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Risk Factors for Mortality in End-Stage Kidney Disease Patients Under Online-Hemodiafiltration: Three-Year Follow-Up Study

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Artigo de Investigação Médica

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Porto, 2015

Risk Factors for Mortality in End-Stage Kidney Disease Patients Under Online-Hemodiafiltration: Three-Year Follow-Up Study

Running Title: Risk Factors for Mortality in ESRD patients under OL-HDF

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Abstract

Purpose: End-stage kidney disease (ESRD) patients under dialysis have high mortality rate. Inflammation, poor nutritional status and disturbances in erythropoiesis and iron metabolism have been reported in these patients. Moreover, there is a growing concern about the health related quality of life (HRQOL) in the context of ESRD. The aim of this work was to study the predictive value of these disturbances, dialysis adequacy and of HRQOL for mortality risk, by performing a three-year follow-up study.

Methods: Clinical, socio-demographical and analytical data (dialysis adequacy, nutritional status, hematological data, lipid profile, iron metabolism and inflammatory markers) were obtained from 236 patients (61.02% male; 67.50 [56.00-75.00] years old) under online-hemodiafiltration. Patient's reported HRQOL score was assessed by using the Kidney Disease Quality of Life-Short Form (KDQOL-SF).

Results: 54 patients died during the 3 years follow-up period. Our data showed that mean cell hemoglobin concentration (MCHC), transferrin and albumin are significant predictors of mortality. The risk of death was higher in patients presenting lower levels of MCHC (Hazard ratio [HR] = 0.70; 95% confidence interval [CI] = 0.500-0.984), transferrin (HR = 0.99; 95% CI = 0.982 – 0.998), and albumin (HR = 0.96; 95% CI = 0.938-0.994).

Conclusions: Our study showed that poor nutritional status and an inflammatory-induced iron depleted erythropoiesis are important factors for mortality in these patients. MCHC, transferrin and albumin may provide useful biomarkers of risk in ESRD patients under OL-HDF.

Keywords: End-stage kidney disease, online-hemodiafiltration, health-related quality of life, mortality, nutritional status, iron metabolism, inflammation

Resumo

Introdução: Indivíduos com doença renal terminal em hemodiafiltração apresentam uma taxa de mortalidade elevada. Inflamação, estado nutricional deteriorado e alterações na eritropoiese e no metabolismo do ferro têm sido descritas nestes doentes. Além disso, a qualidade de vida relacionada à saúde (QVRS) tem vindo a ser uma preocupação crescente no contexto desta doença. O objectivo deste trabalho foi o de identificar possíveis associações entre mortalidade e as alterações anteriormente descritas e com a QVRS, realizando um follow-up de três anos.

Métodos: Dados sociodemográficos, clínicos e analíticos (marcadores de adequação de diálise, nutricionais, hematológicos, inflamatórios, perfil lipídico e metabolismo do ferro) foram obtidos de 236 doentes [61.02% homens; 67.50 (56.00-75.00) anos de idade] que estavam em terapêutica dialítica pela técnica de hemodiafiltração online. A QVRS reportada pelos doentes foi avaliada através do instrumento *Kidney Disease Quality of Life-Short Form*.

Resultados: 54 pacientes morreram durante os 3 anos de seguimento. Os dados do nosso estudo mostraram que a concentração de hemoglobina corpuscular média (CHCM), transferrina e albumina são preditores significantes de mortalidade. O risco de morte foi maior em pacientes que se apresentaram com valores menores de CHCM (Razão de riscos [RR] = 0.70; 95% intervalo de confiança [IC] = 0.500-0.984), transferrina (RR = 0.99; 95% IC = 0.982 – 0.998) e albumina (RR = 0.96; 95% IC = 0.938-0.994).

Conclusões: O nosso estudo demonstrou que o mau estado nutricional e uma eritropoiese deficiente em ferro induzida pelo estado inflamatório são factores associados com mortalidade nestes doentes. A CHCM, transferrina e albumina poderão ser biomarcadores de risco importantes nos doentes renais terminais em hemodiafiltração

Palavras Chave: Doença renal terminal, hemodiafiltração, qualidade de vida relacionada à saúde, estado nutricional, metabolismo do ferro, inflamação

Introduction

End-stage kidney disease (ESRD) is a growing health public problem, given the increasing prevalence worldwide and its socio-economic consequences. By 2020, it is estimated that the number of ESRD patients rise by 60%, as compared to the number of patients registered in 2005.[1] Mortality rate for ESRD patients is 10 to 20 fold higher[2, 3] when compared with general population.[4]

Hemodialysis (HD), widely used during the past half-century, brought forth a way to lengthen ESRD patients' lives. Nonetheless, it is poorly suited to the effective removal of larger solutes, such as β_2 microglobulin. In recent years, online-hemodiafiltration (OL-HDF) was introduced as an alternative to standard HD, as it was claimed that OL-HDF would be more biocompatible, would increase dialysis efficacy and reduce the inflammatory response – features that would diminish the risk of morbidity and mortality in ESRD.[5] However, a recent meta-analysis of randomized trials, comparing the effect of OL-HDF with traditional HD on ESRD patient's mortality, showed that the potential benefits of convective modalities over standard HD, for mortality, remain unproved.[6]

In spite of the technological and pharmacological advances achieved in the last decade for the treatment of ESRD patients, survival is still low. Cardiovascular disease (CVD) has been considered the most common cause of death in these patients[7] and this may be connected with the high prevalence of classic cardiovascular risk factors, which include 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, oxidative stress, anemia, endothelial dysfunction, left ventricular hypertrophy, insulin resistance, hyper-homocysteinemia, high levels of lipoprotein(a) and an enhanced inflammatory state.[8-11] Cardiac arrest and congestive heart failure are more prominent causes of cardiovascular death than acute myocardial infarction in patients with uremia.

Dialysis is associated with symptoms that affect the daily life, and there is evidence that patients consider the health-related quality of life (HRQOL) more important than survival itself.[12] HRQOL is a multidimensional concept and its assessment through disease specific questionnaires has reported HRQOL as a strong and independent predictor of mortality in ESRD patients.[13-15]

In order to evaluate potential predictors of mortality, useful in clinical setting, we evaluated the global mortality in a group of Portuguese ESRD patients under OL-HDF, by performing a follow-up study of three years. In this work several variables were included as potential predictors of mortality, including clinical, socio-demographic and analytical data (dialysis adequacy, nutritional status, hematological data, lipid profile, iron metabolism and inflammatory markers), as well as the different domains and component

summaries of HRQL by using Kidney Disease Quality of Life Short Form (KDQOL-SF) instrument.

Material and Methods

Patients and study design

This is a three-year follow-up study, which started in April 2012, and included 236 ESRD patients (61.02% males; 65.29 ± 13.38 years old) from 5 dialysis clinics in the northern region of Portugal, under OL-HDF three times per week, for 3-5 hours. The main causes of renal failure, in this group of patients, were diabetic nephropathy (n=100), hypertensive nephrosclerosis (n=41) and other diseases and/or uncertain etiology (n=95).

Patients were excluded if they: (1) did not accept to participate in the study; (2) were cognitively impaired; (3) were under 18 years old; (4) had a severe speech or hearing impairment; (5) were in the dialysis program for less than three months; (6) presented with malignancy, autoimmune diseases, and inflammatory or infectious diseases.

Patients were clinically evaluated and blood samples were collected at the beginning of the study; KDQOL-SF questionnaire was self-administered to all patients. The questionnaire was completed by the patients themselves, except blind or disabled patients; in these cases the questionnaire was filled with the help of a family member or of their physician at the dialysis clinic.

For OL-HDF procedure, Fresenius Medical Care dialysis machines (model 5008) and synthetic high-flux polysulfone dialyzers (Fresenius Medical Care, Bad Hamburg, Germany) were used.

The study was approved by the Ethics Committee of Fresenius Medical Care - Portugal, and written informed consent was obtained from all participants.

Assays

Blood samples were obtained immediately before the OL-HDF 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 and serum. Aliquots were immediately stored at -80ºC, whenever necessary, until assayed.

Erythrocyte count, hematocrit, hemoglobin concentration and red blood cell indices [mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)] were measured 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).

Serum iron concentration was determined using a colorimetric method (Iron, Randox Laboratories Ltd., Northern Ireland, UK); serum ferritin and transferrin were measured by immunoturbidimetry (Ferritin, Randox Laboratories Ltd., Northern Ireland UK; Transferrin,

Randox Laboratories Ltd., Northern Ireland, UK); transferrin saturation (TS) was calculated using the formula: TS (%) = 70.9 x serum iron concentration (mg/dL)/serum transferrin concentration (mg/dL).

C-reactive protein (CRP) was evaluated by immunoturbidimetry, using commercially available kits [CRP (latex) High-Sensitivity, Roche Diagnostics, Mannheim, Germany].

Serum total cholesterol and triglycerides concentrations were evaluated by enzymatic colorimetric tests (cholesterol oxidase-phenol aminophenazone and glycerol-3-phosphate oxidase-phenol aminophenazone methods, Roche, Basel, Switzerland). Low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc) levels were measured using enzymatic colorimetric tests, after selective separation of LDLc and HDLc fractions (Direct LDLCholesterol and Direct HDLCholesterol, Roche, Basel, Switzerland).

Serum albumin levels were measured using a colorimetric assay end-point (Albumin Plus; Roche GmbH, Mannheim, Germany). Body mass index (BMI) was calculated using the formula: BMI = mass (kg)/height^2 (m).

All remaining analytical parameters were measured using standard laboratorial techniques.

Kidney Disease Quality of Life Questionnaire-Short Form

The KDQOL-SF has become the most widely used QOL assessment tool for ESRD patients. Since its development in the USA[16], it has been translated into several other languages and applied in other countries, namely in Portugal.[17-19]

HRQOL was measured using the KDQOL-SF 1.3 for the Portuguese population, which includes a kidney disease-specific part (KDQOL) and a generic 35 question-part (SF-36 version 2). The latter is divided into eight domains: patient's physical functioning (10 items); role physical (4 items); pain (2 items); general health (5 items), emotional well-being (5 items); role-emotional (3 items); social functioning (2 items); and energy and fatigue (4 items). Each domain is scored from 0 to 100, increasing the score with a better quality of life. Mental component summary (MCS) and physical component summary (PCS) result from the scores of these eight different SF-36 domains: PCS includes physical functioning, role-physical, pain and general health; MCS includes vitality, social functioning, role-emotional and mental health.

The KDQOL part is made up by 43 kidney disease-targeted questions, divided by 11 domains: symptom/problems (12 items), effects of kidney disease (8 items), burden of kidney disease (4 items), work status (2 items), cognitive function (3 items), quality of social interaction (3 items), sexual function (2 items), sleep (4 items), social support (2 items), staff encouragement (2 items) and patient satisfaction (1 item).

Statistical analysis

Kolmogorov-Smirnov test was used to test the variable distributions for normality; normally distributed variables are presented as mean ± standard deviation (SD), whereas non-normal data are presented as median [interquartile range (IQR)]. Patients were categorized at the end of the three years of follow-up as "Alive after follow-up" and "Deceased during follow-up". Differences between groups were analyzed by using Student t-test or Mann-Whitney test, according to distribution of the variables, evaluated by Kolmogorov-Smirnov test. Adjustment for confounding factors (age and previous time in dialysis) was performed using ANCOVA. The association between categorical variables was analyzed using the chi-squared test or Fisher's exact test.

Mental and physical component summaries of SF-36 were calculated according to Ferreira *et al* methodology.[20] It included a Z-score transformation for each dimension, followed by an arithmetic component (*10+50), added after the sum of the Z-transformed corresponding dimensions.

Survival analysis, considering the competing risk and the event of interest, was performed to analyze patient's survival. The event of interest was death and the competing risk event was renal transplantation. Cumulative incidence function was estimated. Regression models, considering the competing risk (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, adjusting for age and previous time in dialysis. The final multivariable model included all of the candidate variables, presenting with p values <0.1. All analyses were performed with SPSS and R software using the packages *cmprsk*, and significance level (α) was set at 0.05.

Results

During the period from April 2012 through April 2015, 54 patients (22.88%) died and 40 (16.95%) received kidney transplant. The probability of death by 6, 12, 24 and 36 months after starting the study was 0.025, 0.073, 0.147 and 0.236, respectively; figure 1 illustrates cumulative incidence curves that estimate the possible events – death, as event of interest, and transplantation as competing risk.

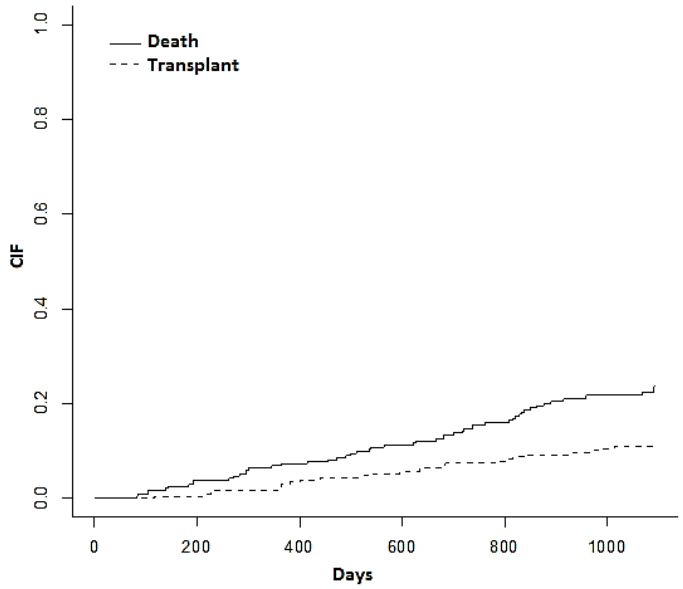

Figure 1: Cumulative incidence curves for all possible events (death as event of interest and kidney transplant as competing risk) for ESRD patients. CIF: cumulative incidence function

Tables I displays sociodemographic and clinical data, as well as markers of dialysis adequacy; in Table II, hematological data, markers of iron metabolism and inflammation, the lipid profile and markers of nutritional status, are presented; the various HRQOL domain values, assessed by using the KDQOL-SF, are presented in Table III. The values at baseline are presented for all patients and for those patients who were alive or deceased, by the end of the follow-up period.

Patients who died along the study showed significantly lower values for creatinine, uric acid, urea, MCHC, transferrin, triglycerides and albumin; significantly higher values were found for RDW and CRP. Moreover, older age and the use of CVC as vascular access for dialysis were also significantly associated with death. As for the HRQOL, lower values of physical functioning, role-physical, pain, emotional well-being, role-emotional,

energy/fatigue and PCS were observed at baseline for the patients who died during the follow-up period; a trend towards a lower quality of social interaction was also observed.

Table I – Sociodemographic, clinical data, and dialysis adequacy, at baseline, for alive and deceased patients, at the end of the follow-up period.

	Baseline values (n=236)	**Alive after follow-up (142 patients)**	**Deceased during follow-up (54 patients)**	***p* value**
Clinical data; biochemical and dialysis markers				
Age, years	67.50 (56.00-75.00)	67.00 (55.25-75.00)	74.00 (68.00-81.00)	<0.001
Gender (male), n(%)	144 (61.02)	82 (57.75)	31 (57.41)	0.925
Previous time on dialysis, months	32.00 (11.25-60.75)	30.00 (10.00-60.25)	35.00 (11.50-91.00)	0.284
Diabetic patients, n (%)	101 (42.80)	64 (45.07)	27 (50.00)	0.797
Hypertensive patients, n (%)	41 (17.37)	21 (14.79)	8 (14.82)	0.996
CVC use, n (%)	55 (23.31)	30 (21.13)	19 (35.20)	0.045
FAV use, n (%)	181 (76.69)	112 (78.87)	35 (64.80)	
URR, %	76.78 ± 5.75	77.37 ± 5.42	75.84 ± 6.35	0.10
KT/Ve	1.52 ± 0.30	1.55 ± 0.30	1.47 ± 0.30	0.08
Creatinine, mg/dL	7.74 ± 3.16	7.82 ±3.13	6.44 ± 3.02	0.01
Potassium, mmol/L	5.21 ± 0.85	5.18 ± 0.81	5.08 ± 0.92	0.45
Sodium, mmol/L	137.00 (135.00-139.00)	137.00 (135.00-139.00)	136.00 (133.00-139.00)	0.30
Phosphorus, mmol/L	4.23 ± 1.31	4.30 ± 1.39	4.08 ± 1.17	0.31
Calcium, mg/dL	8.58 ± 0.60	8.60 ± 0.65	8.51 ± 0.48	0.28
Calcium phosphorus product	36.29 ±11.55	36.96 ± 12.43	34.76 ± 9.96	0.25
Uric Acid, mg/dl	6.72 ± 1.31	6.75 ±1.17	6.15 ± 1.29	0.01
Urea, mg/dl	136.00 (111.50-161.50)	137.00 (116.00-168.00)	127.00 (104.50-150.50)	0.02
PTHi, pg/ml	327.00 (179.50-520.50)	344.00 (188.50-525.00)	297.50 (170.25-530.75)	0.89
Aluminum, µg/L	4.05 (3.00-5.50)	4.20 (3.00-5.50)	4.80 (3.28-6.00)	0.22
Darbopoeitin, µg/kg/week	0.34 (0.19-0.54)	0.33 (0.19-0.49)	0.39 (0.19-0.73)	0.249

Data are presented as mean (± standard deviation) or as median (interquartile range). CVC: Central venous catheter; AVF: Arteriovenous fistula; URR: urea reduction ratio; KT/Ve: dialyzer clearance of urea by dialysis time/volume of distribution of urea; PTHi: intact parathyroid hormone; CRP: C-reactive protein.

Table II - Hematological data, markers of iron metabolism and inflammation, lipid profile and markers of nutritional status, at baseline, for alive and deceased patients, at the end of the follow-up period.

	Baseline values (n=236)	Alive after follow-up (142 patients)	Deceased during follow-up (54 patients)	*p* value
Hematological data				
Erythrocytes, x10^{12}/L	3.74 (3.50-4.14)	3.73 (3.56-4.11)	3.78 (3.61-4.22)	0.12
Hemoglobin, g/dL	11.68 ± 1.36	11.65 ± 1.31	11.62 ± 1.43	0.88
Hematocrit, %	35.95 ± 4.23	35.70 ± 4.04	36.14 ± 4.40	0.51
MCV, fL	95.09 ± 6.03	95.05 ± 5.79	94.44 ± 6.86	0.53
MCHC, g/dL	32.49 ± 1.13	32.62 ± 1.08	32.12 ± 1.11	<0.001
MCH, pg	30.50 ± 4.12	30.57 ± 4.24	29.77 ±4.84	0.26
RDW, %	14.55 (13.70-15.60)	14.20 (13.50-15.40)	15.30 (14.00-16.50)	0.01
Platelets, x10 ^{9}/L	179.29 ± 60.89	179.44 ± 57.44	173.09 ± 67.48	0.23
Leukocytes, x10 ^{9}/L	6.00 (4.95-6.37)	5.84 (5.00-7.28)	6.18 (5.11-7.88)	0.21
Neutrophils, x10 ^{9}/L	3.75 (3.02-4.66)	3.66 (3.00-4.53)	3.87 (3.07-5.04)	0.35
Lymphocytes, x10 ^{9}/L	1.45 (1.15-1.95)	1.47 (1.17-1.94)	1.45 (1.09-2.04)	0.79
Neutrophil/lymphocyte ratio	2.50 (1.80-3.40)	2.50 (1.80-3.28)	2.70 (2.05-3.80)	0.22
Iron metabolism markers				
Transferrin, mg/dL	209.47 ± 96.37	223.55 ± 117.05	179.94 ± 46.14	0.01
Transferrin saturation, %	24.00 (19.31-32.92)	25.00 (19.37-33.00)	23.00 (18.40-36.00)	0.97
Iron, µg/dL	65.00 (50.00-84.25)	66.50 (51.75-85.25)	62.50 (41.25-82.00)	0.16
Ferritin, ng/mL	350.75 (211.00-474.25)	324.90 (206-480.50)	389.80 (327.35 -480.70)	0.66
Inflammatory markers				
CRP, mg/dL	4.40 (2.10-10.23)	4.00 (1.90-9.10)	6.65 (3.55-19.95)	<0.001
Lipid profile				
Total cholesterol, mg/dL	149.00 (125.00-177.00)	148.00 (125.00-181.00)	147.50 (124.25-172.75)	0.56
Triglycerides, mg/dL	122.00 (87.00-188.00)	122.00 (90.00-182.00)	100.00 (76.50-166.00)	0.04
HDLc, mg/dL	43.00 (35.00-50.00)	43.00 (35.00-47.00)	43.00 (35.00-54.00)	0.42
LDLc, mg/dL	80.20 (66.00-100.00)	82.40 (65.40-106.40)	79.20 (63.80-93.40)	0.38
Nutritional markers				
BMI, Kg/m^{2}	25.69 ± 4.53	25.74 ± 4.74	25.53 ± 4.22	0.78
Albumin, g/dL	40.00 (36.85-48.50)	41.00 (37.20-47.00)	37.80 (35.00-40.30)	<0.001
nPCR, g/kg/day	1.17 (1.07-1.50)	1.18 (1.08-1.40)	1.11 (1.02-1.28)	0.11
Total protein, g/dL	7.20 (6.50-66.00)	7.20 (6.50-67.75)	7.00 (6.28-64.00)	0.10

Data are presented as mean (± standard deviation) or as median (interquartile range). MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; HDLc: high-density lipoprotein cholesterol; LDLc: low-density lipoprotein cholesterol; nPCR: normalized protein catabolic rate.

Table III - HRQOL domains based on the KDQOL-SF, at baseline, for alive and deceased patients, at the end of the follow-up period.

	Baseline values (n=236)	**Alive after follow-up (142 patients)**	**Deceased during follow-up (54 patients)**	***p* value**
ESRD–targeted Areas				
Symptoms/problem list	74.93 ± 16.60	76.72 ±15.53	72.05 ± 17.47	0.07
Effects of kidney disease	65.53 ± 21.69	67.93 ± 20.88	64.62 ± 20.20	0.33
Burden of kidney disease	24.44 ± 23.66	26.16 ±24.20	22.34 ± 22.31	0.32
Work status	12.45 ± 27.77	11.79 ± 27.88	10.38 ± 22.70	0.74
Cognitive function	77.37 ± 19.70	78.53 ± 17.95	72.22 ± 24.63	0.10
Quality of social interaction	80.89 ± 19.19	81.12 ±18.80	75.82 ±19.95	0.09
Sexual function	79.48 ± 24.10	83.93 ± 20.73	79.17 ± 26.02	0.70
Sleep	40.53 ± 15.50	41.33 ± 14.30	42.48 ± 17.80	0.67
Social support	82.12 ± 27.01	83.33 ±25.96	78.21 ± 27.92	0.23
Dialysis care				
Staff encouragement	88.37 ± 21.77	90.04 ± 15.59	87.97 ± 21.51	0.53
Patient satisfaction	56.37 ± 22.45	57.50 ± 22.38	51.85 ± 21.88	0.12
36-item health survey (SF-36)				
Physical functioning	43.89 ± 30.92	40.09 ± 30.76	28.33 ± 27.27	<0.001
Role-physical	24.34 ±31.15	27.13 ±32.11	16.09 ± 27.89	0.03
Pain	63.32 ±29.24	66.13 ± 27.13	53.69 ± 34.23	0.02
General health	35.52 ±20.98	35.84 ± 21.14	35.11 ± 20.37	0.83
Emotional well-being	60.30 ± 24.83	62.25 ± 23.89	54.17 ± 25.77	0.04
Role-emotional	44.64 ± 32.23	47.90 ±31.35	35.93 ± 33.98	0.02
Energy/ Fatigue	46.99 ± 19.35	48.59 ± 18.54	41.11 ± 20.30	0.02
Social function	67.98 ± 34.12	68.79 ± 33.09	60.88 ±37.07	0.15
Physical and mental components summaries of SF-36				
PCS	49.82 ± 9.14	50.80 ± 9.55	46.79 ± 7.83	0.003
MCS	49.82 ± 9.67	50.22 ± 9.75	48.03 ± 10.01	0.168

Data are presented as mean (± standard deviation). PCS: physical component summary; MCS: mental component summary

Univariate analysis

Univariate analysis using the Fine and Gray regression model, adjusted for age and previous time under OL-HDF (Table IV), shows that lower values of creatinine, urea, MCHC, transferrin, albumin, role-physical, pain, emotional well-being, role-emotional, energy/fatigue, cognitive function and symptom/problems, as well as a higher value of RDW were significantly linked with mortality.

Table IV- Survival regression model for ESRD patients' mortality, adjusted for age and previous time under OL-HDF (univariate analysis)

	Adjusted hazard ratio	**95% CI**	***p* value**
Role physical	0.989	0.977-1.00	0.043
Pain	0.989	0.980-0.997	0.009
Emotional well being	0.988	0.978-0.998	0.015
Role emotion	0.989	0.980-0.998	0.015
Energy fatigue	0.982	0.967-0.997	0.021
Cognitive function	0.987	0.974-1.00	0.046
Symptom/problems	0.986	0.972-0.999	0.040
Urea	0.990	0.982-0.999	0.027
MCHC	0.645	0.486-0.885	0.002
RDW	1.360	1.147-1.600	<0.001
Transferrin	0.991	0.983-0.999	0.025
Albumin	0.962	0.935-0.999	0.008
Creatinine	0.875	0.797-0.961	0.005

MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; CI: confidence interval.

Multivariate analysis

Survival regression models, adjusted for age and previous time in HD, are presented in Table V. This analysis showed that MCHC, transferrin and albumin are significant predictors of the event of interest, death. Indeed, the risk of death is higher in patients that presented lower levels of MCHC, transferrin and albumin.

Table V- Survival regression model for ESRD patients' mortality, adjusted for age and previous time under OL-HDF (multivariate analysis)

	Adjusted hazard ratio	**95% CI**	***p* value**
MCHC	0.702	0.500-0.984	0.040
Transferrin	0.990	0.982-0.998	0.010
Albumin	0.966	0.938-0.994	0.018

MCHC: mean cell hemoglobin concentration; CI: confidence interval.

Discussion

In this 3-year follow-up study, 236 ESRD patients under OL-HDF were evaluated with the intent of identifying parameters that might me associated with mortality in the context of that disease and, therefore, provide useful biomarkers in the clinical setting.

During the follow-up period, 54 patients (22.88%) died and 40 (16.95%) received kidney transplant. Several variables were remarkably different, when comparing the baseline values presented by patients who were alive at the end of the study, with those of the patients who died during the follow-up study.

We found that the patients who died showed lower levels of creatinine, urea and uric acid. In general population, a slight or moderate increase in serum creatinine has been shown to be an independent risk factor of cardiovascular disease. In ESRD patients under dialysis, serum creatinine concentration is dependent on muscle mass, meat ingestion and on the degree of dialysis efficiency In accordance with our results, it has been reported that creatinine is inversely correlated with the risk for death (i.e., those dialysis patients with a higher serum creatinine live longer).[21, 22] Actually, our data suggests that the reduction in creatinine results, particularly, from malnutrition. This hypothesis is strengthened by the significantly lower values of TG and albumin concentrations, also observed in these patients. Moreover, these patients also showed erythropoietic disturbances, namely iron metabolism, as showed by the significantly lower values of transferrin and MCHC, as well as a higher RDW value; these erythropoietic changes are probably due to an enhanced inflammatory state, as showed by the significantly higher CRP value in these patients, when compared to the living patients. The presence of inflammatory features are well documented in literature are known to reduce iron absorption and iron mobilization, explaining the lower erythrocyte hemoglobinization, the reduction in transferrin and the trends towards a reduction in transferrin saturation and serum iron, as well as the increase in ferritin. The increase in RDW probably reflects these disturbances and the severity of the disease. Actually, these patients also presented a trend *(p*=0.08) towards lower KT/Ve, showing a lower dialysis efficacy.

Considering HRQOL, patients who died during the follow up showed remarkably poor scores for most of the SF-36 domains. The low scores for physical functioning, role-physical, pain, emotional well-being, role-emotional, energy/fatigue and PCS reflects a decreased functional capacity and physical limitations in daily activities, which might contribute to a lower quality of social

interaction (p=0.09). These results are in accordance with previous studies on HRQOL in ESRD patients.[23]

By performing a multivariate analysis, we observed that the values for MCHC, transferrin and albumin were independently associated with all-cause mortality.

Serum albumin concentration has been shown to be a powerful predictor for mortality in patients under dialysis[24] and our results match with previous reports in literature. The lower albumin values are also reflective of a poor nutritional status that may be linked to a malnutrition-inflammatory complex syndrome[25] or to a catabolic state associated with dialysis. Meanwhile, it has been reported that nutritional interventions to increase serum albumin may lead to considerable improvements in mortality rate.[26]

An increase in the inflammatory state of patients under dialysis has been proposed as an important mortality risk factor in these patients. [27] Inflammatory markers can be used, therefore, as predictors of death. In our study, CRP values were higher in deceased patients; however, survival regression models did not show CRP as an independent risk factor. As already referred, inflammation interferes with iron metabolism through hepcidin[28], reducing the mobilization and absorption of iron, needed for erythropoiesis. This leads to worsening of anemia, a common finding in these patients. Our survival regression models showed MCHC and transferrin as independent risk factors for mortality, and, therefore, that inflammatory-induced iron depleted erythropoiesis, should be carefully monitored in ESRD patients.

Another recently reported study29 from our research group, involving a lower number of ESRD patients and a smaller follow-up period, evaluated the predictive value of several analytical parameters; in that study, inflammatory markers and nutritional status were also found as predictors for mortality in ESRD patients.

The bulk of literature concerning risk factors for mortality in ESRD patients usually assesses standard analytical data; indeed, only a few studies considered HRQOL as a possible death predictor. In the present study, we brought together both areas and, while univariate analysis results showed several HRQOL domains significantly associated with mortality, none of them was identified as an independent risk factor after the multivariate analysis.

Evaluation of the patients' perception of HRQOL was, actually, a key aspect of our study, and strongly showed that HRQOL is more important for the patient than survival itself. A previous study reported that HRQOL should be considered an independent factor for mortality in ESRD patients[30]. In opposition, our data showed that the patients who died presented poorer HRQOL, as showed by the scores in the KDQOL-SF, reflecting the worsening of the general health status of ESRD

patients. Moreover, according to our results, HRQOL domains might be used to gauge the progression of the disease, but they are not independently linked to death. They are particularly important to access how the changes brought by ESRD affect the HRQOL, perceived by the patients.[31]

This study presented some limitations, namely the reduced number of patients included and a relatively limited follow-up period. Plus, CRP was the only major inflammatory marker measured and, if other markers were evaluated, we might have assessed inflammation more thoroughly, in ESRD patients.

In conclusion, our study showed that the nutritional status and an inflammatory-induced iron depleted erythropoiesis are important factors for the survival of these patients, and that MCHC, transferrin and albumin may provide useful biomarkers of risk in ESRD patients under OL-HDF.

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