

Research Article

Risk Factors for Mortality in Hemodialysis Patients: Two-Year Follow-Up Study

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Received 24 January 2013; Accepted 2 May 2013

Academic Editor: Sudhir Srivastava

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Background. End-stage renal disease (ESRD) patients under hemodialysis (HD) have high mortality rate. Inflammation, dyslipidemia, disturbances in erythropoiesis, iron metabolism, endothelial function, and nutritional status have been reported in these patients. Our aim was to identify any significant association of death with these disturbances, by performing a two-year follow-up study. **Methods and Results.** A large set of data was obtained from 189 HD patients (55.0% male; 66.4 ± 13.9 years old), including hematological data, lipid profile, iron metabolism, nutritional, inflammatory, and endothelial (dys)function markers, and dialysis adequacy. **Results.** 35 patients (18.5%) died along the follow-up period. Our data showed that the type of vascular access, C-reactive protein (CRP), and triglycerides (TG) are significant predictors of death. The risk of death was higher in patients using central venous catheter (CVC) (Hazard ratio [HR] = 3.03, 95% CI = 1.49–6.13), with higher CRP levels (fourth quartile), compared with those with lower levels (first quartile) (HR = 17.3, 95% CI = 2.40–124.9). Patients with higher TG levels (fourth quartile) presented a lower risk of death, compared with those with the lower TG levels (first quartile) (HR = 0.18, 95% CI = 0.05–0.58). **Conclusions.** The use of CVC, high CRP, and low TG values seem to be independent risk factors for mortality in HD patients.

1. Introduction

Patients with end-stage renal disease (ESRD) have a high mortality rate [1, 2] that far exceeds the mortality rate for the non-ESRD population [3]. In the past half-century, the widespread use of hemodialysis (HD) to prolong life of ESRD patients has been a remarkable achievement, preventing death from uremia in these patients. Nowadays this therapy has expanded widely and is being used by an increasing

elderly patient population, leading to significant economic consequences to patients and to healthcare systems.

Our present knowledge of the mechanisms leading to increased death in this context is incomplete. In the last years, this medical field has known significant technological and pharmacological improvements. Although some evidence may suggest that mortality rate among dialysis patients has decreased over the last few years, actually, patient's survival is still low. Cardiovascular disease (CVD) has been considered

the most common cause of death in these patients [4]. Cardiac arrest and congestive heart failure are more prominent causes of cardiovascular death than acute myocardial infarction in patients with uremia. A higher mortality rate within the first year after initiation of HD has been described (the period of highest risk for death extends to approximately 120 days after starting dialysis).

The high risk of cardiovascular morbidity and mortality in ESRD patients is associated with a high prevalence of classic cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia, smoking, and advanced age). In addition, several uremia-related factors may also play an important role, namely, the presence of multiple comorbid conditions, fluid overload, hyperphosphoremia, high calcium-phosphorous product, anemia, left ventricular hypertrophy, inflammation, oxidative stress, endothelial dysfunction, insulin resistance, excess sympathetic tone, hyper-homocysteinemia, high levels of lipoprotein(a), and increased asymmetrical dimethylarginine [5–8]. Recent reports have highlighted the importance of noncardiovascular mortality in HD patients and that it has been underestimated [3].

Systemic inflammation is frequently present in ESRD patients, and the use of central venous catheter (CVC) has been associated with an enhanced inflammatory state [9].

The aim of the present study was to evaluate the global mortality in Portuguese ESRD patients under HD, by performing a follow-up study of two years, in order to identify any significant association of death with systemic parameters, including dialysis adequacy, nutritional status, hematological data, lipid profile, iron metabolism, and inflammatory and endothelial (dys)function markers, as well as the type of vascular access, presence of comorbidities, and with associated therapies. The parameters associated with mortality in this context may provide biomarkers to be used in the clinical setting.

2. Methods

2.1. Patients. This research protocol was approved by the Ethics Committee of Fresenius Medical Care, Portugal. All participants gave their written informed consent to participate in this study.

In this two-year follow-up study, starting from April 2009, 189 HD patients, from 3 dialysis clinics in the Northern region of Portugal, were included in the study. These patients were under HD for at least 90 days. At the start of the study, the patients were clinically evaluated and blood was collected for the analytical studies; afterwards, a clinical followup was performed during two years, in order to identify the cases of death, transplant, and transfer to peritoneal dialysis. Data regarding demographic characteristics, chronic kidney disease and medical history, dialysis, and medical prescriptions, as well as laboratory data, was also collected at the start of the study.

Diabetes was defined by the current guidelines [10] or by the use of insulin or oral hypoglycemic agents. Hypertension was defined by the current guidelines (blood

pressure > 130/85 mm Hg) [11] or by the use of antihypertensive medication. Therapy with recombinant human erythropoietin (rhEPO) and with intravenous iron was based on the current guidelines. The classification of ESRD patients as responders or nonresponders to rhEPO therapy was performed in accordance with the European Best Practice Guidelines [12]. Patients with autoimmune disease, malignancy, and acute or chronic infection were excluded.

A group of 25 healthy volunteers was selected as control, based on normal hematological and biochemical values, and no history of kidney or inflammatory diseases, in order to better define the changes occurring in HD patients. This group was matched, as far as possible, for age and gender with HD patients.

2.2. Assays. Blood samples were obtained immediately before the HD procedure, in the midweek dialysis day, and processed within 2 hours after collection. Blood was collected to tubes with (EDTA) and without anticoagulant, in order to obtain whole blood, plasma, buffy-coat, and serum. Aliquots were immediately stored at -80°C , whenever necessary, until assayed.

Erythrocyte count, hematocrit, hemoglobin concentration, and hematimetric indices (mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)) were measured by using an automatic blood cell counter (Sysmex K1000; Sysmex, Hamburg, Germany). Leukocyte differential counts were evaluated in Wright-stained blood films. Reticulocyte count was made by microscopic counting on blood smears after vital staining with new methylene blue (reticulocyte stain; Sigma, St. Louis, MO, USA). The reticulocyte production index (RPI) was calculated, as an appropriate way to measure the effective erythrocyte production, by correcting for both changes in hematocrit (degree of anemia) and for premature reticulocyte release from the bone marrow.

Serum albumin levels were measured using a colorimetric assay end-point method (Albumin Plus; Roche GmbH, Mannheim, Germany). Serum iron concentration was determined using a colorimetric method (Iron, Randox Laboratories Ltd., North Ireland, UK), whereas serum ferritin and serum transferrin were measured by immunoturbidimetry (Ferritin, Laboratories Ltd., North Ireland, UK; Transferrin, Laboratories Ltd., North Ireland, UK). Enzyme-linked immunosorbent assays were used for measurement of plasma soluble transferrin receptors (s-TfR) (Human sTfR immunoassay, R&D systems, MN, USA); transferrin saturation (TS) was calculated by the formula: $\text{TS (\%)} = 70.9 \times \text{serum iron concentration (mg/dL)} / \text{serum transferrin concentration (mg/dL)}$.

Plasma concentration of adiponectin, interleukin (IL)-6, tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) were evaluated by using standard commercial enzyme-linked immunoassays (adiponectin, IL-6 ELISA High-Sensitivity, tPA, and PAI-1, all from eBioscience). D-dimer and C-reactive protein (CRP) were evaluated by immunoturbidimetry, using commercially available kits (BCS XP system, Siemens, Germany; CRP (latex) High-Sensitivity, Roche Diagnostics, resp.).

The activity of paraoxonase 1 (PON1) was assessed spectrophotometrically and expressed in nmol of p-nitrophenol/mL/min. Briefly, PON1 activity was measured by adding serum to 1 mL Tris/HCl buffer (100 mmol/L, pH 8.0) containing 2 mmol/L CaCl₂ and 5.5 mmol/L paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma Chemical Co.). The rate of generation of p-nitrophenol was determined by reading the absorbance at 412 nm, 37°C, with the use of a continuously recording spectrophotometer (Beckman DU-68).

The evaluation of serum hepcidin concentration was performed by using an enzymatic immunoassay (Hepcidin-25, EIA Kit Extraction-Free, Peninsula Laboratories, LLC, San Carlos, CA, USA).

Serum lipids, lipoproteins, and apolipoprotein analysis were performed in an autoanalyser (Cobas Mira S, Roche, Basel, Switzerland) using commercially available kits.

Serum total cholesterol and triglycerides concentrations were performed by enzymatic colorimetric tests (cholesterol oxidase-phenol aminophenazone and glycerol-3-phosphate oxidase-phenol aminophenazone methods, Roche, resp.). High-density lipoprotein cholesterol (HDLc) and low-density lipoprotein cholesterol (LDLc) levels were measured using enzymatic colorimetric tests, after selective separation of HDL and LDL fractions (Direct HDL cholesterol and Direct LDL cholesterol, Roche). Serum levels of apolipoprotein (Apo) A-I and Apo B were evaluated by immunoturbidimetric assays (uni-kit apolipoprotein A-I and B specific antisera, Roche). Serum Lp(a) was quantified by using an immunoturbidimetric method (Lp(a), Roche Diagnostics). Oxidized LDL (ox-LDL) was measured directly in plasma by using a two-site enzyme immunoassay (oxidized LDL ELISA, Mercodia, Uppsala, Sweden).

2.3. Statistical Analysis. Kolmogorov-Smirnov test was used to test for normality of the variable distributions. Patients were categorized according to their status at the end of the 2 years of follow-up: alive (group 1) or dead (group 2). Differences between groups were analyzed by using Student's *t*-test or Mann-Whitney test, according to the results obtained in the Kolmogorov-Smirnov test. Normally distributed variables are presented as mean \pm SD and those nonnormally distributed are presented as median (interquartile range). Proportions were compared between groups using chi-square tests.

Survival analysis taking competing risks into account was performed to analyze patient's survival. The event of interest was death and the competing risk event was renal transplantation. Patients transferred to peritoneal dialysis were excluded (given the small number involved ($n = 2$)). Estimates of cumulative incidence function were calculated. Regression models taking competing risks into account (Fine and Gray model based on subdistribution hazard model) were carried out, to analyze the effect of covariates in patient's survival. To decide which variables should be included in the final multivariable model, an exploratory analysis was performed by fitting models for each variable in turn, adjusting for age and previous time in HD. The final multivariable model included all of these candidate variables with *P* values < 0.05 . All analyses were performed with SPSS and R software

using the packages *cmprsk*, and significance level (α) was set at 0.05.

3. Results

3.1. Patient's Demographic and Clinical Data. We studied 189 ESRD patients under HD (104 male, 85 female; mean age: 66.4 years, SD 13.9 years, range 27.6–92.0 years). The patients were under therapeutic HD three times per week, for 3–5 hours, and were under HD for 2.13 (0.82–5.28) years, when they were included in this study. All patients used the high-flux polysulfone FX-class dialyzers (1.4–2.2 m²) of Fresenius (Bad Hamburg, Germany). Arteriovenous fistula (AVF) was used by 146 patients (77.2%), whereas 43 patients (22.8%) had a CVC. The main causes of renal failure in these patients were diabetes mellitus ($n = 68$), followed by arterial hypertension ($n = 25$), glomerulonephritis ($n = 16$), polycystic renal disease ($n = 12$), reflux nephropathy ($n = 8$), obstructive uropathy ($n = 4$), hereditary nephropathy ($n = 3$), chronic interstitial nephritis ($n = 2$), nephrolithiasis ($n = 3$), other diseases ($n = 7$), and uncertain etiology ($n = 40$). Sixteen (8.5%) patients were classified as resistant to rhEPO therapy. A total number of 108 patients (63.9%) had arterial hypertension, 103 patients (54.5%) were under antihypertensive treatment, 75 (39.7%) were using statins, 127 (67.2%) were under intravenous iron therapy, 71 (39.0%) were under anti platelet therapy, and 8 (4.6%) were under oral hypocoagulation.

3.2. Analytical Data. The analytical results for the patients at starting of the follow-up study, and for the control group, are presented in Table 1. The hematological study showed that the HD patients experience a significant decrease in hemoglobin concentration, hematocrit, erythrocyte, platelets and lymphocyte counts, and a significant increase in MCV, as compared with the control; several significant changes were also observed in iron metabolism (an increase in transferrin saturation, sTfR, ferritin and hepcidin, and a decrease in iron and transferrin), in inflammatory markers (increase in adiponectin, IL-6, and CRP and a decrease in PON1), in the lipid profile (increase in oxLDL/LDL ratio and a decrease in total cholesterol, HDLc, LDLc, Apo A, Apo B and ox-LDL), in endothelial (dys)function markers (an increase in tPA, tPA/PAI-1 ratio and D-dimers, and a decrease in PAI-1); a significant increase in BMI, was also observed in patients, when compared with the control group.

3.3. Outcome. During the follow-up period (24 months), 35 patients died (18.5%) and 18 patients received a kidney transplant. Table 2 shows the values of the parameters that presented significant differences, when considering dead and alive patients at the end of the 2-year follow-up period. Statistically significant differences were found, concerning dialysis markers, nutritional status, hematological data, iron metabolism, inflammation, lipid profile, and endothelial function. The patients that died during the study period showed significantly lower values for calcium, calcium phosphorus product, creatinine, ultrafiltration volume, transferrin, transferrin saturation, triglycerides, VLDL, Apo A, BMI,

TABLE 1: Baseline values of the 189 ESRD patients under HD therapy.

	Controls (<i>n</i> = 25)	HD patients (<i>n</i> = 189)
Biochemical and dialysis markers		
URR, %	—	75.9 ± 6.56
KT/Ve	—	1.48 ± 0.31
Ultrafiltration volume, L	—	2.43 ± 0.94
Creatinine, mg/dL	—	8.14 ± 2.81
Potassium, mmol/L	—	5.40 (4.80–5.88)
Sodium, mmol/L	—	137 (135–139)
Phosphorus, mmol/L	—	4.48 (3.60–5.40)
Calcium, mg/dL	—	8.60 (8.10–9.10)
Calcium phosphorus product	—	37.58 (31.15–44.86)
Darbopoeitin, µg/kg/week	—	0.42 (0.20–0.75)
Hematological data		
Erythrocytes, ×10 ¹² /L	4.9 ± 0.45	3.8 ± 0.53*
Hemoglobin, g/dL	14.9 ± 0.86	11.7 ± 1.44*
Hematocrit, %	44.3 ± 2.80	36.5 ± 4.59*
MCV, fL	88.1 ± 11.72	95.8 ± 6.01*
MCHC, g/dL	33.8 ± 0.46	32.3 ± 1.19
RDW, %	15.1 ± 0.54	15.1 ± 1.91
Platelets, ×10 ⁹ /L	257.1 ± 58.85	184.1 ± 55.4*
Leukocytes, ×10 ⁹ /L	6.7 ± 1.66	6.4 ± 2.0
Neutrophils, ×10 ⁹ /L	4.3 ± 1.48	4.0 ± 1.49
Lymphocytes, ×10 ⁹ /L	2.0 ± 0.37	1.7 ± 0.70*
Neutrophil/lymphocyte ratio	2.2 ± 0.78	2.7 ± 1.51
Iron metabolism markers		
Transferrin, mg/dL	310.8 ± 44.04	184.4 ± 35.8*
Transferrin saturation, %	12.7 ± 4.73	15.1 (11.8–19.3)*
Iron, µg/dL	55.0 ± 17.97	38.0 (30.0–53.5)*
sTfR, nmol/L	13.6 ± 3.72	23.3 ± 11.9*
Ferritin, ng/mL	102.5 ± 60.61	400.8 ± 152.9*
Hepcidin-25, ng/mL	218.4 (124.5–318.6)	1599.1 (863.6–2409.0)*
Inflammatory markers		
IL-6, pg/mL	0.43 (0.28–0.61)	2.29 (1.40–4.26)*
CRP, mg/dL	0.75 (0.44–1.91)	5.13 (2.33–13.05)*
PON1, nmol p-nitrofenol/mL/min	466.8 (383.8–535.8)	361.1 (323.3–463.7)*
Adiponectin, mg/L	4.93 ± 2.30	9.27 ± 4.71*
Lipid profile		
Total cholesterol, mg/dL	202.2 ± 26.25	154.2 ± 43.5*
Triglycerides, mg/dL	103.0 (86.5–154.0)	117.0 (90.5–176.5)
HDLc, mg/dL	48.7 ± 12.94	42.2 ± 13.5*
LDLc, mg/dL	105.9 ± 25.29	73.4 ± 29.3*
VLDL, mg/dL	20.6 (17.3–30.8)	23.4 (18.0–35.4)
Lp(a), mg/dL	27.7 (17.3–69.9)	45.4 (25.6–89.0)
Apo A1, mg/dL	145.2 ± 26.91	122.7 ± 30.4*
Apo B, mg/dL	91.4 ± 14.49	72.4 ± 21.7*
Ox-LDL, U/L	40.8 ± 10.97	33.7 (27.7–40.5)*
OxLDL/LDL ratio, U/mg	0.040 ± 0.013	0.052 ± 0.018*

TABLE 1: Continued.

	Controls (<i>n</i> = 25)	HD patients (<i>n</i> = 189)
Endotelial (dys) function markers		
tPA, ng/mL	3.93 (2.95–5.93)	5.35 (3.72–8.18)*
PAI-1, ng/mL	31.0 (15.3–59.2)	18.7 (12.1–32.4)*
tPA/PAI-1 ratio	0.16 (0.09–0.20)	0.29 (0.18–0.39)*
D-dimers, ng/mL	0.25 (0.18–0.34)	0.71 (0.38–1.56)*
Nutritional markers		
BMI, Kg/m ²	22.2 ± 2.26	25.9 ± 4.63*
Albumin, g/dL	—	3.9 ± 0.38

* *P* < 0.05 versus controls. Data are presented as median and interquartile range (parameters with nonnormal distribution), mean and standard deviation (parameters with normal distribution), or number and respective percentage. URR: urea reduction ratio; MCV: mean cell volume; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; sTfR: soluble transferrin receptor; IL-6: interleukin-6; CRP: C-reactive protein; PON 1: paraoxonase 1; HDLc: high-density lipoprotein cholesterol; LDLc: low-density lipoprotein cholesterol; VLDL: very low-density lipoprotein; Lp(a): lipoprotein (a); Apo A: apolipoprotein A; Apo B: apolipoprotein B; Ox-LDL: oxidized LDL; PAI-1: plasminogen activator inhibitor-1; tPA: tissue plasminogen activator.

TABLE 2: Baseline values in ESRD patients under HD therapy for variables in which significant differences were found when the patients were divided in two groups (alive or dead 2 years later).

	Alive at followup (135 patients)	Dead during followup (35 patients)	<i>P</i>
Biochemical and dialysis markers			
Calcium, mg/dL	8.70 (8.20–9.10)	8.40 (8.00–8.90)	0.046
Calcium phosphorus product	38.6 (31.4–46.5)	35.8 (29.2–39.2)	0.025
Creatinine, mg/dL	8.63 ± 2.86	6.45 ± 2.10	<0.001
Ultrafiltration volume, L	2.47 ± 0.85	2.29 ± 1.22	0.037
Darbopoeitin- α , μ g/kg/week	0.40 (0.77–0.66)	0.87 (0.36–1.47)	<0.001
Hematological data			
RDW, %	14.5 ± 1.43	16.9 ± 2.32	<0.001
Iron metabolism markers			
Transferrin, mg/dL	187.6 ± 34.1	172.5 ± 41.6	0.050
Transferrin saturation, %	15.8 (12.3–19.7)	13.5 (9.67–19.1)	0.032
Inflammatory markers			
IL-6, pg/mL	2.21 (1.37–3.60)	4.27 (2.10–6.57)	<0.001
CRP, mg/dL	3.93 (2.09–9.73)	14.9 (5.15–30.4)	<0.001
Lipid profile			
Triglyceride, mg/dL	120.0 (95.0–180.0)	103.0 (82.0–125.0)	0.015
VLDL, mg/dL	24.0 (18.8–36.0)	20.6 (16.4–25.0)	0.021
Apo A1, mg/dL	124.3 ± 29.7	109.4 ± 26.2	0.014
Endotelial (dys) function markers			
PAI-1, ng/mL	17.9 (12.3–30.4)	27.4 (14.2–46.9)	0.034
D-dimers, ng/mL	0.70 (0.40–1.29)	1.47 (0.63–2.28)	0.005
Nutritional markers			
Body mass index, kg/m ²	25.4 (23.0–28.5)	23.3 (20.5–27.3)	0.008
Albumin, g/dL	4.00 (3.78–4.20)	3.70 (3.37–3.94)	<0.001

Data are presented as means and standard deviation or median (interquartile range). RDW: red blood cells distribution width; IL-6: interleukin-6; CRP: C-reactive protein; VLDL: very low-density lipoprotein; Apo A: apolipoprotein A; PAI-1: plasminogen activator inhibitor 1.

and albumin; significantly higher values were found for RDW, IL-6, CRP, PAI-1, and tPA. Moreover, dead patients required significantly higher rhEPO doses to achieve the target hemoglobin levels and presented a significantly higher percentage of patients using CVC (38% versus 15%, *P* < 0.001) as the vascular access for the HD procedure.

Figure 1 summarizes the cumulative incidence curves estimating all possible outcomes, taking competing risks into

account. The probability of death by 6, 12, 18, and 24 months after starting the study was 0.053, 0.106, 0.154, and 0.176, respectively.

Survival regression models, all adjusted for age and previous time in HD, are presented in Table 3. This analysis showed that the type of vascular access (the use of CVC), CRP, and tryglicerides are significant predictors of the event of interest, death. Indeed, the risk of death was higher for

TABLE 3: Survival regression models for ESRD patient's mortality.

	Model 1*			Final model**		
	Adjusted hazard ratio	95% CI	P	Adjusted hazard ratio	95% CI	P
Type of vascular access						
CVC versus AVF	2.65	1.33–5.30	0.006	3.03	1.49–6.13	0.002
C-reactive protein						
Q2 versus Q1	6.01	0.79–46.7	0.083	4.91	0.62–38.6	0.130
Q3 versus Q1	8.04	1.04–62.2	0.046	8.31	1.09–63.1	0.041
Q4 versus Q1	18.1	2.51–130.0	0.004	17.3	2.40–124.9	0.005
Triglycerides						
Q2 versus Q1	0.46	0.20–1.02	0.057	0.48	0.19–1.21	0.120
Q3 versus Q1	0.48	0.20–1.13	0.093	0.48	0.17–1.34	0.160
Q4 versus Q1	0.22	0.07–0.75	0.015	0.18	0.05–0.58	0.004

*Model 1: hazard ratio adjusted for age and previous time on HD; **Final model: hazard ratio adjusted for all variable in the model. CVC: central venous catheter; AVF: arteriovenous fistula; Q: quartile; CI: confidence interval.

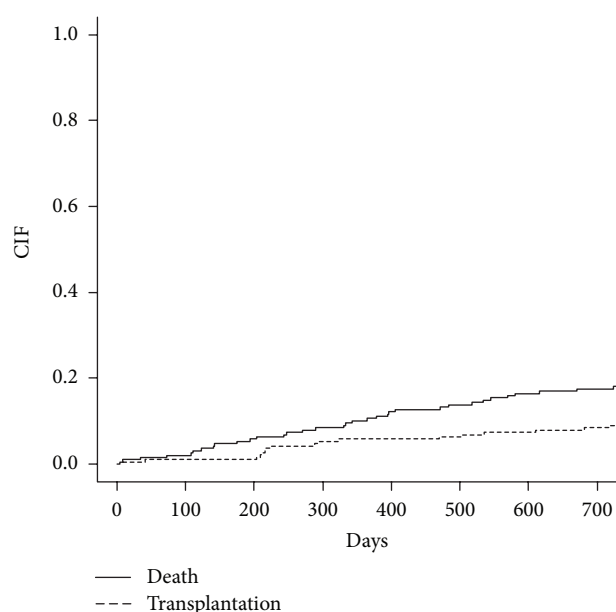


FIGURE 1: Cumulative incidence curves for all possible events (death as event of interest and transplantation as competing risk) for ESRD patients.

CVC (hazard ratio (HR) = 3.03, 95% CI = 1.49–6.13) and for patients with higher levels of CRP (4th quartile), as compared with the patients with lower level (1st quartile) (HR = 17.3, 95% CI = 2.40–124.9) (Table 3). Patients with tryglicerides levels in the 4th quartile had a lower risk of death, as compared with those patients in the 1st quartile (HR = 0.18, 95% CI = 0.05–0.58).

4. Discussion

In the present study, 189 ESRD patients under HD therapy were studied at the start of the study and followed for 2 years, in order to identify parameters that could be associated with mortality and, therefore, to provide biomarkers of risk.

The mortality rate in HD remains high [3, 13], in spite of the considerable improvements in medical and technical

support to these patients in the last years. CVD events are the main causes of mortality in ESRD patients [4]. However, it has been argued that noncardiovascular causes of death seem to be increasing to the same extent as cardiovascular mortality in ESRD patients [3]. Shastri et al. studied the patterns of death in a large cohort of HD patients and showed that sudden cardiac death accounted for 22% of the fatal events, whereas nonsudden cardiac death and noncardiac death accounted for 17% and 61%, respectively [14].

In our study, during the follow-up period, 35 patients died (18.5%) and 18 patients received a kidney transplant. Considering dead and living patients at the end of the 2-year follow-up period, we found that several variables were significantly different for the two groups of patients. We found that the patients who died during the follow period of the study, as compared to living patients, had lower HD efficacy, as showed by lower values of calcium, calcium phosphorus product, creatinine, and ultrafiltration volume; higher disturbances in iron metabolism and erythropoiesis, as shown by lower values of transferrin and transferrin saturation and higher values of RDW; a poor nutritional status, as reflected by the lower values of triglycerides, VLDL, Apo A, BMI, and albumin; a higher inflammatory condition, as shown by the higher values of IL-6 and CRP; endothelial and hemostatic disturbances, as suggested by the higher values of PAI-1 and D-dimers; and the development of resistance to rhEPO therapy, as they needed higher rhEPO doses to achieve hemoglobin concentrations within the target range; a higher percentage of them used CVC for vascular access. After performing a multivariate analysis, we observed that the use of CVC as the vascular access for the HD procedure, high CRP levels, and low triglycerides levels were independently associated with mortality.

The present work, showing an association between mortality in ESRD patients under HD treatment and both the type of vascular access (CVC) and CRP, shows that inflammation plays an important role in the mortality of these patients [15] and that their values can be used as predictors of death. Whether inflammation has any significant role in the physiopathology of ESRD, leading to death, or is merely a risk marker remains to be established. Zimmermann et al.,

studying 280 HD patients, showed CRP as an independent predictor of both overall death and cardiovascular death [15], which is in agreement with our findings.

In a previous report [9], we showed that ESRD patients with a CVC had lower levels of hemoglobin and albumin and higher levels of CRP, IL-6, D-dimers, and tPA, when compared to ESRD patients using AVF [5]. This association between CVC use and the enhancement of the inflammatory process and endothelial dysfunction could justify the high mortality rate found in ESRD patients using CVC. Several other reports [16, 17] have shown an association between the type of vascular access and mortality in HD patients.

Low values for plasma triglycerides were also associated with mortality in this study. This finding must be seen in the context of a significant importance of malnutrition in this type of patients [18]. Owen et al. showed serum albumin concentration as a powerful predictor of death in a cohort of 13, 473 HD patients [18]. In our study, albumin presented lower values in patients that died during followup; however, the survival regression models did not show albumin as an independent risk factor. The low levels of triglycerides might reflect a “malnutrition-inflammation complex syndrome” [19] or, more probably, a hemodialysis-catabolic state, associated with increased mortality.

Most of the changes (Table 1) observed in the characterization of HD patients are in accordance with the literature. HD patients presented with anemia, as shown by a decreased hemoglobin concentration, hematocrit, and erythrocyte count. They also presented a decrease in platelet and lymphocyte counts. The lymphocytopenia seems to result, at least in part, from a decrease in total circulating CD3+ T-lymphocytes and affects both the CD4+ and the CD8+ T-cell subsets [20]. The presence of inflammatory features (increased levels of IL-6 and CRP and decreased PON1 activity) were consistent with findings reported in HD patients [21–23]; however, the causes for the inflammatory response are not well clarified. Indeed, there are several potential sources, including bacterial contamination of the dialyser, incompatibility with the dialyser membrane, and the vascular access. It is known that some inflammatory players, for example, hepcidin, have an important impact in iron metabolism, reducing the mobilization and absorption of iron for erythropoiesis [24], leading, therefore, to an iron depleted erythropoiesis. In accordance with this, we found an increase in sTfR, ferritin, and transferrin saturation and a decrease in transferrin and in iron serum levels. IL-6, which is significantly increased in HD patients (Table 1), is known to strongly induce hepcidin mRNA expression [25, 26]. Thus, the high levels of hepcidin found in HD patient are explained by the underlying chronic inflammation. Recent studies have reported that higher body mass index (BMI) plays a protective role in HD patients [27–29]. Actually, the increased BMI in our HD patients was associated with higher adiponectin serum levels. Adiponectin is an adipokine that exerts a variety of anti-inflammatory activities, interfering with macrophage function by inhibiting phagocytosis, the production of IL-6 and TNF α , T-cell function, and by promoting the release of IL-10 and IL-1 receptor antagonists [30, 31]. The increase in adiponectin suggests that the adipose

tissue in HD patients may influence or modulate the inflammatory response. Several changes in the lipid profile were also found, namely, a decrease in total cholesterol, HDLc, LDLc, ApoA, ApoB, and Ox-LDL. In spite of the decrease in Ox-LDL, a higher ox-LDL/LDL ratio was found, showing a higher proportion of oxidized LDL particles in HD patients. It is known that ox-LDLs present important proinflammatory and procoagulant properties [32], and, therefore, it may further contribute to inflammatory changes. Endothelial function was also disturbed, as shown by the increase in D-dimers and tPA/PAI-1 ratio. It is uncertain if the higher values of fibrin fragment D-dimers in HD patients result from a higher activation of coagulation and/or of fibrinolysis, though the higher tPA values and tPA/PAI-1 ratio suggest an increase in fibrinolysis and a disturbance in endothelial function.

This study presented some limitations, namely, the number of patients included in this study and the follow-up period that were relatively limited.

In conclusion, our study showed that the type of vascular access for HD procedure, inflammation, and the nutritional status of the patients are important factors for the survival of these patients and that the use of CVC, CRP, and TG values may provide good biomarkers of risk in ESRD patients under HD.

Acknowledgments

This study was supported by “Fundação para a Ciência e Tecnologia” (FCT: PIC/IC/83221/2007) and cofinanced by FEDER (FCOMP-01-0124-FEDER-008468).

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