Article

Not peer-reviewed version

Novel Biomarkers in Evaluating Cardiac Function in Patients on Hemodialysis – a Pilot Prospective Observational Cohort Study

Lazar A. Chisavu , Viviana M. Ivan , Adelina Mihaescu * , Flavia Chisavu , Oana Schiller , Luciana Marc , Flaviu Bob , Adalbert Schiller

Posted Date: 28 February 2024

doi: 10.20944/preprints202402.1615.v1

Keywords: hemodialysis; mortality; cardio-vascular disease; novel biomarkers; cardiac function and structure

Article

Novel Biomarkers in Evaluating Cardiac Function in Patients on Haemodialysis – A Pilot Prospective Observational Cohort Study

Lazar A. Chisavu [1,2], Viviana M. Ivan [1,3], Adelina Mihaescu [1,2,*], Flavia Chisavu [1,4], Oana Schiller [5], Luciana Marc [1,2], Flaviu Bob [1,2] and Adalbert Schiller [1,2]

[1] Centre for Molecular Research in Nephrology and Vascular Disease, Faculty of Medicine 'Victor Babes', 300041 Timisoara, Romania.
[2] Nephrology Discipline, University of Medicine 'Victor Babes' Timisoara, Romania.
[3] Cardiology Discipline, University of Medicine 'Victor Babes' Timisoara, Romania.
[4] Louis Turcanu' Emergency County Hospital for Children, 300011 Timisoara, Romania
[5] AVITUM BBraun Romania
* Correspondence: adekmi@yahoo.com

Abstract: Chronic kidney disease patients treated by hemodialysis present a high cardio-vascular morbidity and mortality. There is an imperative need for novel biomarkers in identifying and to offer possible therapeutically intervention in these patients. We performed a prospective observational cohort study on 77 patients in the period October 2021 – October 2023. We measured serum plasma levels of Interleukin 1-Beta, Galectin 3, human suppression of tumorigenicity factor 2, bone morphogenetic protein 2 and fibroblastic growth factor 23 at the inclusion. We evaluated the correlations of these biomarkers with cardiac function and structure evaluated by echocardiography. Mean age was 61.02 (±11.81) years, 45 (56.2%) males and with a dialysis vintage of 4.95 (2.4-7.8) years. Median ejection fraction was 51 (43-54 %) and more than two thirds of the patients presented valvular calcifications. Overall mortality was 22%. Interleukin 1-Beta correlated positively with ejection fraction and global longitudinal strain and negatively with left atrium diameter and left ventricle tele systolic diameter. Galectin 3 values negatively correlated with aortic valve fibrosis and mitral valve calcifications and human suppression tumorigenity factor 2 negatively correlated with mitral valve calcifications. Some of these novel biomarkers could be used to better asses the cardio-vascular disease in patients on maintenance hemodialysis.

Keywords: hemodialysis; mortality; cardio-vascular disease; novel biomarkers; cardiac function and structure

1. Introduction

Chronic kidney disease (CKD) patients present a high cardio-vascular disease (CVD) risk and CVD related mortality [1]. This risk is increasing with the decrease of kidney function and CVD mortality becomes the main cause of death in kidney failure patients treated by HD [2, 3]. Even though more than 50 years have passed since the hemodialysis became a reliable method of renal replacement therapy, the cardiovascular events remain the leading cause of death among these patients. [4]

In the recent years, in order to detect and revers the mechanisms involved in CVD alterations and mortality in CKD patients, biomarkers have been used to explore inflammation, accelerated atherosclerosis, oxidative stress, cardiovascular calcifications associated to mineral bone disorder (CKD-MBD), and so on. [5-7] Many of these researches used clinical hard end-points and biological quantifiers to prove and validate the utility, predictability and at the end the usefulness of the explored biomarkers. Less research was addressed to explore the influence of the above-cited mechanisms (i.e. inflammation, CKD-MBD and so on) on cardiac structural and functional alterations detectable by cardiac ultrasound (known to be associated with CVD mortality in CKD), using biomarkers.

 doi:10.20944/preprints202402.1615.v1

Interleukin 1-beta (IL-1) is a pro-inflammatory cytokine involved in tissue repair, cell growth and inflammatory response. [8] It is a well-known fact that patients on HD have a pro-inflammatory status and the increased IL-1 level is well documented in CKD and HD patients. [9-12] Some studies explored the connections between IL-1 and cardiac structure with promising results. [13-16].

Galectin 3 is a soluble β-galactoside-binding lectin (250 amino acids) that has regulatory roles in cell proliferation, tissue repair, inflammation and fibrogenesis. [16] Several studies suggested that increased levels of Galectin 3 are associated with renal fibrosis. [17, 18] In addition, there are proves that Galectin 3 plays a central role in heart failure pathophysiology through myocardial inflammation and fibrosis, thus becoming a predictor of heart failure. [19, 20] Some studies evaluated the impact on all-cause mortality and cardiovascular events and showed that higher levels of Galectin 3 are independent risk factors for death in HD patients. [21-23]

Suppression of tumorigenicity 2 (ST2) gene was discovered in 1989 and is part of IL-1 gene cluster. [24] The main products of transcription of this gene are ST2L (or IL1RL1-b) which is a membrane receptor for IL-1 family and sST2a (or IL1RL1-a) which is the soluble receptor that can be identified in plasma. [24] Several articles proved the prognostic utility of sST2a in heart failure and its association with left ventricle hypertrophy (LVH) in CKD, hypertension and in metabolic-syndrome patients. [25-27] In a recent meta-analysis regarding sST2a levels in HD patients, it seems that increased levels are associated with higher all-cause mortality. [28]

Bone morphogenetic protein (BMP) is a member of the super family of transforming growth factor. The most important isoform is BMP2, which regulates cell growth and differentiation. [29] The role of BMP2 in vascular calcification, atherosclerosis and inflammation is well established, but only one study evaluated the BMP-2 levels in HD patients. [30, 31]

Fibroblastic growth factor 23 (FGF23) is an osteocytic hormone with effect on calcitriol production and increased phosphate excretion. Besides that, it appears that FGF23 is an independent risk factor for cardiovascular disease. [32] It seems that beside the bone production of FGF23, the myocytes also produce FGF23 in certain conditions. [32] The impact of FGF23 on the myocardium is still debated with some studies suggesting a negative impact of elevated FGF23 and others suggesting a positive one. [32]

In the face of these scarce data of specific biomarkers and their implication in CVD in HD patients, we conducted a prospective observational cohort study between October 2021 and October 2023 where we evaluated all the aforementioned biomarkers and their relationship with cardiac structure and function in HD patients.

2. Material and methods:

This observational prospective cohort study was conducted between October 2021 and October 2023. All the patients signed an informed consent form at the inclusion and the Ethics Committee of the Dialysis Centre approved the study. The study respected the declaration of Helsinki regarding human studies and was in accordance with the Ethics Code of the World Medical Association. The inclusion criteria in this study was, beside the informed consent of the patients, the history of at least 3 months on stable hemodialysis.

The parameters were retrieved in the same day with the dialysis session, before the beginning of hemodialysis, after the longest weekly period between dialysis sessions (for patients who perform dialysis on days of Monday, Wednesday and Friday, the data were recorded on Monday and for patients who perform dialysis on Tuesday, Thursday and Saturday, the data were retrieved on Tuesday). All the patients perform three dialysis sessions per week of at least 4 hours long, using high flux dialyzers.

The cardiac assessment was performed during the 2nd and 3rd hour of dialysis (Pulse Doppler, M-mode continuous and two-dimensional), on Monday or Tuesday respectively. A single cardiologist using the same echocardiography device performed the measurements in order to limit the bias. Echocardiographies were performed in concordance with European Association of Cardiovascular Imaging (EACI) recommendations. The left ventricular ejection fraction (LVEF) was assessed using Simpson method, we noted hearth valve calcifications and fibrosis, interventricular

 doi:10.20944/preprints202402.1615.v1

septum (IVS), left ventricle tele diastolic diameter (LVTDD), right ventricle diameter (RV), global longitudinal strain (GLS), endomiocardial calcifications, aortic atheroma, left ventricle tele systolic diameter (LVTSD), left ventricle mass (LVM), left atrium diameter (LA), and E/A rapport.

The samples for special biomarkers were collected as the manual instructions for each biomarker.

Human Bone Morphogenetic Protein 2 (BMP2) was quantified using ELISA Kit with the Catalog No: E-EL-H0011 and Product Size: 96T/48T/24T/96T*5 from Elabscience. The sensitivity of the kit is 37.5 pg/ml with a detection range of 62.5-4000 pg/ml. All the samples were performed from the patient's plasma. Plasma was collected using EDTA-Na2 as an anticoagulant. The samples were centrifuged for 15 minutes at 1000xG at a temperature raging from 2 to 8 degrees Celsius in the first 30 minutes after collection. The supernatant was collected for the assay.

Fibroblastic growth factor 23 (FGF-23) was quantified using ELISA Kit with the Catalog No: E-EL-H1161 and Product Size: 96T/48T/24T/96T*5 from Elabscience. The sensitivity of the kit is 9.38 pg/ml with a detection range of 15.63-1000 pg/ml. All the samples were performed from the patient's plasma. Plasma was collected using EDTA-Na2 as an anticoagulant. The samples were centrifuged for 15 minutes at 1000xG at a temperature raging from 2 to 8 degrees Celsius in the first 30 minutes after collection. The supernatant was collected for the assay.

Interleukin 1 Beta (IL-1B) was quantified using ELISA Kit with the Catalog No: E-EL-H0149 and Product Size: 96T/48T/24T/96T*5 from Elabscience. The sensitivity of the kit is 4.69 pg/ml with a detection range of 7.81-500 pg/ml. All the samples were performed from the patient's plasma. Plasma was collected using EDTA-Na2 as an anticoagulant. The samples were centrifuged for 15 minutes at 1000xG at a temperature ranging from 2 to 8 degrees Celsius in the first 30 minutes after collection. The supernatant was collected for the assay.

BMP2, FGF23 and IL-1B analysis was performed at the laboratory of the Clinical Emergency County Hospital from Timisoara, Romania.

Galectin 3 and suppression of tumorigenicity 2 (ST2) analysis was performed in an outer laboratory. The blood samples were collected on vials with EDTA-Na2 anticoagulant. After the samples were centrifuged for 15 minutes at 1000xG at a temperature ranging from 2 to 8 degrees Celsius in the first 30 minutes after collection, the supernatant was collected for the assay. Galectin 3 and ST2 analysis was performed in an outer laboratory.

3. Statistical analysis:

Data are presented as average ± standard deviation for numerical variables with Gaussian distribution, median and interquartile range for numerical variables with non-Gaussian distributions and percentage from the sub-group total and number of individuals. Shapiro-Wilk test was performed for normality assessment for continuous variables distributions and Levene's test for equality of variances. Multivariable regression and logistic regression models assessed the individual impact of several confounding factors. A repeated backward-stepwise algorithm was performed in order to find the most appropriate theoretical model (exclusion criteria $p>0.1$ and inclusion criteria $p<0.05$). Kaplan-Meyer survival curves were assessed to evaluate the impact on mortality. In addition, we performed cox-regression hazard curves to assess the impact of cofounding factors on mortality. A p value<0.05 was considered for statistical significance. The software package used for statistical analysis was MedCalc® Statistical Software version 22.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023).

4. Results

The baseline characteristics of the 77 patients included in the study are presented in Table 1. The mean age of the cohort was 61.02 (SD of 11.81years), with a median dialysis history of 4.95 (IQR: 2.4-7.8 years) and male majority (56.2%). The nutritional status was good with serum albumin of 4.1 (IQR: 3.9-4.3 g/dl), predialysis creatine of 8.54 (SD 2.05 mg/dl) and reduced inflammation with a C reactive protein of 0.43 (IQR: 0.26-1.2 mg/dl). The patients presented an in-target hemoglobin (10.85 with IQR: 10.3-11.6 g/dl), in target mineral-bone disease management with calcium phosphorus product below

 doi:10.20944/preprints202402.1615.v1

55 mg^2/dl^2 (47.86 with SD of 13.06 mg^2/dl^2), parathyroid hormone between 2 and 9 times maximum normal value (428 with IQR 197-713.5 ng/ml). The patients were not acidotic (serum bicarbonate of 22 with a SD of 2.27 mmol/l) and presented light hyperkalemia (5.37 with SD of 0.64 mmol/l).

Table 1. – baseline characteristics.

Parameter	Value N=77 patients
Age (A+SD) years	61.02 (11.81)
Dialysis vintage (M+IQR) years	4.95 (2.4-7.8)
Sex (male)	45 (56.2%)
Dry weight (A+SD) kg	82.61 (19.52)
BMI (A+SD) kg^2/m^2	28.93 (6.46)
Albumin (M+IQR) g/dl	4.1 (3.9-4.3)
Serum bicarbonate (A+SD) mmol/l	22 (2.27)
Predialysis creatinine (A+SD) mg/dl	8.54 (2.05)
Predialysis urea (A+SD) mg/dl	122.63 (25.9)
Haemoglobin (M+IQR) g/dl	10.85 (10.3-11.6)
C reactive protein (M+IQR) mg/dl	0.43 (0.26-1.2)
Serum sodium (M+IQR) mmol/l	139 (137.7-140.4)
Serum potassium (A+SD) mmol/l	5.37 (0.64)
Serum calcium (A+SD) mg/dl	8.66 (0.51)
Serum phosphorus (M+IQR) mg/dl	5.39 (4.67-6.3)
Calcium phosphorus product (A+SD) mg^2/dl^2	47.86 (13.06)
Parathyroid hormone (M+IQR) ng/ml	428 (197-713.5)
Thrombocytes (M+IQR) N/mm^3	219500 (174000-254000)
eKT/V (A+SD)	1.6 (0.2)
Mean ultrafiltration volume (A+SD) ml	2343 (587)
Deaths N (%)	17 (22.1%)

Legend: A=average, SD=standard deviation, M=median, IQR=interquartile range, kg=kilograms, m=meters, g=grams, dl=decilitres, mmol=millimols, l=litres, mg=milligrams, ng=nanograms, mm=millimetre, N=number.

Cardiac parameters for all the patients are presented in Table 2. Overall, they presented a slightly increased left atrium diameter (41.87 with SD of 4.7mm), 76.9% with aortic atheromatosis, a high incidence of calcifications (endomyocardial – 70.5%, aortic valve – 67.9%, mitral valve – 78.2%), important valvular fibrosis incidence (aortic – 80.8%. mitral – 78.2%), a normal ejection fraction (51% with IQR of 43-54%) and increased interventricular septum (13 IQR 12-14mm) and left ventricle mass (254.5, IQR 203-304g).

Table 2. – echocardiography parameters.

Parameter	Value
Left atrium diameter (A+SD) mm	41.87 (4.7)
Right ventricle diameter (M+IQR) mm	27 (26-28)
Aortic atheromathosis N (%)	60 (76.9%)
Endomyocardial calcifications N (%)	55 (70.5%)

 doi:10.20944/preprints202402.1615.v1

Kinetics dysfunction N (%)	33 (42.3%)
Aortic valve calcifications N (%)	53 (67.9%)
Aortic valve fibrosis N (%)	63 (80.8%)
Mitral valve calcification N (%)	61 (78.2%)
Mitral valve fibrosis N (%)	61 (78.2%)
Let ventricle tele diastolic diameter (M+IQR) mm	53 (49-58)
Left ventricle tele systolic diameter (A+SD) mm	38.78 (6.51)
Waves E/A rapport (M+IQR)	0.7 (0.4-1)
Ejection fraction (M+IQR) %	51 (43-54)
Global longitudinal strain (M+IQR) % (absolute value)	14 (12-17)
Interventricular septum (M+IQR) mm	13 (12-14)
Left ventricle mass (M+IQR) g	254.5 (203-304)

Legend: A=average, SD=standard deviation, M=median, IQR=interquartile range, N=number, mm=millimetre, g=gram.

The values of specific biomarkers are presented in Table 3. FGF-23 values are near the upper normal limit (47.93 with IQR 21.89-104.29 pg/ml), Galectin 3 presented normal values, human ST2 higher than normal values (68.35 with IQR of 34.5 – 121.1 ng/ml) and higher values for IL-1B (44.74 with IQR of 42.92-48.49 pg/ml).

Table 3. – special parameters analysis.

Parameter	Value
FGF-23 (M+ IQR) pg/ml	47.93 (21.89-104.29)
Galectin 3 (M+ IQR) ng/ml	3.9 (2.62-9.62)
Human ST2 (M+ IQR) ng/ml	68.35 (34.5-121.1)
Human BMP2 (M+ IQR) pg/ml	712.7 (451-725.9)
IL-1B (M+IQR) pg/ml	44.74 (42.92-48.49)

Legend: FGF=fibroblastic growth factor, ST= suppression of tumorigenicity, BMP=bone morphogenetic protein, IL-1B= interleukin 1Beta, M=median, IQR=interquartile range, pg=picograms, ml=millilitre, ng=nanograms.

We were able to compare the values of FGF-23, human BMP2 and IL-1Beta between our dialysed cohort and 10 healthy subjects (the same statistical age). The results are presented in Table 2. Dialysed patients presented higher values of Human BMP2 and lower values of FGF-23. There was no statistical differences regarding IL-1Beta values, but the dialysed patients presented a higher heterogeneity regarding this parameter with a bigger standard deviation.

Table 4. Comparison between dialysed cohort and healthy subjects for FGF-23, Human BMP2 and Il-1Beta.

Parameter	Dialysed patients	Healthy controls	P value
FGF-23 (A+SD) pg/ml	75.98 (76.97)	290.31 (173.91)	**<0.001**

 doi:10.20944/preprints202402.1615.v1

Human BMP2 pg/ml	591.18 (341.69)	242.11 (22.82)	**0.0061**
IL-1Beta	65.14 (82.53)	46.03 (2.84)	0.468
Age (years)	61.02 (11.81)	59.12 (7.86)	0.587

Legend: FGF=fibroblastic growth factor, BMP=bone morphogenetic protein, IL-1B= interleukin 1Beta, A=average, SD=standard deviation, pg=picograms, ml=millilitre.

In order to evaluate the connections between the special markers and cardiac parameters, we performed several multiple regressions and logistic regressions models (as appropriate) with our special markers as the independent variables. The results are presented in Tables 5 and 6. IL-1B positively correlated with EF values and global longitudinal strain and negatively with left atrium diameter and left ventricle telesystolic diameter. Galectin 3 values negatively correlated with aortic valve fibrosis and mitral valve calcifications and human ST2 negatively correlated with mitral valve calcifications.

None of the specific biomarkers correlated with mortality during the follow-up.

Table 5. –multiple regression models with specific biomarkers as independent variables.

Parameter	EF	GLS (absolute value)	LA	LVTSD
IL-1B pg/ml	Coef=0.022, p<0.0001	Coef=0.011, p=0.003	Coef=-0.024, p=0.031	Coef=-0.021, p<0.0001
Galectin 3 ng/ml	-	-	-	-
Human ST2 ng/ml	-	-	-	-
Human BMP2 pg/ml	-	-	-	-
FGF-23 pg/ml	-	-	-	-
R square adjusted	0.689	0.109	0.054	0.73

Legend: FGF=fibroblastic growth factor, ST= suppression of tumorigenicity, BMP=bone morphogenetic protein, IL-1B= interleukin 1Beta, M=median, IQR=interquartile range, pg=picograms, ml=millilitre, ng=nanograms.

Table 6. - logistic regression models with specific biomarkers as independent variables.

Parameter	Aortic valve fibrosis	Mitral valve calcifications
IL-1B pg/ml	-	0.98 (0.97-1.00), p=0.071
Galectin 3 ng/ml	0.92 (0.85-0.99), p=0.042	0.88 (0.79-0.98), p=0.025
Human ST2 ng/ml	0.99 (0.98-1.00), p=0.084	0.98 (0.97-0.99), p=0.01
Human BMP2 pg/ml	-	-
FGF-23 pg/ml	-	1.01 (0.99-1.02), p=0.085
Nagelkerke R square	0.154	0.37
AUC (95%CI)	0.655 (0.531-0.765)	0.809 (0.697-0.894)

Legend: FGF=fibroblastic growth factor, ST= suppression of tumorigenicity, BMP=bone morphogenetic protein, IL-1B= interleukin 1Beta, M=median, IQR=interquartile range, pg=picograms, ml=millilitre, ng=nanograms.

 doi:10.20944/preprints202402.1615.v1

5. Discussions

After more than 50 years since hemodialysis become a reliable method of renal replacement therapy, the first cause of death among HD patients is the cardiovascular related one. In the face of CKD and cardiovascular disease continuum, the research of better predictors and possible intervention means is mandatory in order to reduce mortality. The aim of this pilot study was to evaluate the correlations between specific biomarkers and echocardiographic parameters in a cohort of HD patients. In addition, we explored the impact on mortality of these specific biomarkers.

The mean age of the cohort was 61.02 years with 56.2% males. Our cohort was younger compared to the ERA-EDTA annual report from 2020 (65.1 years) and similar to several European countries like Finland (61 years), United Kingdom (61 years), Serbia (60.3 years), etc. [33] In Romania, mean age of the patients on HD in 2020 was 62.2 years. [34] The management of CKD complications was conducted according to KDIGO guidelines and as a result, the patients had in-target haemoglobin levels, good nutritional status, reduced inflammation and proper calcium phosphorus product.

Regarding echocardiographic parameters, the EF was within normal range. The valvular and endomyocardial calcification rate was high as a result of the mineral-bone disease evolution. For instance, in a study published by Kraus on 243 HD patients, all of them presented aortic or mitral valve calcifications. [35] Most of the cohort presented left ventricle hypertrophy. This is commonly encountered in the dialysis patients, as previously published data. [36] The cause of this comorbidity among HD patients is plurifactorial and is linked besides arterial hypertension and fluid overload to specific CKD complications factors (vitamin D, eritropoetin, calcifications, etc.) [36].

IL-1B is a pro-inflammatory cytokine involved in inflammatory response, cell growth and tissue repair. In patients with myocardial infarction, increased levels of IL-1B are associated with worst cardiac outcome. [17] Patients on maintenance HD have a pro-inflammatory state with higher IL-1B levels. [14, 15] 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 44.74 pg/ml, being lower than the mean values in a cohort of 390 patients evaluated by Yu (84.82 ± 94.38 pg/ml). [37] These differences are most likely due to a lower inflammation status in our cohort (CRP 0.43mg/dl vs 8.46mg/dl in Yu cohort). [37] In addition, in another recently published study by Lisowska 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. [38] These differences may be in the context of several factors: inflammation and nutrition status, haemoglobin levels and anemia management, dialysis vintage, comorbidities, and the number of patients.

Interestingly, the IL-1B values were positively associated with GLS values in our cohort. Each unit increase in IL-1B translated in 0.011% decrease in the GLS (increase in the absolute value). For instance, in a study that evaluated the effects on anakinra (interleukin-1 receptor antagonist) in 80 patients with rheumatoid arthritis on coronary and left ventricular function, the patients with higher IL-1B levels presented the highest reduction in GLS after anakinra administration. In addition, the GLS values in our cohort were higher compared with the one in aforementioned (-17% vs -33%). One should mention that we did not evaluate the incidence of coronary artery disease in our patients. To our knowledge, no studies evaluated the relationship between IL-1B and GLS in patients on maintenance HD. Some authors consider that GLS could be a better predictor of left ventricle systolic dysfunction compared to the ejection fraction measurement in patients on dialysis. [39]

In our study, IL-1B was positive associated with the EF values. Each unit increase of IL-1B generated a 0.022% increase in the EF. No study evaluated so far the correlation between EF and IL-1B in hemodialysis patients. A nice study by Orn on patients following myocardial infarction evaluated the correlation between IL1-B and the EF fraction values in different time-points. [40] Their findings show a negative correlation between EF and IL-1B values, with patients that presented higher IL-1B levels having lower EF values. [40] One should mention that this negative correlation was present at 2 months and one year after angiographic intervention, thus not only in acute settings. Our findings seems to be opposite compared with Orn cohort, with higher levels of IL-1B being associated with higher levels of EF. In the dialysis settings, there are already some known facts

 doi:10.20944/preprints202402.1615.v1

regarding reversed epidemiology. HD patients live longer at higher body mass indexes and, to some authors, if they present higher cholesterol levels. [41, 42, 43] These facts are explainable due to the nutritional status, malnutrition being associated with higher mortality among HD patients. [42, 43] Thus, lower body mass indexes and lower cholesterol levels are most likely associated with a malnutrition status in the HD population. Our results are unnatural, as one would expect that higher IL-1B levels would be associated with lower EF. Even though in our cohort, values of IL-1B had no influence on mortality, perhaps patients with higher EF values presents higher valvular regurgitation values and the EF is artificially increased (this is just an assumption due to the fact w]that we did not evaluate the valvular regurgitation status).

Moreover, IL1-B levels were negatively correlated with left atrium diameter and left ventricle telesistolic diameter. So far, increased left atrium diameter is associated with increased all-cause mortality among general population. [44] To our knowledge, no studies evaluated the impact of left atrium diameter on IL-1B levels. Even though left atrium diameter larger than 40mm is associated with higher risk of major adverse cardiac events (MACE), [45] in our study left atrium diameter did not increased the risk of death (results not shown). The study of Orn is the only one that evaluated the impact of IL-1B measured after myocardial infarction on left ventricular modelling evaluated through cardiac magnetic resonance at one year after infarction. [40] He showed that increased IL-1B levels at 2 months following myocardial infarction were associated with left ventricle end systolic index. In our cohort, it seems that the correlation is opposite from Orn, with the respect of the differences regarding cohort size, non-infarct patients in our cohort and different timing in cardiac evaluation. [40]

Galectin-3 is a relatively new biomarker that is well studied as a promoter of cardiac inflammation and fibrosis leading to heart failure. [19, 20] In hemodialysis treated patients, higher levels Galectin-3 seems to be associated with increased cardiac and all-cause mortality. [21-23] In our study, Galectin-3 levels did not influenced mortality during the two years of follow-up. One should mention that the study by Liu on 506 HD patients showed that Galectin levels above 8.65 ng/ml increased mortality and the study by Hogas on 88 HD patients found a Galectin-3 cut-off value of 23.73ng/ml as increased mortality risk. [22, 23] The interesting result is that Galectin-3 levels negatively correlates with aortic valve fibrosis and mitral valve calcifications. Previously published papers showed a direct link between Galectin-3 and cardiac fibrosis and cardiac remodelling. [19, 20] No study from the literature evaluated this relationship among dialysis or non-dialysis patients. It is possible, at first view, to consider these results unnatural. One should expect that a patient with proved fibrosis to present higher Galectin-3 levels. The valvular disease progress during the hemodialysis. Patients with increased Galectin-3 levels probably are at higher risk of valvular fibrosis and maybe valvular calcification. The interplay between fluid overload, myocardial HD associated stunning, left ventricle hypertrophy, progression of mineral-bone disease is complex and Galectin-3 may play an important role. It is possible that our results could be attribute to chance, or can be explained by the reduced number of patients, but they should not be overlooked. There is definitely an imperative need of future studies to validate our results.

As Galectin-3, sST2 proves its efficiency in predicting MACE, even in CKD patients. [24, 25] There are proved links between elevated sST2 levels and cardiac remodelling. [26] The relationship between sST2 and cardiac remodelling is less studied among HD patients, but a meta-analysis on HD patients that gathered more than 1300 patients, showed that increased sST2 levels increase all-cause mortality. [28] No study evaluated so far the possible connection with valvular calcifications. Some studies evaluated the imbrication of sST2 and atherosclerotic plaque calcifications. For instance, Luo showed that in patients with non-ST elevated acute coronary syndrome, higher levels of sST2 were associated with spotty atherosclerotic calcifications and those with lower levels exhibited large plaque calcifications. [46] In our study, there is a negative connection between sST2 levels and mitral valve calcifications. Previous studies showed that sST2 may supress the M2 phenotype macrophages differentiation from macrophages. [47] M2 macrophages present mostly anti-inflammatory effects and they are related to macrocalcification in atherosclerotic plaques. There is a possibility that patients with elevated sST2 present less often mitral calcifications following the same

 doi:10.20944/preprints202402.1615.v1

pathophysiological mechanisms from atherosclerotic plaques calcification. These results require further confirmation in prospective studies with larger number of patients in order to reduce bias and/or positive results due to chance.

In our cohort, BMP2 values were not correlated with neither cardiac parameter. One should mention that healthy controls presented lower BMP2 levels compared to HD patients. A study by Dalfino ahowed that petients with chronic kidney disease presented higher levels of BMP2 compared to healthy controls. [48] In addition he suggested that BMP2 may contribute to vascular calcification due to increased oxidative stress and arterial stiffness. [48]. Our results confirm the previous findings.

In conclusion, some of these biomarkers could be used for a better assessment of cardiac function and structure in patients on hemodialysis.

There are several limitations in our study. First, the small number of patients and the relative reduce period of follow-up is a drawback. Due to the small number of cases, the results should be interpreted with caution. To our knowledge, this is the first study that explores the interrelation between some specific biomarkers and cardiac remodelling in patients on maintenance haemodialysis. There is an imperative need for future prospective studies with a larger number of patients in order to validate our results.

Author Contributions: Conceptualization, L.C. and A.S.; methodology, A.M.; software, L.C. and F.C.; validation, V.I, F.B. and O.S.; formal analysis, L.M.; investigation, L.C., V.I., A.M., F.B, L.M and O.S.; resources, O.S..; data curation, L.C. and F.C.; writing—original draft preparation, L.C. and F.C.; writing—review and editing, A.M., V.I., O.S., F.B. and L.M..; visualization, L.M.; supervision, A.S.; project administration, O.S.; funding acquisition, A.M., .I. and L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of AVITUM BBraun Romania.

Data Availability Statement: Anonymized data will be available after request to the corresponding author – Adelina Mihaescu, e-mail: adekmi@yahoo.com.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jankowski J, Floege J, Fliser D, et al. Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options. Circulation. 2021 Mar 16;143(11):1157-1172. doi: 10.1161/CIRCULATIONAHA.120.050686.
2. Matsushita K, van der Velde M, Astor BC, et al for the CKD Prognosis Consortium. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet 2010; 375: 2073–81. doi: 10.1016/S0140-6736(10)60674-5
3. Van der Velde M, Matsushita K, Coresh J, et al, for the Chronic Kidney Disease Prognosis Consortium. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int 2011; 79: 1341–52. doi: 10.1038/ki.2010.536
4. United States Renal Data System. 2022 USRDS Annual Data Report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2022.
5. Park M, Hsu CY, Go AS, et al. "Urine kidney injury biomarkers and risks of cardiovascular disease events and all-cause death: the CRIC study." Clinical Journal of the American Society of Nephrology 12.5 (2017): 761-771. doi: 10.2215/CJN.08560816.
6. Yan, Zhonghong, Guanran Wang, and Xingyang Shi. "Advances in the progression and prognosis biomarkers of chronic kidney disease." Frontiers in Pharmacology 12 (2021): 785375. doi: 10.3389/fphar.2021.785375
7. Bolignano D, Coppolino G, Lacquaniti A, Buemi M. From kidney to cardiovascular diseases: NGAL as a biomarker beyond the confines of nephrology. Eur J Clin Invest. 2010 Mar;40(3):273-6. doi: 10.1111/j.1365-2362.2010.02258.x.

8. Xu D., Mu R., & Wei X. (2019). The Roles of IL-1 Family Cytokines in the Pathogenesis of Systemic Sclerosis. Frontiers in Immunology, 10. doi:10.3389/fimmu.2019.02025
9. Descamps-Latscha B, Herbelin A, Nguyen AT, et al.: Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. J Immunol 154: 882–892, 1995
10. Dinarello CA, Koch KM, Shaldon S: Interleukin-1 and its relevance in patients treated with hemodialysis. Kidney Int Suppl 24: S21–S26, 1988
11. Kimmel PL, Phillips TM, Simmens SJ, et al: Immunologic function and survival in hemodialysis patients. Kidney Int 54: 236–244, 1998
12. Hung AM, Ellis CD, Shintani A, Booker C, Ikizler TA. IL-1β receptor antagonist reduces inflammation in hemodialysis patients. J Am Soc Nephrol. 2011 Mar;22(3):437-42. doi: 10.1681/ASN.2010070760.
13. Hilde L. Orrem, Christian Shetelig, Thor Ueland, et al: Soluble IL-1 receptor 2 is associated with left ventricular remodelling in patients with ST-elevation myocardial infarction, International Journal of Cardiology, Volume 268, 2018, Pages 187-192, ISSN 0167-5273, https://doi.org/10.1016/j.ijcard.2018.05.032.
14. Van Tassell BW, Arena RA, Toldo S, et al. Enhanced interleukin-1 activity contributes to exercise intolerance in patients with systolic heart failure.PLoS One. 2012; 7:e33438. doi: 10.1371/journal.pone.0033438
15. Toldo S, Mezzaroma E, O'Brien L, et al. Interleukin-18 mediates interleukin-1-induced cardiac dysfunction.Am J Physiol Heart Circ Physiol. 2014; 306:H1025–H1031. doi: 10.1152/ajpheart.00795.2013
16. Henderson NC, Mackinnon AC, Farnworth SL, et al. Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. Am J Pathol. 2008;172:288–298. doi: 10.2353/ajpath.2008.070726
17. Van Tassell B. W., Trankle C. R., Canada J. M., et al. (2018). IL-1 Blockade in Patients With Heart Failure With Preserved Ejection Fraction. Circulation: Heart Failure, 11(8). doi:10.1161/circheartfailure.118.005036
18. Henderson NC, Mackinnon AC, Farnworth SL, et al. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. Proc Natl Acad Sci U S A. 2006;103:5060–5065. doi: 10.1073/pnas.0511167103
19. de Boer RA, Voors AA, Muntendam P, van Gilst WH, van Veldhuisen DJ. Galectin-3: a novel mediator of heart failure development and progression. Eur J Heart Fail. 2009;11:811–817. doi: 10.1093/eurjhf/hfp097
20. Lin YH, Lin LY, Wu YW, et al. The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. Clin Chim Acta. 2009;409:96–99. doi: 10.1016/j.cca.2009.09.001.
21. Obokata M, Sunaga H, Ishida H, et al. Independent and incremental prognostic value of novel cardiac biomarkers in chronic hemodialysis patients. Am Heart J. 2016 Sep;179:29-41. doi: 10.1016/j.ahj.2016.05.018.
22. Liu S, Wu Q, Zhang S, et al. Serum Galectin-3 levels and all-cause and cardiovascular mortality in maintenance hemodialysis patients: a prospective cohort study. BMC Nephrol. 2022 Jan 3;23(1):5. doi: 10.1186/s12882-021-02636-z.
23. Hogas S, Schiller A, Voroneanu L, et al. Predictive Value for Galectin 3 and Cardiotrophin 1 in Hemodialysis Patients. Angiology. 2016;67(9):854-859. doi:10.1177/0003319715623397
24. Kakkar R., Lee R.T. The IL-33/ST2 pathway: Therapeutic target and novel biomarker. Nat. Rev. Drug Discov. 2008;7:827–840. doi: 10.1038/nrd2660
25. Plawecki M, Morena M, Kuster N, et al. SST2 as a new biomarker of chronic kidney disease-induced cardiac remodeling: impact on risk prediction. Mediators Inflamm. 2018;2018: 3952526. doi: 10.1155/2018/3952526.
26. Ojji DB, Opie LH, Lecour S, et al. Relationship between left ventricular geometry and soluble ST2 in a cohort of hypertensive patients. J Clin Hypertens. 2013;15(12):899–904. doi: 10.1111/jch.12205.
27. Celic V, Majstorovic A, Pencic-Popovic B, et al. Soluble ST2 levels and left ventricular structure and function in patients with metabolic syndrome. Ann Lab Med. 2016;36(6):542–9. doi: 10.3343/alm.2016.36.6.542.
28. Wang S, Wei F, Chen H, et al. The Prognostic Value of Soluble ST2 in Maintenance Hemodialysis Patients: A Meta-Analysis. Blood Purif. 2020;49(1-2):114-120. doi: 10.1159/000503601.
29. Park Y, Kim JW, Kim DS. The bone morphogenesis protein-2 (BMP-2) is associated with progression to metastatic disease in gastric cancer. Cancer Res Treat. 2008;40:127–132. doi: 10.4143/crt.2008.40.3.127
30. Simões Sato AY, Bub GL, Campos AH. BMP-2 and −4 produced by vascular smooth muscle cells from atherosclerotic lesions induce monocyte chemotaxis through direct BMPRII activation. Atherosclerosis. 2014;235:45–55. doi: 10.1016/j.atherosclerosis.2014.03.030.
31. Zhang M., Sara J.D., Wang Fl. et al. Increased plasma BMP-2 levels are associated with atherosclerosis burden and coronary calcification in type 2 diabetic patients. Cardiovasc Diabetol 14, 64 (2015). https://doi.org/10.1186/s12933-015-0214-3
32. Stöhr R., Schuh A., Heine G. H., & Brandenburg V.. FGF23 in Cardiovascular Disease: Innocent Bystander or Active Mediator? Frontiers in Endocrinology, 2018 9. doi:10.3389/fendo.2018.00351
33. ERA Registry: ERA Registry Annual Report 2020. Amsterdam UMC, location AMC, Department of Medical Informatics, Amsterdam, the Netherlands, 2022.
34. https://www.registrulrenal.ro/resurse_statistica/Raport_RRR_2020_covid.pdf

35. Kraus M. A., Kalra P. A., Hunter J., Menoyo J., & Stankus N. The prevalence of vascular calcification in patients with end-stage renal disease on hemodialysis: a cross-sectional observational study. Therapeutic Advances in Chronic Disease, 2015, 6(3), 84–96. doi:10.1177/2040622315578654
36. Di Lullo L, Gorini A, Russo D, Santoboni A, Ronco C. Left Ventricular Hypertrophy in Chronic Kidney Disease Patients: From Pathophysiology to Treatment. Cardiorenal Med. 2015 Oct;5(4):254-66. doi: 10.1159/000435838.
37. Jinbo Yu, Xiaohong Chen, Yang Li, et al. (2021) Pro-inflammatory cytokines as potential predictors for intradialytic hypotension, Renal Failure, 43:1, 198-205, DOI: 10.1080/0886022X.2021.1871921
38. Lisowska K.A.; Storoniak H.; Soroczynska-Cybula M, et al. ′Serum Levels of α-Klotho, Inflammation-Related Cytokines, and Mortality in Hemodialysis Patients. J. Clin. Med. 2022, 11, 6518. https://doi.org/10.3390/jcm11216518
39. Carrasco-Ruiz MF, Ruiz-Rivera A, Soriano-Ursúa MA, et al. Global longitudinal strain is superior to ejection fraction for detecting myocardial dysfunction in end-stage renal disease with hyperparathyroidism. World J Cardiol. 2022 Apr 26;14(4):239-249. doi: 10.4330/wjc.v14.i4.239.
40. Ørn S, Ueland T, Manhenke C, et al. Increased interleukin-1β levels are associated with left ventricular hypertrophy and remodelling following acute ST segment elevation myocardial infarction treated by primary percutaneous coronary intervention. J Intern Med. 2012 Sep;272(3):267-76. doi: 10.1111/j.1365-2796.2012.02517.x.
41. Degoulet P, Legrain M, Reach I, et al. "Mortality risk factors in patients treated by chronic hemodialysis: Report of the Diaphane collaborative study." Nephron 31.2 (1982): 103-110.
42. Kalantar-Zadeh K, Cano NJ, Budde K, et al. "Racial and survival paradoxes in chronic kidney disease." Nature clinical practice Nephrology 3.9 (2007): 493-506. doi: 10.1038/nrneph.2011.60
43. Tetsuo S, Yoshiharu T, Shigeru N, Yoshiki N, Reverse epidemiology in hemodialysis patients. Lessons from Japanese registries, Néphrologie & Thérapeutique, Volume 4, Issue 4, 2008, Pages 223-227, ISSN 1769-7255, https://doi.org/10.1016/j.nephro.2008.01.005.
44. Bouzas-Mosquera A, Broullón FJ, Álvarez-García N, et al. Left atrial size and risk for all-cause mortality and ischemic stroke. CMAJ. 2011 Jul 12;183(10):E657-64. doi: 10.1503/cmaj.091688.
45. Gao F, Huo J, She J, et al. Different associations between left atrial size and 2.5-year clinical outcomes in patients with anterior versus non-anterior wall ST-elevation myocardial infarction. Journal of International Medical Research. 2020;48(4). doi:10.1177/0300060520912073
46. Luo G, Qian Y, Sheng X, et al. Elevated Serum Levels of Soluble ST2 Are Associated With Plaque Vulnerability in Patients With Non-ST-Elevation Acute Coronary Syndrome. Front Cardiovasc Med. 2021 Jul 22;8:688522. doi: 10.3389/fcvm.2021.688522.
47. Ono Y, Yoshino O, Hiraoka T, et al. IL-33 exacerbates endometriotic lesions via polarizing peritoneal macrophages to M2 subtype. Reprod Sci. (2020) 27:869–76. doi:10.1007/s43032-019-00090-9
48. Dalfino, G., Simone, S., Porreca, S. et al. Bone morphogenetic protein-2 may represent the molecular link between oxidative stress and vascular stiffness in chronic kidney disease. Atherosclerosis 2010, 211(2), Pages 418-423, https://doi.org/10.1016/j.atherosclerosis.2010.04.023