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ANALYSIS OF TOXINS REMOVAL, INFLAMMATION MARKER AND CARDIOVASCULAR OUTCOME IN PATIENTS WITH END STAGE RENAL DISEASE

Presentata da: Fulvia Zappulo

Coordinatore Dottorato

Niccolò Daddi

Supervisore

Gaetano La Manna

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ABSTRACT

Introduction

Patients with End-Stage Renal Disease (ESRD) have a significantly higher risk of cardiovascular (CV) disease due to traditional risk factors (age, male gender, hypertension, diabetes, dyslipidemia, and physical inactivity) and uremic toxins, which independently contribute to CV damage. Chronic inflammation further exacerbates this risk.

Methods

This study evaluates the impact of hemodialysis (HD) on toxin removal, inflammatory status, and cardiac outcomes in ESRD patients starting chronic HD at the Nephrology Unit of IRCCS-AOU Bologna. Baseline clinical, biochemical, and echocardiographic data were collected at enrollment (T0) and after three years (T1). Cardiac outcomes included CV-related mortality and major adverse cardiovascular events (MACE: myocardial infarction, heart failure (HF) hospitalization, arrhythmias, and atrial fibrillation). Moreover correlation between uremic toxins retention and cardiac modification was made.

Results

Fifty-five patients (mean age 65.5 years, BMI 25.8) were enrolled. The overall MACE incidence was 47%. At follow-up, four patients (7.27%) died due to CV events, seven (12.7%) were hospitalized for HF, and 12 (23.5%) developed atrial fibrillation. Regarding cardiac remodeling the prevalence of pulmonary hypertension ($p=0.041$) and pericardial effusion ($p=0.037$) decreased. Significant correlations emerged between β2-microglobulin and ejection fraction (EF) ($r=-0.462$, $p=0.035$), as well as between Kt/V and EF ($p=0.001$) and ferritin level and interventricular septum thickness ($r=0.383$, $p=0.031$). Higher UF rate correlated with EF reduction ($r =- 0.205$, $p=0.049$). Multivariate logistic regression analysis showed a significant association between high UF rate and mortality.

Conclusions

Cardiovascular risk remains high in HD patients, potentially due to uremic toxin

retention and dialysis-related factors. This study highlights the association between UF rate, toxins clearance, and cardiac function in ESRD patients undergoing chronic HD.

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1 INTRODUCTION

Chronic Kidney Disease (CKD) is a worldwide phenomenon who affects over than 10% of the general population worldwide, amounting to >800 million individuals (1). End Stage renal Disease (ESRD) is present in more than 3.7 milions people with an incidence of 20.000 cases per year (2). The onset on Kidney Disease (KD) and the progressive deterioration of renal function increases the risk of cardiovascular disease and of premature death (3). Infact ESRD frequently results from hypertension and diabetes mellitus, which also are the main risk factors for CVD. There is higher prevalence of many traditional factors for CV risk (age, male gender, hypertension, diabetes, dyslipidaemia and physical inactivity) (4).

Moreover, ESRD represents *per se* a CVD risk factor independently by traditional risk factors (5). Infact, HD patients have disease-related risk factors such as anaemia, hyperhomocysteinaemia, CKD–mineral bone disorder (CKD-MBD), oxidative stress, malnutrition and chronic inflammation (6). There is evidence that uraemic factors may be implicated in the pathogenesis of CV disease in HD patients, since CV survival improves after kidney transplantation even in high-risk patients (7).

Uremia is a condition that is characterized by retention of toxins as a consequence of the reduction of renal function (8). In 2003 the *European Uremic Toxin Work Group* (9) has classified uremic toxins according to chemic and phisical properties. In particular small water-soluble molecules (Molecular Weight < 500 Da), protein bound middle molecules (MW > 500 Da). In last years the physiopathological effect and removal pattern of molecules have been considerated for classification of Uremic Toxins (10). Infact these molecules can influence the function of different biological system.

In particular B2-microglobulin (β2M), complement factor D, serum Free Light Chain (FLC), Endothelin and FGF 23 (fibroblast growth factor 23) have negative effects of cardiovascular system: these molecules can enhance inflammatory state and increase fibrosis (11,12)

Furthermore the accumulation of uremic toxins is enhanced by an inflammatory state: patients affected by ESRD experienced lot of comorbidities (diabetes,

peripheral vasculopathy, obesity, advanced age). Moreover the dialytic process itself can enhance the inflammatory state throught the biocompatibility of the dialysis membrane, the water dialysis solution and vascular access (13,14). Infact the blood–membrane contact during dialysis triggers a foreign body reaction with the recruitment of neutrophils and monocytes, which release pro-inflammatory cytokines; therefore, the measurement of neutrophils and monocytes and of their activation or apoptosis provides useful information on dialyzer biocompatibility (15,16).

The removal of Uremic Toxins is important to determine patient survival and reduce the cardiovascular burden.

B2-microglobulin is considered the marker of middle molecules and the it is currently used as a marker of dialysis adequacy. In previous observational study, removal rate of middle molecules during HD treatment in related to lower mortality and morbidity rates (17). Finally dialytic adequacy is responsible for depurative capacity and removal of uremic toxins.

1.1 Inflammation in ESRD

The genesis of chronic inflammatory state in HD patients is heterogenous. Most factors are related to the dialytic treatment itself: the biocompatibility of the dialysis membranes, the ultrapure dialysate, the vascular access and its disfunctions.

In dialysis patients the dialyzer membrane can generate an inflammatory response which is very complex: it involves leucocytes, cytokines and chemokines, platelets, complement system ad coagulation factors. These phenomena lead to chronic inflammation, fibrosis, endothelial activation and increasing cardiovascular risk (18). The persistence of inflammatory state in HD patients has an important role in the onset of atherosclerosis (19) and it determine a worse outcome of the patient. This is determined by the increased cardiovascular risk, all-cause mortality, vascular calcification, protein energy wasting syndrome (PEW).

1.2 Cardiovascular Risk assessment in dialysis patients

The most common cardiac alteration in ESRD are Myocardial hypertrophy, cardiac calcifications, pericardial effusion and feart Failure (HF). The genesis of these alteration is complex and it involves: CKD Mineral Bone Disease (MBD), Anemia (20), Inflammation, Uremic toxins retention (21), Oxidative stress (22), Endothelial disfunction and Renin Angiotensin System (RAS) activation (23).

Echocardiography is commonly used as the primary method for evaluating cardiac function and structure. As a result, it is also considered one of the most accessible and robust examinations for evaluating heart failure in patients with kidney failure (24).

The pathogenesis of the increased cardiovascular disease and cardiac events in Kidney disease and in ESRD in not fully understand. The aim to this study is to analyze factor that are related to dialytic treatment and that can contribute to cardiovascular outcome.

2 AIM OF THE STUDY

This is a prospective, observational, monocentric study with the aim to evaluate the influence of dialytic treatment on toxins removal, inflammatory state and cardiac outcome in patients with ESRD who started Chronic HD treatment in the Nephrology Unit of IRCCS-AOU of Bologna.

The primary endpoint of this study was the evaluation of cardiovascular death and incidence of major adverse cardiovascular events (MACE).

The secondary endpoint were:

- The evaluation of changes in inflammatory markers, middle molecules removal and UF rate;
- The correlation between inflammatory markers, middle molecules removal, UF rate and cardiac remodelling.

3 PATIENTS AND METHODS

Patients suffering from ESRD who started Chronic Hemodialytic treatment (HD) were enrolled in this study.

Inclusion criteria were:

- Stable HD treatment for 3 months at most;
- HD scheduled 3 times/week
- Compliance to prescribed therapy.

Exclusion criteria were:

- Age < 18 years or > 90 years;
- Previuos renal transplant;
- Severe cardiac Heart Failure (HF) with EF < 20%;
- Active cancer;
- Inability to consent;
- Pregnancy.

For each patients the following baseline characteristic were recorded: age, sex, body weight, BMI, comorbility (nephropathy, smoke use, diabetes, hypertension, dyslipidemic, chronic obstructive peripheral artery disease, aritmic disease, atrial fibrillation, coronary artery disease, cardiomiopathy, history of cancer), concomitant medications, dialysis prescription and vascular access. Moreover the following haematological, biochemical and depurative parameters have been evaluated: White Blood Cells count (WBC), Heamoglobin (Hb), Urea, serum creatinine, electrolytes (Na, K, P, Ca), Parathyroid Hormone (PTH), inflammation markers (albumin, iron, Ferritin, transferrin Saturation, C-reactive protein), middle molecules and uremic toxins (β2-microglobulin, κ and λ free light chain).

In addition, cardiac outcome has been evaluated: death for cardiovascular disease and onset of MACE (Major Adverse Cardiovascular Events: (myocardial infarction, hospitalization for heart failure (HF), onset of arrhythmia and atrial fibrillation (AF), stroke). Finally ecocardiographic changes have been analysed: ejection fraction (EF), interventricular septum (IV) thickness, aortic stenosis, mitral stenosis, aortic calcification, pulmonary hypertension, pericardial effusion. These

evaluations have been recorded within 3 months from the beginning of chronic HD treatment (TO) and after 3 years (T1).

3.1 Statistical methods

Continuous variables are expressed as mean and standard deviation if normally distributed, or as a median with IQ range if unusually distributed. Categorical variables are presented as number (n) and percentage (%). The Student's t-test was used to assess differences between means. Differences between categorical variables were analysed by the chi-square test. Correlation analyses were performed computing Pearson's correlation coefficients. Univariate and multivariate conditional logistic regression models were used to assess the impact of the exposure to risk factors and risk of death ad hospitalization for Heart Failure. Statistical analysis have been performed by Jamovi software (version 2.38.28), with a significance level set at $p < 0.05$.

4 RESULTS

Fifty-five patients, fulfilling the inclusion criteria, have been enrolled in this study. Mean Age was 65.5 years. Mean Body weight was 70.1 Kg and BMI 25.8. Twenty-seven patients were male (49,1%). The table 1 shows the main epidemiological and clinical features of the analysed cohort.

The majority of patients (36.4%) didn't have a specific diagnosis of the disease which lead to ESRD, the other specific causes presented in the cohort were Glomerulonephitis (20%), Diabetic Kidney Disease (DKD 14.5%), Nephroangiosclerosis (10.9%), Tubulointerstitial Nephritis (7.3%), Autosomal Dominant Policistic Kidney Disease (ADPKD, 3.6%), other Inherited Disease (3.6%), Multiple Myeloma (3.6%) (table 2). At baseline 27% of the cohort was treated with Renin Angiotensin System (RAS) Blockers and only 5.5% with Mineral Receptor Antagonist (MRA) blocker. Table 3 shows baseline concomitant medications in the considered cohort.

Dialysis modality was High Flux Haemodialysis (HF-HD) in 31 patients (56.4%), online Haemodiafiltration (olHDF) in 21 patients (38.2%) and Haemodiafiltration with endogenous reinfusion (HFR) in 3 patients (5.5%). OlHDF was carried out with a substitution fluid of almost 16 Lt per session. Dialysis tecnique was established considering blood pressure, intoxication by middle molecules, the performance of vascular access and inflammatory state.

Vascular access was arterovenous fistula (AVF) in 32 patients (59.3%), a tunnelled Central Venous Catheter (CVC) in 18 patients (33.3%) and arterovenous graft (AVG) in 4 patients (7.4%). The mean ultrafiltration rate was 5.93 ± 3.65 ml/Kg/hour. In table 4 baseline biochemical and haematological features are shown.

Regarding cardiac characteristics at baseline mean EF at baseline was 58.4 ± 10.9 %, mean interventricular septum was 1.2 ± 0.17 cm. Seven patients (12.7%) presented aortic stenosis, 2 (3.7 %) subjects had a mitral stenosis, 20 patients (37%) had aortic calcifications. 7 patients (12.7%) presented pulmonary hypertension while 8 patiens (14.8%) had mild pericardic effusion (table 5).

During the 3-years follow-up the dialysis prescription was changed increasing convective techniques: HF-HD (17%), olHDF (56.6%), HFR (18.9%), Expanded

Haemodialysis (HDx 5.7%), Acetate Free Biofiltration (AFB 1.9%). The reasons for changing the dialytic tecnique were: intradialytic hypotension (37%), need for increase dialytic dose (40%), chronic inflammation (21%) and hypercapnia (1%). The mean time for change the dialytic treatment was 11.6 months.

Haematological and Biochemical parameters at 3-years follow-up showed a significant decrease of Urea 124 mg/dl (p 0.001) and Phosphate (p 0.013). Moreover a significant increase of serum creatinine 8.15 mg/dl (p 0.001), Ferritin 449 mg/dL (p 0.001), Trasferrin saturation (p 0.024) and ERI-index (p 0.003) was found. CRP level (mean 2.46 mg/dL) was higher (p 0.056). Similarly an higher Mean UF rate was found 8.49 vs 5.93 ml/kg/hour (p 0.003). Data are shown in table 4 and figure 1 – 6.

At follow up time cardiac EF was 57.4% (p 0.334), mean interventricular septum was 1.24 (p 0.084). 13 (23%) patients presented aortic stenosis (p 0.157), 46 subjects (73.2%), 5 (9%) had a mitral stenosis (p 0.452), 23 patients (41.18%) had aortic calcifications (p 0.048). Finally there was a significative reduction of patients who presented pulmonary hypertension (p 0.041) and of patients who had pericardial effusion (p 0.037) (table 5).

Considering β2-microglobulin as markers of middle molecules, we have analysed the effect of retention of this toxin on cardiac function, measured with EF: we found a significant association between β2-microglobulin and EF (r Pearson -0.462, p 0.035) (figure 7). Moreover, a significant association has been found between Kt/V and EF (p 0.001) (figure 8).

Since ferritin was considerable higher at 3-year follow up we analysed the correlation existing between Ferritin level and cardiac changes: we found a correlation between Ferritin level and EF at the follow-up (r -0.462, p 0.065) (figure 9) and between Ferritin level and interventricular septum (IV) thickness (r 0.383, p 0.031) (figure 10).

Similarly a correlation has been found between UF rate and EF (r -0,205, p 0.049) (figure 11) and between UF rate and IV septum thickness (p 0.243) (figure 12). Nevertheless a correlation has been found between ERI-index and EF even if not statistically significant (p 0.400) and between ERI index and IV septum (0.945).

4.1 Survival and outcome

At 3 years follow- up 4 patients (7.27%) died because of cardiovascular event: 2 patients Non ST Elevation Myocardial Infarction NSTEMI, 1 patient Heart Failure (HF) and 1 patient Ischemic Stroke. Seven patients (12.7%) were hospitalized because of HF. Four patients (7.7%) experienced a coronary heart disease (CAD) while 12 patients (23.5%) Atrial Fibrillation (AF). One patient had a non fatal Ischemic Stroke. The incidence of MACE is 47%.

Mutivariate logistic regression analysis was perfomed to determine the effect of UF rate, ERI-index, Ferritin level, Kt/V value on risk of cardiovascular death. It found significant association between high UF-rate and patients mortality (table 6).

Moreover multivariate logistic regression analysis was performed to determine the effect of UF rate, ERI-index, Ferritin level, Kt/V value on risk of hospitalization: the results show that no significant association with risk for hospitalization for Heart Failure (table 7).

5 TABLES AND FIGURES

Variables	Results
Age, years mean (sd)	65.5 (14.2)
Body weight, kg mean (sd)	70.1 (17.6)
Body Mass Index (BMI), kg/cm^2 mean (sd)	25.8 (6.2)
Smoke use, n (%)	26 (47.3)
Diabetes, n (%)	25 (45.5)
Hypertension, n (%)	49 (89.1)
Dyslipidemic, n (%)	41 (74.5)
Chronic obstructive peripheral artery disease, n (%)	20 (36.4)
Aritmic disease, n (%)	6 (10.9)
Atrial fibrillation (AF), n (%)	8 (14.8)
Coronary artery disease, n (%)	15 (27.3)
Cardiomiopathy, n (%)	9 (16.3)
History of cancer, n (%)	4 (7.3)
Diuresis, ml/day, mean (SD)	1250 (566)

Table 1 - Baseline Clinical and epidemiological characteristics of patients

Primary nephropathy	N (%)
Unknown, n (%)	20 (36.4)
Glomerulonephritis, n (%)	11 (20)
Diabetic kidney disease DKD, n (%)	8 (14.5)
Nephroangiosclerosis, n (%)	6 (10.9)
Tubulointerstitial nephritis, n (%)	4 (7.3)
ADPKD, n (%)	2 (3.6)
Inherited disease, n (%)	2 (3.6)
Multiple myeloma, n (%)	2 (3.6)

Table 2 - Primary Nephropaty of patients

Medication	T0	T1	p
RAS BLOCKERS n (%)	15 (27.3)	6 (11.76)	0.046
B-BLOCKERS n (%)	30 (54.5)	30 (58.82)	0.210
MRA n (%)	3 (5.5)	1 (1.96)	0.420
K-binders, n (%)	9 (16.8)	21 (41.17)	0.014
ESAs, n (%)	24 (43.6)	48 (94.11)	0.001
Intravenous iron, n (%)	2 (3.6)	35 (68.62)	0.001
Antiaggrengant, n (%)	33 (60)	31 (60.78)	0.341
Oral anticoagulants, n (%)	6 (11)	21 (41.17)	0.004

Table 3 - Concomitant medications at baseline and at 3-years follow-up (T1) RAS: Renin Angiotensin System, MRA: Mineral Receptor Antagonist, ESAs Erithropoiesis Stimulating Agents

Variables	T0	T1	p
WBC, 10^9 cells, mean (sd)	6578 (2895)	7215.53 (2855)	0.409
Hb, gr/dl median (IQR)	10.30 (1.85)	10.30 (1.57)	0.384
PLT, 10^9 cells, mean (sd)	224,000 (79525)	203,000 (85500)	0.658
Urea, mg/dl mean (sd)	165.65 (47.33)	125.41 (40.79)	0.001
Creatinine, mg/dl mean (sd)	7.14 (1.93)	8.66 (2.09)	0.001
Na, mmol/L mean (sd)	143.19 (38.41)	138.9 (3.02)	0.405
K, mmol/L mean (sd)	4.84 (0.751)	5.15 (0.41)	0.171
Ca, mg/dl mean (sd)	8.68 (0.50)	8.63 (0.98)	0.363
P, mg/dl mean (sd)	5.46 (1.54)	5.02 (1.45)	0.013
PTH, pg/ml mean (sd)	254.26 (187.28)	271.33 (197.27)	0.816
Albumine, gr/dl mean (sd)	34.8 (5.81)	40.41 (37.33)	0.773
β2-microglobulin, mg/l mean (sd)	26.61 (12.24)	43.63 (67.31)	0.243
Iron, microg/dl mean (sd)	51.58 (27.34)	48.43 (26.86)	0.867
Ferritin, ng/dl mean (sd)	235.25 (221.5)	499.03 (424)	0.001
Transferrin saturation, % mean (sd)	15.8 (10.13)	28.66 (25.62)	0.024
K-FLC, mg/L mean (sd)	131.27 (65.92)	152.38 (145.7)	0.864
Λ-FLC, mg/L mean (sd)	315.98 (1152.23)	95.37 (48.95)	0.347
CRP,mg/dl mean (sd)	0.82 (0.8)	2.46 (1.15)	0.056
Kt/, mean (min – max)	1.21 (0.7 -2)	1.5 (0.7 – 2.38)	0.141
ERI-INDEX, U/Kg/week, mean (sd)	9.14 (16.9)	25.6 (14.6)	0.003
Uf rate, ml/kg/hour, mean (sd)	5.93 (3.65)	8.46 (2.83)	0.002

Table 4 - Laboratory variables at baseline and at 3-years follow-up (T1) WBC White Blood Cells, PLT PLatelets, PTH ParaTyroid Hormone, FLC Free Light Chain, ERI Erithropoietin Resistence Index, UF UltraFiltration

	T0	T1	p
EF (%) mean, SD	58.4 (10.9)	57.4 (6.8)	0.334
IVS, SD	1.20 (0.176)	1.24 (0.62)	0.084
Aortic stenosis n (%)	7 (12.7)	13 (25.49)	0.157
Mitral Steosis n (%)	2 (3.7)	5 (9.8)	0.452
Aortic Valve Calcifications, n (%)	20 (37)	23 (45.09)	0.347
Pulmonary hypertension, n (%)	7 (12.7)	5 (9.8)	0.048
Pericadial Effusion, n (%)	8 (14.8)	3 (5.88)	0.037

Table 5 - Cardic Characteristics of the cohort at baseline and at 3-years follow-up (T1) EF: Ejection fraction, IVS InterVentricular Septum

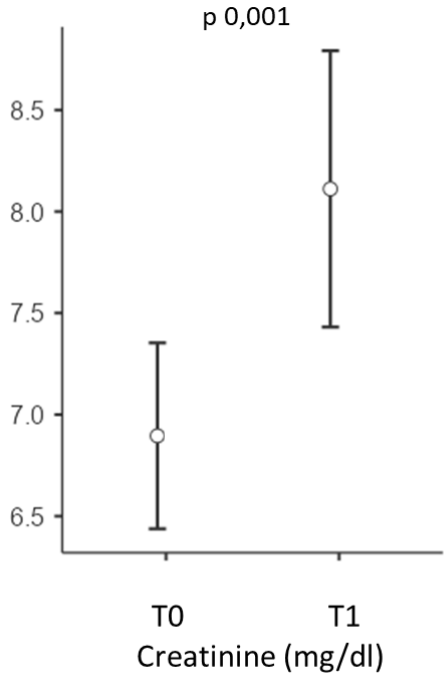

Figure 1 - Changes of Creatinine level at baseline and at 3years follow up (T1)

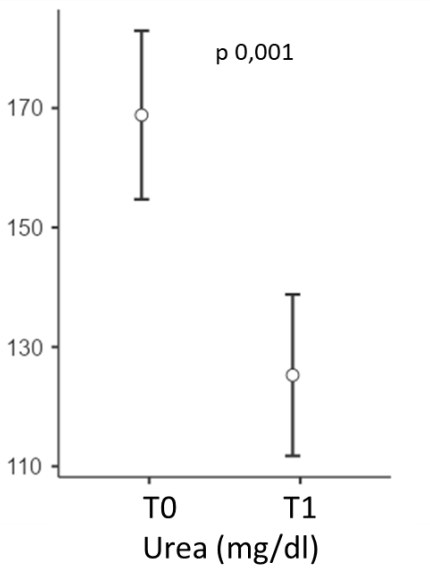

Figure 2 - Changes of urea level at baseline and at 3years follow up (T1)

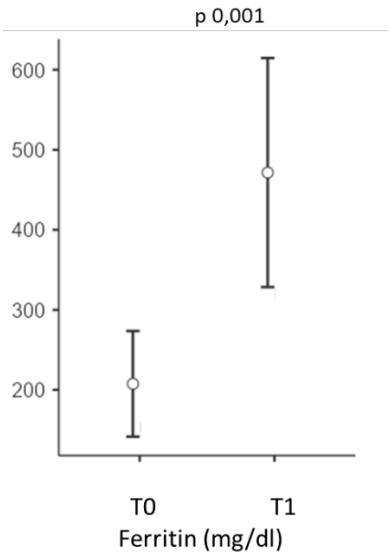

Figure 3 - Changes of Ferritin level at baseline and at 3years follow up (T1)

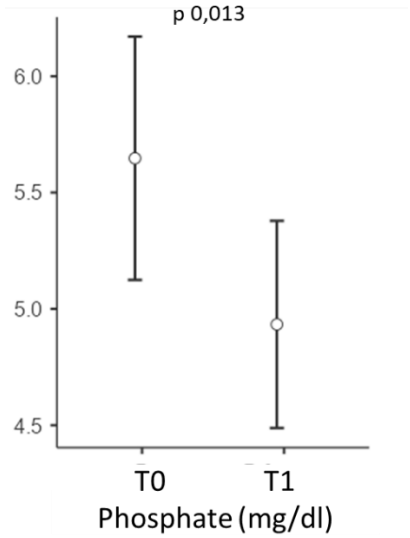

Figure 4 - Changes of phosphate level at baseline and at 3years follow up (T1)

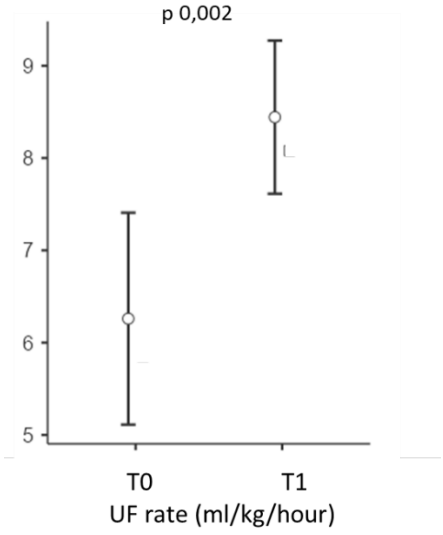

Figure 5 - Changes of UF rates at baseline and at 3years follow up (T1)

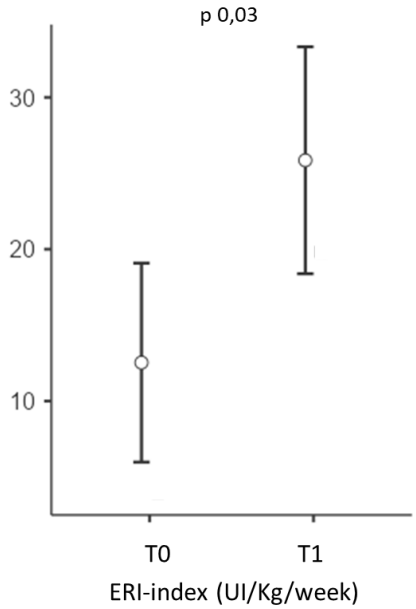

Figure 6 - Changes of ERI-index level at baseline and at 3years follow up (T1)

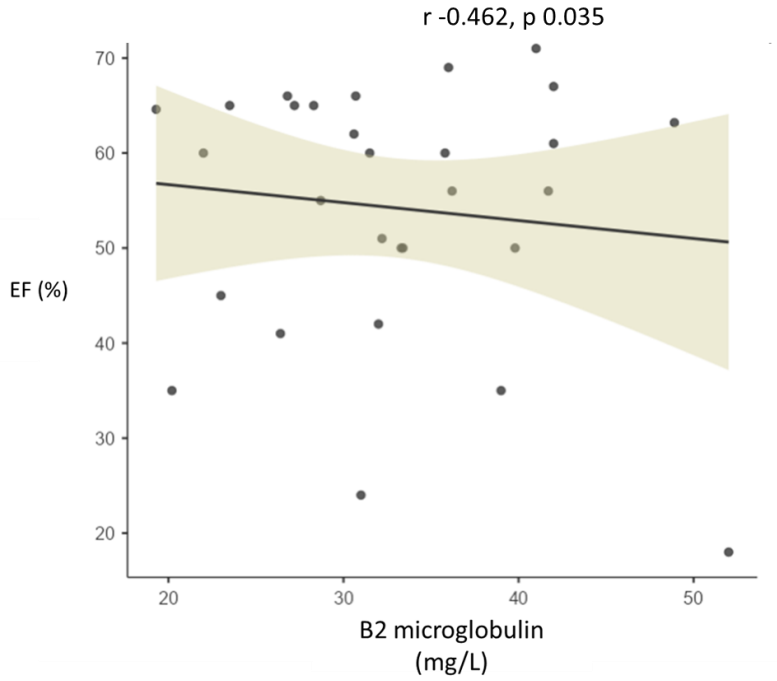

Figure 7 - Correlation between B2microglobulin level and Ejection Fraction (EF)

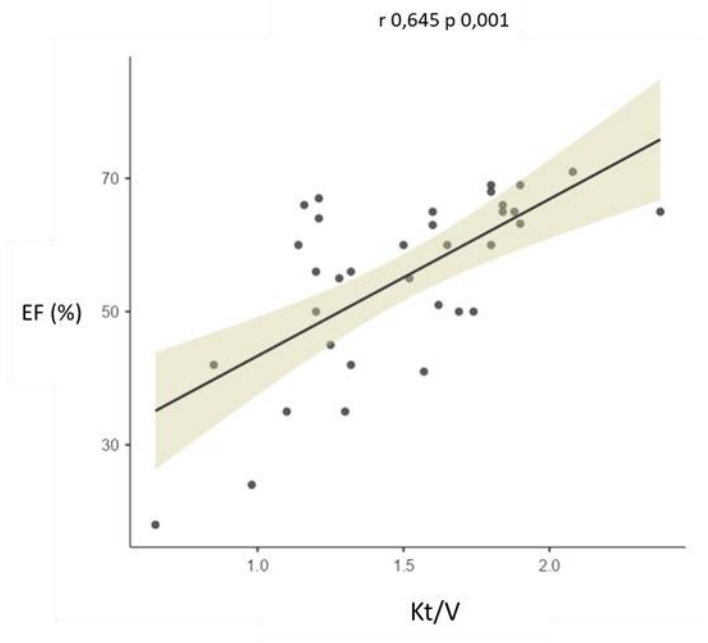

Figure 8 - Correlation between Kt/v value and Ejection Franction (EF)

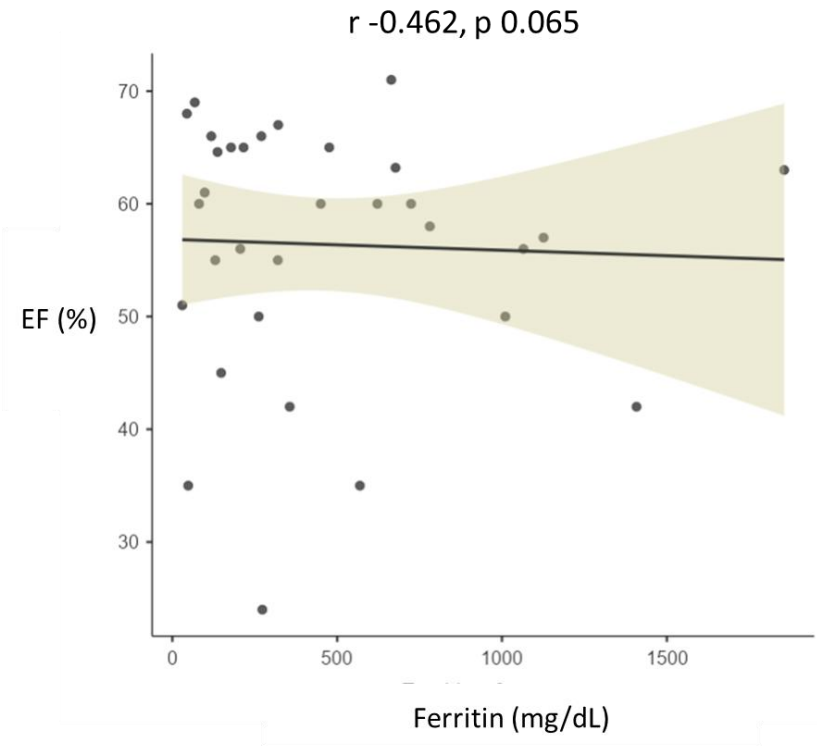

Figure 9 - Correlation between Ferritin level and Ejection Fraction (EF)

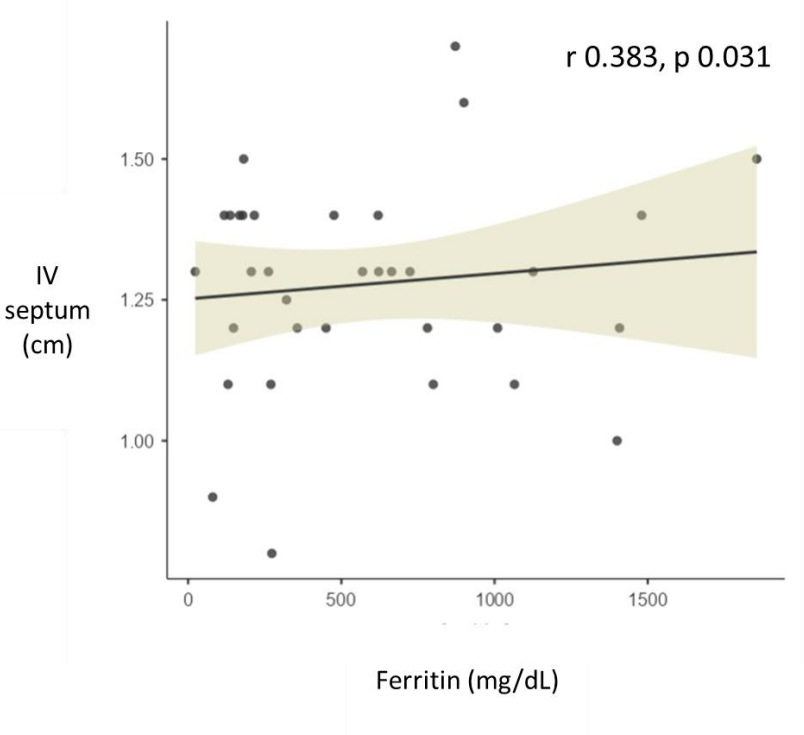

FIgure 10 - Correlation between Ferritin level and Interventricular Septum tickness

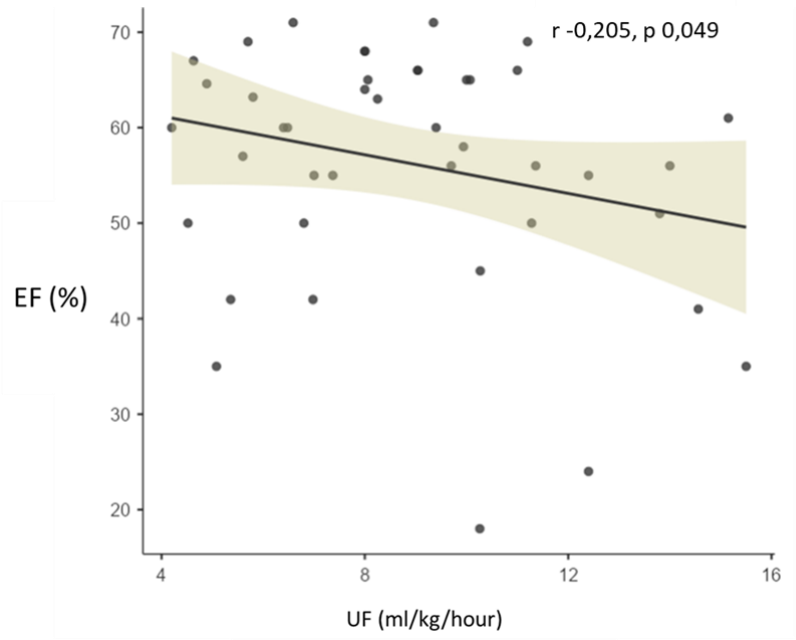

FIgure 11 - Correlation between UF rate and Ejection Fraction

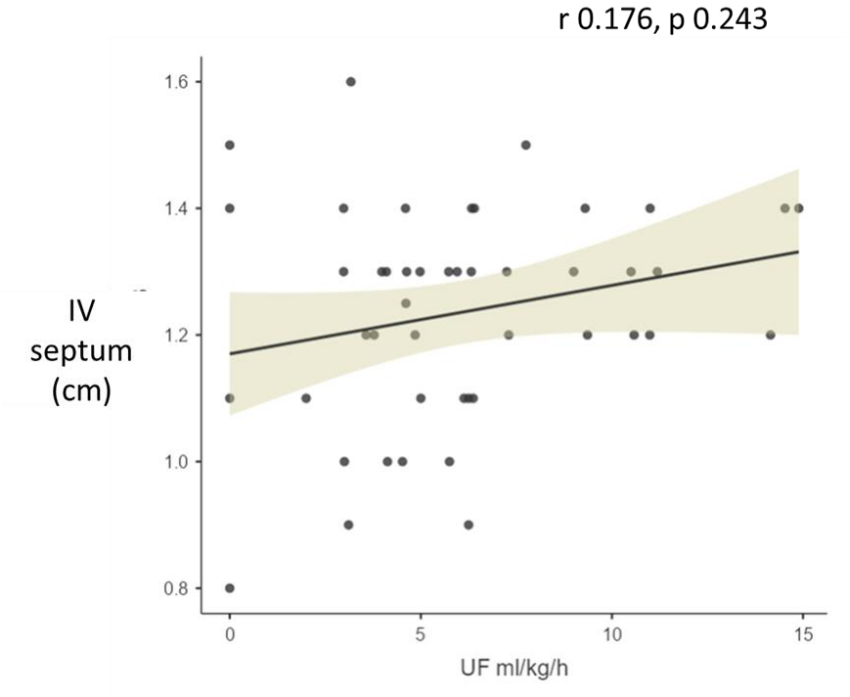

FIgure 12 - Correlation between UF rate and Interventricular Septum thickness

	OR	95% CI	p
UF rate (ml/Kg/hour)	2.37	0.89 – 4.29	0,004
ERI-index (U/Kg/week)	1.04	0,91 – 1,18	0,621
Ferritin (mg/dL)	1.04	0,98 - 1,01	0,571
Kt/V	2.33	0.001- 4.04	0.747

Table 6 - Multivariate logistic regression analysis of predictive factor of death.

	OR	95% CI	p
UF rate (ml/Kg/hour)	1.01	0.82 – 1.25	0.541
ERI-index (U/Kg/week)	1	0.92 – 1.08	0.965
Ferritin (mg/dL)	1.08	0.91 – 1.19	0.541
Kt/V	0.66	0.18 – 2.52	0.547

Table 7 - Multivariate logistic regression analysis of predictive factor of hospitalization for heart failure

6 DISCUSSION

This study investigates the outcome of patients with ESRD undergoing chronic HD treatment. It examines the effect of HD treatment on removal of middle molecules and its impact on inflammatory markers. The findinds indicate a significant reduction in certain uremic toxins such as urea and phosphate, while increase in ferritin, transferrin saturation and ERI index are noted at 3 year follow up. Furthermore the mean UF rate is also increased. These changes in uremic toxins and inflammatory markers may also provide insight into the complex interplay affecting cardiovascular putcomes in patients receiving chronic HD. Additionaly, the study explores cardiac remodeling in relation to uremic toxins and inflammatory markers, evaluation potential correlation among these factors.

Previosly Port et al, demonstrated that lower Kt/V and higher BMI were associated with greater risks for all-cause mortality (25). Similary the HEMO study, which analyse cardiac event in ESRD population, found that cardiac deaths accounted for 39.4% of all-cause mortality. Notably, the HEMO Study did not show a significant benefit of increased dialytic dose in reducing all-cause mortality or cardiac events. This result was consistent across various cardiac outcomes, with the average relative risk of the high dose goal compared with the standard dose goal being close to unity. However, in the same study, high flux HD was associated with a significant reduction in cardiac death (20%) and 13% of reduction in the composite outcome of first cardiac hospitalization or cardiac death. In patients with older dialytic age (over 3.7 years before the study) there was a greater (37%) reduction of the risk of cardiac death associated with high flux HD (26).

A previous meta-analysis of 11 studies (total number of patients 3396) has compared different convective dialysis tecniques (hemofiltration, hemodiafiltration, and acetate-free biofiltration) with standard HD : the data showed a reduction in cardiovascular mortality but no effect on death from any cause, nonfatal cardiovascular events, or hospitalization (27).

Recently the CONVINCE study has demonstrated a lower risk of death from any cause among patients who were receiving high-dose hemodiafiltration compared to

those on HF-HD, with notable benefits in infection-related and cardiovascular deaths showed a beneficial effect for hemodiafiltration (28).

Cardiovascular disease in ESRD, as well as in the general population, is a complex process. Atherosclerosis and arterial occlusive lesions (coronary, peripheral artery disease and cerebrovascular) are the most frequent causes of cardiovascular morbidity in ESRD (29).

Looking at cardiac outcome 4 patients died because cardiovascular events, but 17 patients experienced a cardiovascul event including AF, ospedalization for HF, ischemic stroke and acute coronary artery disease). In our study, middle molecules retention has been associated to a worse patient cardiac involvement as shown throught the correlation between B2microglobulin and EF and Kt/v and EF. It is well know that heart failure is prevalent among patients with CKD and ESRD (30). Previosly predialytic EF was found to correlate negatively with myocardial calcification, PTH level, and serum calcium-phosphorus product (31).

In this study, a significant reduction in certain uremic toxins, such as urea and phosphate, was observed after three years of treatment; we hope that dialysis tecniques with greater capacity for reducing these molecules may therefore contribute, to improve cardiovascular outcome in patients with ESRD.

Similary the prevalence of Left Ventricular (LV) hypertrophy increases with the progression of CKD and rise 90% in patients with an eGFR less than 15 mL/ min (32). The main stimulus to myocardial hypertrophy is volume overload (33). As demonstrated in our study increased UF rate, which are the consequence of volume overload, are associated to the developpement of worsening hypertrophy (evaluated as IV septum thickness). Some studies have highlight that higher UF rates are associated with worse patient's outcome.

In a retrospective study of 1846 patients receiving thrice-weekly chronic dialysis higher ultrafiltration rates were significantly associated with increased all-cause and cardiovascular-related mortality. Nevertheless, Flythe et al have found a linear dose–response relationship between the UF rate and the risk of incident atrial fibrillation (34). In the analyzed cohort, the UF rate was also significantly

associated with cardiovascular mortality (OR 2.37, p 0.004). However it is not associated with hospitalizzation for heart failure in the multivariate analysis.

The activation of the RAS and sympathetic system determines persistent arterial pressure elevation and accelerating atherosclerosis, contributing to to myocardial hypertrophy. Althought there are no RCT on RAS inhibitors use in dialysis patients a review published in 2009 have reported that the blockade of the RAS with ACE inhibitors or ARBs might be beneficial for patients with ESRD in terms of the prevention of cardiovascular events.

Another interesting findigs of the study was that ferritin level has been associated to worsening of cardiac function (EF) and increasing risk of hypertrophy.

It as alredy been demonstrated the markers of iron metabolism alteration (ferritin, transferrin saturation) have been indicated as important prognostic and treatment values (35).

Infact in the 2021 guidelines for the diagnosis and treatment of heart failure (ESC) advocated screening for iron deficiency (including ferritin and transferrin saturation) with class IC recommendation (36). Moreover, HF heart failure patients with EF < 50% should be considered for iron supplementation if iron deficiency is found. Nevertheless ferritin could be regarded as a marker of both iron metabolism and inflammation. Ferritin decreases in iron deficiency and increases in inflammatory conditions (35). Iron metabolism is also involved in atherosclerosis process: infact it has been documented that serum ferritin enhanced low-density lipoprotein oxidation, which represent a risk factor for atherosclerosis progression (37). In our population, the reduction of ferritin value determines an amelioration of sistolic cardiac function (EF) in patient with preserved EF. Previosly high ferritin concentrations were linked to an accentuated EF decline in STEMI patients treated with PCI (38).

Beyond its effect on Hb, iron plays an important role in oxygen transport and in the metabolism of cardiac muscle. Mitochondria are the most important sites of iron utilization and energy production. These factors can have roles in the reduced functional capacity in HF. Simillary a pro-inflammatory state has been observed in overweight and obese patients : in these cases ferritin acted more as marker of

inflammation than a marker of iron metabolism (39). These factors can exlplain the adverse clinical events associated with both lower and higher ferritin level when it indicates iron deficiency, and a pro-inflammatory condition (35).

Regarding ERI index a previous multicenter prospective study included 4034 patients undergoing maintenance HD, has shown that baseline ERI at six months predicted only all-cause mortality; however, time-dependent ERI was a predictor of cardiac events, all-cause mortality, major cardiovascular event and HF (40). Similary the results of Lu et al (41), demonstrated a relationship between comorbidity factors and erythropoietin resistance in HD patients and a negative impact on survival. In this study ERI was used to evaluate Erytropoitin hyporesponsiveness. The result of the study found that ERI had a statistically positive correlation with CRP, ferritin, and a negative correlation with Albumine and creatinine. In the same study ERI was a significant predictor of all-cause mortality and cardiovascular mortality.

In conclusion, this study describes cardiovascular outcome of HD patients, exploring the relationship with uremic toxins and dialytic treatment. Even in a small size cohort, cardiac remodelling has been analyzed: this could represent the base to modulate HD treatment according CV risk of patients.

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