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Oxidative stress, inflammation and cardiovascular mortality in haemodialysis—role of seniority and intravenous ferrotherapy: analysis at 4 years of follow-up

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Abstract

Background. Cardiovascular disease is the principal cause of morbidity and mortality in haemodialysis patients. The classic risk factors do not account for all cases of elevated cardiovascular disease in this patient population and it is becoming increasingly clear that other cardiovascular risk factors are implicated. The objective of this study was to analyse whether or not C-reactive protein (CRP) and plasma copper oxidized anti-lipoprotein (oxLDL) antibody titre are risk factors for cardiovascular mortality during 4 years of follow-up.

Methods. A prospective follow-up study was carried out in 94 stable, chronic haemodialysis patients for 48 months (July 1999–July 2003) (gender: 50 males and 44 females; mean age: 67 ± 14 years). Eighty-four per cent of these patients were receiving intravenous erythropoietin and 63% were receiving intravenous ferrotherapy (iron gluconate). Basal markers of inflammation and oxidative stress were determined at the beginning of the study. CRP levels were determined by chemiluminescent enzyme-labelled immunometric assay. The oxLDL antibody titre was measured by enzyme-linked immunosorbent assay using native LDL and oxLDL as antigens.

Results. Fifty deaths occurred during the study, 66% ($n = 33$) of which were due to cardiovascular disease. Patients presented with basal CRP and oxLDL levels indicative of chronic inflammation and elevated oxidative stress [CRP median: 5.16 mg/l (25–75% percentile: 0.35–88.7 mg/l); oxLDL antibodies median: 153 (optical density at 495 nm × 1000) (25–75% percentile: 112–214)]. A positive correlation was found between CRP and age ($r = 0.33$, $P = 0.003$). Study of the risk factors demonstrated that age ($P = 0.007$), oxLDL antibody titre ($P = 0.04$) and albumin ($P = 0.02$) were the only predictors of cardiovascular mortality at 4 years of follow-up in this patient population. The Cox proportional hazards model for cardiovascular mortality showed that of the markers studied, oxLDL antibody titre was an independent risk factor for cardiovascular mortality.

Conclusions. Oxidative stress (oxLDL antibody titre) is one of the principal risk factors for cardiovascular mortality in this population of haemodialysis patients. Intravenous ferrotherapy, due to its pro-oxidant properties, probably favours oxidative stress. Serum concentration of CRP was not a good predictive factor of cardiovascular mortality during 4 years of follow-up, possibly because of the slight positive correlation that exists between CRP and age.

Keywords: C-reactive protein; cardiovascular mortality; haemodialysis; oxidative stress

Introduction

The prevalence of cardiovascular mortality is highly elevated in patients undergoing dialysis and is the principal cause of mortality in this patient population. The Registre de Malalts Renals de Catalunya (RMRC) coincides with other registries of patients undergoing renal substitution therapy (United States, Europe, Australia and Japan) and verifies that the primary cause of death in these patients in 2002 was cardiovascular disease [http://www10.gencat.net/catsalut/ocatt/ca/htm/est_pub_trans_renal.htm].

Although we are in a time when cardiovascular mortality is decreasing in the general population, this reduction has not been observed in patients with chronic renal insufficiency. This patient population shows an elevated prevalence of cardiovascular risk factors. In addition to the classic risk factors

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(Framingham), and situations typical to chronic renal insufficiency that contribute to cardiovascular disease (hypervolaemia, anaemia, AVF (arteriovenous fistula) and alterations in calcium–phosphate metabolism), haemodialysis patients also present an elevated prevalence of what are known as 'emergent' factors [lipoprotein (a), homocysteine, oxidative stress, chronic inflammation, etc.] [1]. In a previous study, we demonstrated that haemodialysis patients show a decrease in antioxidant vitamins (vitamin E) and an increase in markers of lipid peroxidation [2], as well as an elevated state of inflammation. The augmentation of C-reactive protein (CRP) levels in haemodialysis patients confirms the existence of a chronic activation of acute-phase reactants. Recent data demonstrate that elevated concentrations of CRP are significantly associated with hypoalbuminaemia, malnutrition, erythropoietin resistance and an increase in morbidity and mortality [3,4]. Himmelfarb *et al.* [5] proposed the hypothesis that increased oxidative stress and its sequelae are major contributors to increased atherosclerosis and cardiovascular morbidity and mortality found in uraemia. In a previous study, we demonstrated that an elevated state of inflammation and an increase in oxidative stress were predictors of global mortality in haemodialysis patients [6]. However, the long-term relative contribution of these new risk factors of cardiovascular morbidity and mortality in patients undergoing haemodialysis has not been evaluated fully. The objective of this study was to analyse the role of elevated oxidative stress [plasma copper oxidized anti-lipoprotein (oxLDL) antibody titres] and chronic inflammation (CRP concentration) in a population of patients undergoing haemodialysis who were followed-up for 4 years.

Subjects and methods

Patients

A prospective follow-up study of 94 stable, chronic haemodialysis patients was carried out during a 48 month period from July 1999 to July 2003 and the incidence and causes of mortality were determined. All patients received a minimum of 12 h per week maintenance haemodialysis therapy (three sessions of 4 h each) with an HCO_3 bath on haemophane membranes.

The 94 haemodialysis patients studied included 50 men (53.2%) and 44 women (46.8%) aged 23–88 years (mean: 66.74 ± 14.32 years), 63.8% of whom were older than 65 years of age and 34.2% of whom were older than 75 years of age. The mean time from starting dialysis to joining the study was 46.90 ± 44.5 months. Eighty-two of the patients (87.2%) were undergoing haemodialysis due to arteriovenous fistula.

The underlying renal diseases included the following: diabetes mellitus (17%), nephroangiosclerosis (17%), chronic glomerular nephritis (13.6%), tubulointerstitial nephropathy (12.5%), polycystic liver and kidney disease (9.1%) and unknown (30.7%).

History of ischaemic cardiopathy (31.4%), peripheral vasculopathy (33.3%), cerebrovascular stroke (14.3%), smoking (34.1%), hypertension (76.1%) and diabetes mellitus (20.9%) were recorded.

Following dialysis, all patients were administered oral vitamin supplements. Eighty-four per cent of the patients were receiving intravenous erythropoietin and 63% were receiving intravenous ferrotherapy (iron gluconate) according to European Dialysis and Transplant Association (EDTA) guidelines [7].

Diagnostic criteria

The diagnostic criteria used to study previous disease activity are described below.

Coronary artery disease was diagnosed by a cardiologist if patients showing symptoms compatible with the disease presented with one or more of the following criteria: (i) a documented myocardial infarction; (ii) stenosis of >70% of at least one major epicardial coronary vessel recorded at the time of coronary angiography conducted according to standard procedure; or (iii) an abnormal cardiac effort test. Peripheral vascular disease was diagnosed if there were diminished pulses on clinical examination combined with measurements of peripheral vascular resistance and/or peripheral angiography. Cerebrovascular disease was suspected on clinical grounds and the diagnosis was confirmed by computerized tomography, magnetic resonance imaging and duplex carotid ultrasonography. Subjects were classified either as non-smokers (if they had never smoked) or ever-smokers (if they were current or ex-smokers).

Hypertension was defined as a blood pressure >140/90 mmHg or, in the presence of a history of hypertension, if the patient was also taking antihypertensive medications. Diabetes mellitus was diagnosed if patients were using insulin or if their fasting glucose concentration was >140 mg/dl (criteria used for the diagnosis of diabetes mellitus at the beginning of the study, 1999).

Methodology

Blood samples were drawn in the morning during fasting conditions before the start of the mid-week haemodialysis session. The samples were kept refrigerated before centrifuging (<1 h). The samples were frozen at −80°C until examination.

Total cholesterol, triglyceride and high-density lipoprotein cholesterol levels were all determined using enzymatic methods. The concentration of low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald's formula. Plasma albumin concentration was determined using Technicon Omnipack® bromocresol green method (Dax System; Bayer Diagnostic, Dublin, Ireland).

Serum CRP levels were measured by chemiluminescent enzyme-labelled immunometric assay, based on ligand-labelled monoclonal antibody and separation by anti-ligand-coated solid phase (Immulite; Dipesa SA, Madrid, Spain). The reference value for ultrasensitive CRP was <3 mg/l. The normal value of CRP in a healthy population is 1.81 mg/l (25–75% percentile: 0.16–12.8 mg/l).

Oxidation of LDL was assessed by measuring immunoglobulin G (IgG) oxLDL antibody titres [2] using a solid-phase immunoassay method that involved isolating plasma LDL from the blood of healthy subjects by sequential ultracentrifugation. Oxidation of LDL was performed by

incubating LDL with Cu^{2+}. Microtitre plates were coated with freshly isolated native LDL and Cu^{2+}-oxidized LDL at a concentration of 8 µg/ml in phosphate-buffered saline (PBS) (pH 7.4). Patient serum was assayed in duplicate at a 1/20 dilution and incubated overnight at 4°C. After the wells were washed three times, 100 µl peroxidase-conjugated rabbit anti-human IgG was added at a dilution of 1:1500 in PBS and incubated for 3 h at 37°C. Phenylenediamine dihydrochloride and H_2O_2 was used as a chromogenic substrate. Plates were read at 495 nm using a Micro Plate Reader A4. Results were expressed as optical density (OD) × 1000. The levels of anti-oxLDL antibodies were calculated by subtracting the value obtained from binding to native LDL from binding to oxLDL. All antibody determinations were conducted on the same day using the same batch of modified LDL and the same batch of reagents. The coefficient of variation was 6%. The normal value of anti-oxLDL antibodies in a healthy population is 107 (OD at 495 nm × 1000) (25–75% percentile: 78–151).

Haematocrit, haemoglobin and red blood cell indices were obtained through a Coulter Gen's counter (Hyaleah, FL, USA). Serum iron (spectrophotometry), transferrin and ferritin (turbidimetry) were obtained through a GernonStar XL-300 analyser (RAL/TRANSASIA, Dabhel, India) using the reactives supplied by RAL (Barcelona, Spain). The percentage of transferrin saturation was calculated with the formula:

$$\text{IS (\%)} = 100 \times \text{serum iron/transferrin}$$

The study received approval from the local ethics committee and all patients gave their informed consent to participate in the study.

Statistical analysis

All data were analysed using the software programs SPSS 11.0 and SAS V8. *P*-values of <0.05 were considered significant.

Initially, the type of distribution the continuous variables followed was verified using the Kolmogorov–Smirnov test. In the case of normal distribution, Student's *t*-test was used for comparing two means, while in the opposite case the non-parametric Wilcoxon–Mann Whitney test was used. To study the correlation between two variables, the Pearson or Spearman correlation was carried out, depending on whether or not the distribution was normal. With respect to the study of the association of two categoric variables, the chi-squared test was used or, when considered appropriate, the Cochran–Mantel–Haenszel test of general association.

Furthermore, a survival analysis was carried out using the Kaplan–Meier estimation. To determine the factors contributing to cardiovascular mortality, the odds ratios, as well as their 95% confidence intervals, were calculated. Adjustment of cardiovascular mortality was carried out through the selection of a logistic model; all previously detected significant risk factors were incorporated into this model. Hazard ratios were carried out in analysing the risk factors for survival of cardiovascular mortality in 4 years of follow-up. Patients who received a renal transplant during the study and those who died of causes other than cardiovascular disease were not included in the study of cardiovascular mortality.

Results

Fifty deaths occurred during the 4 year follow-up, 66% of which ($n = 33$) were deaths due to cardiovascular disease (acute myocardial infarction and non-coronary vascular disease). Fifteen of the patients in the study were receiving renal grafting.

CRP concentrations

The mean serum CRP level was 11.63 ± 16.43 mg/l. The median CRP level was 5.16 mg/l (25–75% percentile: 0.35–88.7 mg/l). A positive correlation was found between CRP and age ($r = 0.33$, $P = 0.003$). As age increased, the percentage of patients showing basal levels of CRP <3 mg/l decreased in a statistically significant way (Cochran–Mantel–Haenszel linear association test, $P < 0.001$) (Figure 1). We did not find a correlation between CRP and albumin levels, CRP and time in haemodialysis or CRP and oxLDL antibody titres.

OxLDL antibody titre and intravenous ferrotherapy

The mean oxLDL antibody titre (IgG) was 163.7 ± 77.65 (OD at 495 nm × 1000). The median oxLDL antibody titre was 153 (OD at 495 nm × 1000) (25–75% percentile: 112–214). There was a significant correlation between oxLDL antibody titre and total cholesterol, LDL cholesterol and triglycerides. Patients undergoing ferrotherapy at baseline showed a greater oxLDL antibody titre ($P = 0.04$); this treatment does not contribute to chronic inflammation (CRP) ($P =$ NS) (Table 1).

Factors predicting cardiovascular mortality

The relationship between clinical parameters and analytical studies and patient survival during the follow-up is highlighted in Tables 2 and 3. The clinical and analytical factors that were predictors of mortality in this group of patients are shown in Table 4. It was found that age ($P = 0.003$), CRP ≥10 *vs* <3 mg/l ($P = 0.025$), oxLDL antibody titre ($P = 0.019$)

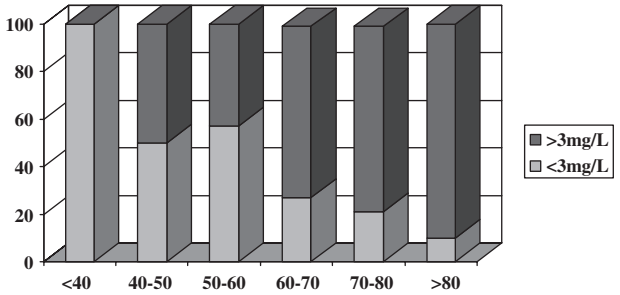

Fig. 1. Relationship between CRP separated into two categories (CRP <3 *vs* ≥3 mg/l) and age. As age increases, the percentage of patients presenting with a CRP <3 mg/l decreases ($P < 0.001$). *P*-value obtained using Cochran–Mantel–Haenszel's test of general association.

Table 1. Relationship between intravenous ferrotherapy (iron gluconate) and baseline markers of oxidative stress and inflammation

	With i.v. ferrotherapy ($n=79$)	Without i.v. ferrotherapy ($n=15$)	*P*-value
IgG oxLDL (OD × 1000)	174 (82.6)	135.3 (62.1)	0.04[a]
CRP (mg/l)	6.5 (2.9–12.6)	4.72 (2.1–20.5)	NS[b]
Haematocrit (%)	32.85 (4.19)	33.2 (4.01)	NS[a]
Serum iron (μg/dl)	9 (7–13)	11 (8–13.25)	NS[b]
Ferritin (ng/ml)	273 (165–361.5)	158 (102.7–442.2)	NS[b]
TSI (%)	43.7 (17)	49.2 (23)	NS[a]

Values are expressed as mean (SD) if they follow a normal distribution and median (27–75% percentile) if the distribution is not normal.
P-value was determined using the [a]*t*-test and [b]Mann–Whitney test. IgG oxLDL, anti-oxLDL antibodies (IgG); NS, not significant; TSI, transferrin saturation index; i.v., intravenous.

Table 2. Clinical parameters of the haemodialysis patients according to their vital status at the end of follow-up

	CV death[a] ($n=33$)	Alive on HD-4a[b] ($n=29$)	*P*-value[c]
Age	74 (8)	63 (14)	0.003
Gender (male *vs* female)	54.5%	51.7%	NS
Ferrotherapy i.v. (%)	78.8%	44.8%	0.006
AH (%)	74.2%	88.9%	NS
DM (%)	24.1%	14.3%	NS
C. ischaemia (%)	43.3%	19.2%	NS
CA (%)	20%	11%	NS

[a]Patients who died due to cardiovascular problems during the 4 years of follow-up.
[b]Patients who are alive and undergoing haemodialysis at 4 years of follow-up. Patients who received a renal graft are not included ($n=15$).
[c]*P*-values determined using the chi-squared test.
AH, arterial hypertension; DM, diabetes mellitus; C. ischaemia, cardiovascular ischaemia; CA, cerebrovascular accident; i.v., intravenous; NS, not significant.

and intravenous ferrotherapy (yes *vs* no) ($P=0.007$) were predictors of mortality in this group of patients. The logistic regression model for the study of cardiovascular mortality is shown in Table 5. When adjusted for age, oxLDL antibody titre and intravenous ferrotherapy in the multivariate analysis, CRP was no longer a significant risk factor for cardiovascular mortality. Table 6 shows the multivariate Cox proportional hazards model for cardiovascular mortality, which demonstrated that only age ($P=0.007$), albumin ($P=0.02$) and oxLDL antibody titres ($P=0.04$) are independent risk factors for cardiovascular mortality in this population.

Figure 2 shows the survival curve for the haemodialysis patients in relation to the oxLDL antibody titre. This parameter was categorized into two groups using the median oxLDL antibody titre (153 OD at 495 nm × 1000) as the cut-off point. Patients with lower lipid peroxidation showed greater survival. In this analysis the value of the long-rank test was $P=0.003$ and the Wilcoxon test was $P=0.003$.

Table 3. Analytical study of the haemodialysis patients according to their vital status at the end of follow-up

	CV death[a] ($n=33$)	Alive on HD-4a[b] ($n=29$)	*P*-value
CRP (mg/l)	9.09 (3.8–15.2)	4.58 (1.8–11.4)	NS[c]
Albumin (g/l)	41 (39–45)	44.5 (40–45.7)	NS[c]
Total cholesterol (mmol/l)	3.74 (0.71)	3.71 (0.8)	NS[d]
Triglycerides (mmol/l)	1.11 (0.42)	1.08 (0.48)	NS[d]
IgG oxLDL (OD × 1000)	178.90 (87.71)	139.69 (68.20)	NS[d]
Haematocrit (%)	33.23 (4.3)	32.83 (3.9)	NS[d]
Serum iron (μg/dl)	9 (6–11.5)	11 (9–13)	NS[c]
Transferrin (g/l)	22.91 (5.49)	23.85 (2.98)	NS[d]
Ferritin (ng/ml)	240 (142–344)	339 (142–512)	NS[c]

Values are expressed as mean (SD) if they follow a normal distribution and median (25–75% percentile) if the distribution is not normal.
[a]Patients who died due to cardiovascular problems during the 4 years of follow-up.
[b]Patients who are alive and undergoing haemodialysis at 4 years of follow-up. Patients who received a renal graft are not included ($n=15$).
P-value was determined using the [c]Mann–Whitney test and [d]*t*-test. IgG oxLDL, anti-oxLDL antibodies (IgG); NS, not significant.

Table 4. Unadjusted cardiovascular mortality-related risk factors

	OR	95% CI	*P*-value
Age	1.100	1.032–1.173	0.003
Gender (male *vs* female)	1.120	0.412–3.044	NS
AH (yes *vs* no)	0.359	0.085–1.524	NS
DM (yes *vs* no)	1.909	0.491–7.422	NS
C. ischaemia (yes *vs* no)	3.212	0.954–10.81	NS
CA (yes *vs* no)	1.916	0.428–8.583	NS
CRP (>3 *vs* ≤3 mg/l)	2.139	0.999–1.014	NS
CRP (≥10 *vs* <3 mg/l)	7.499	1.287–43.68	0.025
Homocysteine	0.993	0.925–1.065	NS
Albumin	0.900	0.792–1.022	NS
Cholesterol	1.062	0.530–2.130	NS
Triglycerides	1.171	0.366–3.746	NS
IgG oxLDL (>153 *vs* ≤153 OD × 1000)	3.562	1.225–10.36	0.019
Serum iron	0.853	0.722–1.008	NS
Transferrin	0.951	0.872–1.093	NS
Ferritin	0.998	0.996–1.001	NS
Ferrotherapy i.v. (yes *vs* no)	4.571	1.507–13.87	0.007

OR, odds ratio; CI, confidence interval; AH, arterial hypertension; DM, diabetes mellitus; C. ischaemia, cardiovascular ischaemia; CA, cerebrovascular accident; IgG oxLDL, anti-oxLDL antibodies (IgG); i.v., intravenous.

Discussion

This study demonstrates that oxidative stress, measured as the function of anti-oxLDL antibody titre, is the

Table 5. Cardiovascular mortality study: significant odds ratios of the multivariate analysis

	OR	95% CI	*P*-value
Age >75 *vs* <65 years	8.371	1.642–42.690	0.01
IgG oxLDL (>153 *vs* ≤153 OD × 1000)	4.010	1.165–13.799	0.03
Ferrotherapy i.v. (yes *vs* no)	4.226	1.218–14.658	0.02
Albumin (≤43 *vs* >43 g/l)	0.593	0.213–1.652	0.31

The dependant variable is cardiovascular mortality (yes/no). The factors introduced are age (categorized as >75 *vs* <65 years), oxLDL antibody titre (with two categories: less or equals the mean and greater than the mean), intravenous ferrotherapy (those who received it *vs* those who did not) and albumin (with two categories: less or equals the mean and greater than the mean). CRP is not introduced because it did not achieve statistical significance.
OR, odds ratio; CI, confidence interval; IgG oxLDL, anti-oxLDL antibodies (IgG); i.v., intravenous.

Table 6. The Cox proportional hazards model for cardiovascular mortality

	Coefficient	Hazard ratio	95% CI	*P*-value[a]
Age	0.06166	1.064	1.017–1.113	0.007
Albumin	−0.11738	0.889	0.803–0.958	0.02
IgG oxLDL (>153 *vs* ≤153 OD × 1000)	0.81031	2.249	1.014–4.987	0.04

[a]*P*-value of the Wald chi-squared test.
The *P*-value of the likelihood ratio test was 0.0001.
The dependant variable is time of survival of cardiovascular mortality and the risk factors in this model are age (continual), albumin (continual) and oxLDL antibody titre (with two categories: as less or equals the mean and greater than the mean). CRP and intravenous ferrotherapy have not been introduced as they did not show statistical significance ($P > 0.05$).
CI, confidence interval; IgG oxLDL, anti-oxLDL antibodies (IgG).

principal risk factor for cardiovascular mortality in a population of elderly patients undergoing haemodialysis who were followed-up for 4 years. It is likely that intravenous ferrotherapy contributes to the augmentation of this oxidative stress. In this patient population, one initial determination of CRP was not an independent risk factor for cardiovascular mortality at 4 years of follow-up.

C-reactive protein

CRP values in haemodialysis patients are elevated and can increase to 5–10 times those of the general population. Inflammation plays a role in the pathogenesis of cardiovascular disease. Currently, some markers of inflammation are considered cardiovascular risk factors. After analysing various parameters of inflammation (CRP, serum amyloid A (SAA), erythrocyte sedimentation rate (ESR), fibrinogen), the College of American Pathologists (CAP) recommends that the value of CRP be used as a clinical marker of inflammation to determine the risk of cardiovascular

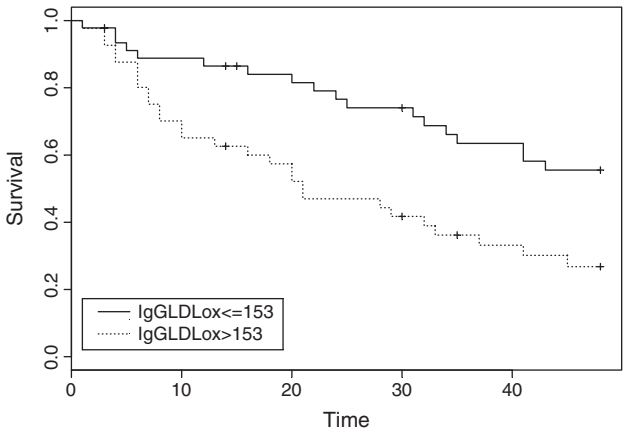

Fig. 2. Kaplan–Meier estimate of survival in haemodialysis patients with plasma copper oxidized anti-LDL antibody levels >153 *vs* ≤153 OD at 495 nm × 1000. The tests that analysed the equality of the curves and demonstrated a statistically significant value included the log-rank test ($P = 0.003$) and Wilcoxon ($P = 0.003$).

disease, considering patients with a CRP level >3 mg/l to have an elevated cardiovascular risk [8]. The role that CRP plays in the pathogenesis of cardiovascular disease in dialysis patients is controversial, and, occasionally, the increase in this protein has been considered to be more of an epiphenomenon then a pathogenic mechanism. In analysing clinical studies that show cardiovascular mortality in haemodialysis patients, we have observed discrepancies. Zoccali *et al.* [9], who analysed non-classic risk factors of cardiovascular disease in haemodialysis patients, demonstrated that adiponectin and homocysteine, but not CRP, were predictors of cardiovascular events. On the other hand, Wanner *et al.* [4] demonstrated that a value of CRP >8 mg/l is a good indicator of cardiovascular and global mortality in a group of haemodialysis patients who were followed-up during 4 years. Firstly, we believe that the fact that Wanner *et al.* [4] uses a serum concentration of CRP >8 mg/l as a cut-off point, the cut-off point recommended by the EDTA guidelines [10], shows that the relationship between CRP and mortality in haemodialysis patients appears in cases of elevated inflammation. Perhaps a CRP level >3 mg/l, the level recommended by the CAP, is adequate in a normal patient population without chronic renal insufficiency, but it is too low in uraemic patients as a screening parameter to stratify cardiovascular risk. In our study, the univariate analysis showed that a CRP concentration >3 mg/l was not a predictive marker of cardiovascular mortality, while a CRP level >10 mg/l did predict cardiovascular mortality in a statistically significant way (Table 4).

Secondly, it is important to keep in mind that the relationship between CRP concentration and cardiovascular pathology has been found in both sexes and in distinct age groups, although one recent study argues that the relationship between CRP and mortality (global and cardiovascular) diminishes in elderly patients [11]. Data cited by the RMRC – CatSalut

shows that after analysing 2443 haemodialysis patients, CRP correlated with age ($P < 0.001$) and the percentage of patients that presented with a CRP <3 mg/l decreased with age ($P < 0.001$) in such a way that the percentage of patients >60 years of age with a CRP <3 mg/l was <35% (unpublished data, cited by RMRC – CatSalut, Date Registry 2003 [http://www10.gencat.net/catsalut/ocatt/ca/htm/est_pub_trans_renal.htm]). These data are in agreement with our study and reinforce the hypothesis that the positive correlation that exists between CRP and age in this elderly population (mean age: 68 ± 14 years) may justify that CRP, in the multivariate analysis after adjusting for age and oxLDL antibody titre, is not a good independent predictor of cardiovascular mortality in 4 years of follow-up (Table 5).

Finally, it should be mentioned that although CRP is one of the most sensitive acute-phase reactants, it does not provide diagnostic specificity. CRP concentrations in plasma increase with inflammation and cardiovascular disease, but enormous increases in CRP are also seen in traumas, surgical interventions, infections and neoplastic processes. Renal patients frequently present with infections and processes that stimulate inflammation. This could explain why in our previous study [6] CRP was a predictor of global mortality, but was not a useful parameter to predict cardiovascular mortality during 4 years of follow-up.

Oxidative stress (lipid peroxidation): role of intravenous ferrotherapy

Various studies indicate that uraemia, in general, is associated with an elevation in oxidative stress and that haemodialysis contributes to an increase in free radical production and a decrease in antioxidant levels in these patients.

The biomolecules that are most susceptible to attack by free radicals are, in general, lipids. Lipid peroxidation and, above all, oxidative modifications of LDL are important factors in the development of atherosclerosis [12]. The oxLDL antibody titre is a specific marker that is used widely to identify oxidative stress. These antibodies are directed against the molecular epitopes present in oxidative LDL and reflect the existence of LDL oxidation *in vivo*. In a previous study, we confirmed the results of Maggi *et al*. [13], by demonstrating that haemodialysis patients showed an elevated level of oxLDL antibodies compared with a control population.

Various works suggest that oxLDL antibody titre predicts the development of atherosclerosis and coronary disease in certain circumstances [14]. There are few studies that analyse the role of oxLDL antibody titre and its relationship with cardiovascular disease in renal patients. In a study of 109 pre-dialysis patients, Stenvinkel *et al*. [15] found that patients with carotid plaques have a statistically significant elevation in antibody titre compared with patients with no plaques. Furthermore, the authors demonstrated an inverse correlation between oxLDL antibody titre and vitamin E levels, suggesting that vitamin E is an important inhibitor of LDL oxidation. In contrast, a recent study by Shoji *et al*. [16] showed that haemodialysis patients with a higher oxLDL antibody titre showed a lower risk for cardiovascular disease, while no relationship was found for non-cardiovascular mortality. In spite of these discrepancies, the majority of observations suggest that the oxLDL antibody titre is elevated in cases of advanced atherosclerosis [13–15]. In our study, the multivariate analysis and the Cox proportional hazards model showed the oxLDL antibody titre to be an independent predictive factor of cardiovascular disease after adjusting for other factors (age, albumin and intravenous ferrotherapy), so that patients with less lipid peroxidation have greater survival at 4 years of follow-up (Table 5).

Intravenous ferrotherapy is necessary in patients undergoing haemodialysis who are receiving erythropoietic stimulators to obtain a better efficacy and achieve an optimal level of haemoglobin. However, the potentially toxic effects of ferrotherapy have been described recently, as an increase in the susceptibility to infections and cardiovascular morbi-mortality has been observed. *In vitro*, all iron preparations have the capacity to cause the production of reactive oxygen species, increase oxidative stress, consume antioxidants and, at very high doses, promote lipid peroxidation and cell death. These observations carried out *in vitro* may signify the possibility of these effects in clinical practice and that ferrotherapy may accelerate atherosclerosis and increase cardiovascular morbidity [17]. Sun *et al*. [18], in a cohort of the 14 886 haemodialysis patients, demonstrated that death rate due to infection and cardiovascular disease was higher in patients who received >5 g/year of iron compared with patients who did not. Although our study has the limitation that the doses of iron administered during the 4 years of follow-up were not determined, we suggest that iron acts as a pro-oxidant favouring lipid peroxidation, since at baseline patients treated with intravenous iron presented with a higher oxLDL antibody titre and, thus, greater lipid peroxidation (Table 1). The logistic regression analysis showed that intravenous ferrotherapy could be an independent risk factor for cardiovascular disease, but the Cox regression model did not confirm its influence on survival.

Based on our results, we believe that the optimization of treatments to avoid negative side effects is necessary. Serum concentration of CRP was not a good predictive factor of cardiovascular risk during 4 years of follow-up, most likely due to the slight positive correlation that exists between CRP and age. We would also like to mention that the utilization of CRP as a marker of inflammation and a screening parameter to stratify cardiovascular risk in the long term is not useful in haemodialysis.

This study suggests that long-term elevated oxidative stress, in contrast to elevated inflammation measured by basal levels of CRP, is a cardiovascular risk factor contributing to the development of atherosclerosis and cardiovascular mortality in haemodialysis patients.

Our results affirm that the oxLDL antibody titre is an independent predictor of mortality and of survival in this population of haemodialysis patients who were followed-up during 4 years.

Conflict of interest statement. None declared.

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