURE

Anemia is widespread among ESRD patients, and contributes to morbidity/mortality, affecting the quality of life of HD patients. Dialysis patients often present with iron depletion and secondary anemia due to frequent blood (and iron) loss. The prevalence of iron deficiency increases with declining kidney function, impairing the effectiveness of treatment with erythropoiesis-stimulating agents. The etiology of anemia in ESRD is multifactorial and is caused by both low production, short erythrocyte lifespan, occult losses, and deficient intake/low absorption. Treatment consists in replenishing iron deposits, and intravenous iron has become the standard treatment in this context.

We know that some older i.v. iron formulas have certain limitations. Thus, this small observational study mainly investigated the safety and efficacy of ferric carboxymaltose (FCM), a modern iron formulation, administered as an iv. bolus in dialysis patients.

Patients aged between 18 and 65 years (n=26) were included, with abnormal biological parameters (Hb \leq 11.0 g/dl and ferritin \leq 200 μ g/l), undergoing maintenance HD three times a week. Ferritin was determined at admission on day 2, 5, 7 and 1 month after FCM administration with both 250 and 500 mg doses. At the same time, patients were assessed for heart failure class according to NYHA classification and completed the HeartQoL questionnaire at admission and at 1 month.

Statistical analysis included Paired t-test and was used to compare serum ferritin with their baseline values after confirmation of normal distribution using Kolmogorow-Smirnow test. We compared the 2 groups (250 mg FCM and 500 mg FCM) using the T test, Chi-square test, or Mann-Whitney U test as appropriate. Ferritin values increased by 211.2 ± 82.25 μ g/l ($P < 0.001$) from baseline to peak and remained significantly elevated until 1 month after 250 mg CMF administration (n = 16). After administration of 500 mg FCM (n = 11), ferritin values increased by 358.5 ± 97.66 μ g/l ($P < 0.001$) and remained significantly elevated until the end of the month. HEARTQoL score values increased by $13.0 \pm 8.7\%$ ($P < 0.001$) and $24 \pm 19\%$ ($P = 0.002$) in patients receiving 250, 500 mg FCM respectively and NYHA reclassification was statistically significantly improved.

The aim of the study was to evaluate the dynamic change and stability over time of laboratory parameters in these patients, by administration of 250 and 500 mg FCM, including those dialysis patients who were admitted to the Cardiology Clinic for worsening cardiovascular pathology.

Although we included a small number of patients, we observed an improvement in NYHA class, symptoms assessed by HeartQoL, and a significant and stable increase over time, up to 1 month, in serum ferritin values after iv. administration of FCM, this regimen of administration being safe and with significant efficacy among such a vulnerable population.

6. PHARMACOKINETICS OF APIXABAN TREATMENT IN DIALYSIS PATIENTS

The last part of the thesis was dedicated to a current controversy - is DOAC treatment suitable for dialysis patients? And if so, which dose is appropriate for them?

Chronic oral anticoagulant treatment is indicated for many dialysis patients, especially due to the increased prevalence of atrial fibrillation and other thromboembolic pathologies. In the last decade, direct oral anticoagulants (DOAC) have been developed for the general population to overcome the limitations of warfarin/acenocoumarol. These

products have been successfully validated in well-designed, randomized controlled trials, and have shown non-inferior efficacy and improved safety compared to warfarin treatment.

In this paper we decided to determine the pharmacokinetics of apixaban in hemodialysis patients, wishing to assess the safety and efficacy of current 'real-life' recommendations.

Nine patients were enrolled in the study. The median age was 60+/-14 years. Six were men and three were women. They had a dialysis age of 4.5+/-2.3 years. All of them had a diuresis <100 ml/24 h. BMI was 31.2+/-6.1 kg/m². Of note, this small group is a preamble to a much larger cohort of patients to highlight the benefits/risks of DOAC treatment in dialysis patients with non-valvular AF. All patients enrolled had permanent atrial fibrillation for more than 1 year.

Thus, in the nine patients, we proceeded as follows: initially we started with a dose of 2.5 mg and after one month of treatment we measured the anti FXa activity on a day without dialysis. We then took a pause of one month, in which time we gave them acenocoumarol, and after that we started treatment for another month with a 5 mg dose, checking at the end of this period the result of the anti FXa activity.

The apixaban dose of 5 mg twice/day resulted in anti-FXa activity above the upper limit (>1.1 U/ml) in 8 out of 9 patients. Only one patient had a value of 1.02 U/ml, being the same patient who developed mild hemorrhoidal bleeding and required specific treatment plus 10-day discontinuation of apixaban, probably justifying the anti-FXa value in the laboratory range.

All other patients had supratherapeutic values above 1.1 U/ml, with a maximum of 1.8 U/ml and a mean of 1.4+/- 0.4 U/ml.

The apixaban dose of 2.5 mg twice/day generated anti-FXa activity within the accepted limits under such treatment (0.5-1.0 U/ml), with only one slightly lower value (0.41 U/ml-accepted by some authors), and a maximum value of 0.94 U/ml(fig 6.1). No minor or major bleeding was recorded with this dose.

We included patients admitted to the Cardiology Clinic, hospitalized in a cardiovascular context, and although the number was relatively small, we can say that we had interesting results using the 2.5 mg apixaban dose twice/day vs. 5 mg twice/day. Apixaban 2.5 mg twice daily resulted in the same anti-FXa factor values, comparable to the standard dose (5 mg twice/day) in patients with preserved renal function, and could be a reasonable alternative to VKA for the prevention of stroke and systemic embolisms in dialysis patients. Apixaban 5 mg twice daily showed supratherapeutic levels (p=0.0002) in these patients and should be avoided. These data suggest that apixaban may be a reasonable alternative to vitamin K antagonists for stroke prevention in patients with ESRD and AF, and a randomized clinical trial is justified in the future.

PERSONAL CONTRIBUTIONS

In this paper we proposed the development of methods for adapting the indications of standard and tissue echocardiography, from dysfunction diagnosis to prognostic estimation, as well as monitoring the effects of potentially cardioprotective therapy and for the reduction of all-cause mortality.

We addressed controversial topics such as furosemide therapy, DOAC therapy, and the effects of FCM on this vulnerable population, as well as prompt intervention with cardioprotective medication to reduce mortality in patients treated with HD.

From the literature, we find very few papers related to the effects of furosemide in dialysis, much more related to echocardiographic quantifiable changes, thus making the work original, and with hemodynamic and clinical impact results.

In the following part, we have chosen a unique dialyzed subpopulation, in which we have proposed modern and simple echocardiographic assessable parameters, which have an impact on the prognosis and quality of life of these patients. Even in a developing country, with an increasing number of specialized physicians and available ultrasound

equipment, we can implement this relatively cheap/effective method of annual follow-up in Romanian dialysis centers.

FCM therapy, quantified by routine biological samples, clinical symptomatology and quality of life, has resulted in improvement of all monitored parameters, making this small analysis a positive preamble for a much larger future study.

The results of using DOAC in dialysis, as shown in the paper described above, appear to be relatively safe when it comes to the appropriate dose, this study being as unique as the previous, both with its simple and effective design, as well as the contrary results to the pharmacological brochure recommendations.

So, as closing words, in order to have detailed knowledge regarding risk factors and pathogenic mechanisms, but also to ensure optimal management of these complications, we need to conduct more randomized trials, and my personal desideratum in this regard, is to continue the research and to put on paper all that is relevant and of prognostic/therapeutic significance, in achieving primary/secondary endpoints, in this highly vulnerable population.