European Journal of Internal Medicine xxx (xxxx) xxx

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original Article

# Endothelin-1 as dual marker for renal function decline and associated cardiovascular complications in patients with chronic kidney disease

Laura González-Rodríguez [a,b], Manuel Martí-Antonio [c], Sonia Mota-Zamorano [d], Celia Chicharro [e,f], Bárbara Cancho [g], Enrique Luna [g], Álvaro Álvarez [g], Zoraida Verde [e,f], Fernando Bandrés [h], Nicolás R Robles [g], Guillermo Gervasini [a,b,i,*]

[a] Department of Medical and Surgical Therapeutics, Medical School, Universidad de Extremadura, 06006 Badajoz, Spain
[b] RICORS2040 Renal Research Network, 2802940 Madrid, Spain
[c] Vascular and Renal Translational Research Group, Institute for Biomedical Research in Lleida, Spain
[d] Department of Medical and Surgical Therapeutics, Plasencia University Center, Universidad de Extremadura, 10600 Plasencia, Spain
[e] Department of Biochemistry, Molecular Biology and Physiology, Universidad de Valladolid, 42005 Soria, Spain
[f] GIR—Pharmacogenetics, Cancer Genetics, Genetic Polymorphisms and Pharmacoepidemiology, University of Valladolid, 47005 Valladolid, Spain
[g] Service of Nephrology, Badajoz University Hospital, 06006 Badajoz, Spain
[h] Biopathology-Toxicology Laboratory, Department of Legal Medicine, Psychiatry and Pathology, Faculty of Medicine, University Complutense of Madrid, 28040 Madrid, Spain
[i] Institute of Molecular Pathology Biomarkers, Universidad de Extremadura, 06006 Badajoz, Spain

ARTICLE INFO

*Keywords:*
Endothelin-1
Chronic kidney disease
Cardiovascular events
Biomarkers
eGFR decline

ABSTRACT

*Background:* Cardiovascular (CV) complications are the leading cause of death in patients with chronic kidney disease (CKD). Endothelin-1 (ET-1), a potent vasoconstrictor involved in both renal and vascular dysfunction, may represent a promising biomarker for the disease.
*Methods:* ET-1 plasma levels were quantified in 692 Spanish CKD patients (stages 1–5) and used to stratify individuals into three clusters (cluster 3 meaning highest concentrations). Associations with CKD progression, CVE, and all-cause mortality were assessed over a mean follow-up of 48.6 ± 27.4 months using linear mixed-effects models and Cox regression analyses adjusted for conventional risk factors.
*Results:* ET-1 levels increased with CKD severity (mean±SD: 1.65 ± 0.71 pg/mL for stages 1–2; 1.82 ± 0.71 pg/mL for stage 3; 2.39 ± 1.08 pg/mL for stages 4–5; $p < 0.001$). Higher ET-1 levels were independently associated with accelerated eGFR decline over 3 years ($\beta = -12.64$, $p < 0.001$ for cluster 2; and $\beta = -11.71$, $p = 0.034$ for cluster 3). Sixty-nine CVE (10.1 %) were recorded. Participants with higher ET-1 levels had significantly lower CV event-free survival [HR = 2.24 (1.12–4.45), $p = 0.022$, and HR = 2.50 (1.09–5.73), $p = 0.03$] for clusters 2 and 3, respectively. ET-1 also predicted all-cause mortality ($p < 0.001$) although the association lost significance after adjusting for age. Random forest models for CV risk and all-cause mortality including the ET-1 cluster produced C-indices of 0.835 and 0.837, respectively.
*Conclusions:* Elevated ET-1 levels are independently associated with both CKD progression and CV complications. ET-1 may serve as a dual biomarker for renal deterioration and CV risk, potentially improving clinical stratification in CKD management.

## 1. Introduction

Chronic kidney disease (CKD), defined by abnormalities in renal function and/or structure lasting over 3 months with associated clinical consequences [1], has become a growing public health challenge. Cardiovascular (CV) complications are the primary cause of death in individuals with CKD [2], and this CV risk is evident across all stages of the disease, underscoring the complex interplay between kidney function and CV health [3]. The disproportionately high rate of CV mortality in this patient population emphasizes the critical need to elucidate the underlying mechanisms driving these complications and to identify potential therapeutic targets.

* Corresponding author at: Department of Medical and Surgical Therapeutics, Medical School, University of Extremadura, Av. Elvas s/n 06071, Badajoz, Spain.
*E-mail address:* ggervasi@unex.es (G. Gervasini).

https://doi.org/10.1016/j.ejim.2025.106542
Received 31 July 2025; Received in revised form 1 September 2025; Accepted 17 September 2025

Among the various factors implicated in the pathogenesis of CV problems, the endothelin family has emerged as a key player, with endothelin-1 (ET-1) recognized as the main isoform involved in both vascular and renal pathophysiology [4]. Elevated levels of ET-1 have been proposed to contribute to renal impairment [5,6] and to play a pathophysiological role in many CV conditions, such as primary pulmonary hypertension, hypertension, atherosclerosis, coronary artery disease, cardiac hypertrophy or heart failure [7]. Given these effects of ET-1 in the kidney and the CV system, it is not surprising that ET-1 receptor antagonists (ERAs) have shown promising results for its use in kidney disease, particularly for the selective ETA blockade [6,8,9].

Despite this background, and somewhat surprisingly, there is very little information on the association of ET-1 levels with the incidence of CV events (CVE) in the CKD setting. Moreover, no studies have addressed the link between ET-1 concentrations and the estimated glomerular filtration rate (eGFR) decline over time, reflecting the worsening of kidney function in patients with CKD. Therefore, in the present work, we have aimed to address these research gaps by determining ET-1 plasma concentrations in a large cohort of patients with different degrees of CKD to establish putative associations with disease progression and the incidence of CV-related complications.

## 2. Methods

### 2.1. Subjects

Between 2017 and 2022, 692 Caucasian Spanish subjects with different stages of CKD (185 with stage 1–2, 181 with stage 3 and 326 with stage 4–5) were recruited at the Nephrology Service and the Advanced CKD Unit of the Badajoz University Hospital during the patients' regular visits. Exclusion criteria for the study included transplantation, pregnancy or breastfeeding, active infection, cancer, and acute kidney injury. All participants were adults (over 18 years of age) and provided written informed consent for their involvement. The study was approved by the Ethics Committees of Badajoz University Hospital and conducted in compliance with the Declaration of Helsinki and its subsequent revisions.

### 2.2. Endothelin-1 plasma levels determination

Blood samples (3 mL) were collected from each participant in EDTA tubes, and plasma was promptly separated by centrifugation at 3000 rpm for 10 min. Plasma samples were then aliquoted into 500 μL portions and stored at −80 °C until further analysis. The quantification of ET-1 was performed using the ELLA™ system (Bio-Techne, Minneapolis, USA), an automated microfluidic ELISA platform designed for low-volume samples and equipped with 32-well cartridges. Briefly, 50 μL of plasma—diluted with an equal amount of sample diluent—was dispensed into each well. Subsequently, 1 mL of wash buffer was loaded into the designated buffer ports. The immunoassay process was automatically conducted using the Simple Plex Runner software (version 3.9.0.28) through several stages, namely system initialization, microfluidic sample distribution, and incubation within glass nano-reactor (GNR) channels. These channels contained immobilized capture antibodies, biotin-labeled detection antibodies, and streptavidin-conjugated fluorescent dyes. Fluorescence signals were then elicited by laser excitation and recorded. The resulting fluorescence intensities, expressed in relative fluorescence units, were translated into ET-1 concentrations via reverse interpolation against a standard calibration curve provided by the manufacturer. As each microfluidic pathway includes three GNRs, every sample was measured in triplicate per well, with mean values reported.

### 2.3. Main clinical variables

Renal patients were stratified diagnostically and prognostically using the KDIGO classification, the progression risk table, and the CONSORTIUM-CKD equation [10]. Kidney function was evaluated with the CKD-EPI equation. Proteinuria was characterized as exceeding 500 mg (or albuminuria >300 mg) in a 24-hour urine collection. When proteinuria surpassed 1 g, a kidney biopsy was performed to confirm the diagnosis. Patient files were examined to extract data on kidney function, general biochemical parameters, and CVE documented during the follow-up period. Participants were monitored until the earliest occurrence of a CVE, death, or the study's endpoint (September 2024). CVE included acute coronary syndrome, acute myocardial infarction, coronary bypass, coronary catheterization requiring angioplasty, death from CV cause, lower limb ischemia, peripheral artery disease, stroke, sudden death, and typical angina with positive stress tests.

### 2.4. Statistical analysis

To study the association between categorical and quantitative variables, T-test/ANOVA or Mann-Whitney/Kruskal-Wallis tests were used, depending on the number of groups and data distribution. Categorical variables were summarized as absolute frequencies and percentages, whereas quantitative variables were expressed as mean ± standard deviation. Chi square or Fisher exact tests were used for the association between categorical variables. Logistic regression models were carried out to establish the association of the analyzed variables with clinical parameters. Clinical and demographic covariates incorporated into each model for adjustment were chosen according to clinical criteria and/or univariate analyses.

We carried out cluster analyses to group the patients according to their ET-1 concentrations. For the whole population, the analysis by the optimal *univariate k-means* method produced three different clusters [mean (range): 1.276 (0.268–1.730), 2.188 (1.740–2.830) and 3.482 (2.850–5.860) pg/mL] that were analyzed in relation to the severity of CKD, eGFR decline over time, CV event-free survival and all-cause mortality. To assess whether ET-1 levels were associated with the eGFR decline, a linear mixed-effects model was fitted. The model included fixed effects for time (3-year follow-up), ET-1 cluster, diabetes, hypertension, body-mass index (BMI) and smoking status, with patients established as random effect. Age and sex were not included as they are already used to calculate eGFR values. For this analysis, only patients with a baseline eGFR> 15 mL/min/1.73 $m^2$ were studied, as the physiological status of subjects with lower eGFR values, i.e., close to dialysis is significantly altered, which, together with the polypharmacy commonly required at this stage results generate confounding factors that could affect the validity of the analysis. Associations with CV risk and mortality were assessed in Kaplan-Meier curves and Cox regression models adjusted for meaningful covariates and expressed as hazard ratios (HR) with 95 % confidence intervals (CI) in parenthesis.

Two fast unified random forests for survival (RF-SRC) by Breiman's method were conducted to predict the risk of CVE and mortality. The variables included in the model were age, sex, body mass index, hypertension, diabetes, smoking, history of CVE, as well as the ET-1 cluster. The number of trees to grow was 1000, and the splitting rule was based on long-rank score method. The variable importance was measured by permutation. The events according to ET-1 cluster were estimated with the model, interpreted as the expected number of events for an individual with the same covariates. The discriminative power of the models was expressed with the concordance index (C-index) for right censored event times.

Statistical power calculations were carried out taking as reference previous studies reporting a significant ET-1 increase (from 1.5 up to 10-fold) in individuals who have experienced CVE compared to healthy subjects [11,12]. Considering the 692 individuals studied, an estimated 2-fold increase in ET-1 circulating concentrations, a CVE incidence in CKD of 10 % and an alpha error of 0.05, the obtained statistical power for the association of ET-1 levels with the main outcome, CVE, was 0.99 (GPower v. 3.1.9.6, Kiel University, Germany).

All the analyses were conducted with different packages in the *R* environment. The threshold for statistically significant associations was set at $p < 0.05$.

## 3. Results

In this study, patients with CKD stage 4–5 were significantly older than those with CKD stage 1–2 ($p < 0.0001$), whilst the proportion of males was higher in the CKD 3 group ($p = 0.021$). The prevalence of classical CV risk factors—including BMI ($p = 0.033$), diabetes mellitus ($p < 0.0001$), hypertension ($p = 0.024$), and smoking ($p = 0.027$) increased with the severity of the disease. Among the most frequent causes for CKD, diabetic nephropathy ranked first (24.4 %), followed by nephroangiosclerosis (17.8 %) and interstitial nephropathy (11.5 %). Causes were unknown in 18.2 % of cases. Table 1 presents demographic and clinical characteristics of the study population.

### 3.1. Association of endothelin-1 levels with renal function

Plasma concentrations of ET-1 differed significantly across CKD stages ($p < 0.001$). Mean (and standard deviation) concentrations were 1.65 (0.71) pg/mL for CKD stages 1–2, 1.82 (0.71) pg/mL for CKD stage 3, and 2.39 (1.08) pg/mL for CKD stages 4–5 (Fig. 1A). Since ET-1 role in diabetes has also been extensively studied, we compared its concentrations between patients with and without diabetes, ET-1 levels were significantly higher in diabetic patients compared with non-diabetic patients ($2.25 \pm 0.97$ and $1.92 \pm 0.94$, $p < 0.0001$).

Based on their ET-1 levels, participants were then clustered into three groups, which were used in further analyses (see below). Individuals with higher values (cluster 3) were predominantly distributed in the more advanced stages of CKD, whereas lower values (cluster 1) were significantly overrepresented among individuals with higher eGFR (Fig. 1B). Statistically significant differences were observed across all CKD stages ($p < 0.001$).

We then assessed whether ET-1 levels can also be indicative of CKD progression. According to the results of the linear mixed-effects model, and after adjusting by meaningful covariates, patients with higher ET-1 levels showed a greater decline in eGFR over 3 years compared with patients with the lowest ET-1 concentrations in cluster 1. Table 2 shows the β coefficients for clusters 2 [−12.64 ($p < 0.001$)] and 3 [−11.71 ($p = 0.034$)].

**Table 1**
Participant characteristics included in the study.

| | CKD 1–2 ($N = 185$) | CKD 3 ($N = 181$) | CKD 4–5 ($N = 326$) | [∫]P-value |
|---|---|---|---|---|
| Males (%) | 105 (56.8)[a] | 128 (70.7) | 209 (64.1) | 0.021 |
| Age (Years) | 56.8 (12.8)[a,b] | 67.1 (10.1)[c] | 68.9 (14.0) | <0.0001 |
| Weight (Kg) | 80.5 (17.2) | 84.2 (50.9) | 81.4 (50.0) | 0.367 |
| BMI | 28.5 (5.1) [a,b] | 29.4 (4.5) | 29.4 (5.3) | 0.033 |
| Glucose (mg/dL) | 106.1 (23.5)[a] | 125.6 (51.0)[c] | 111.3 (37.0) | 0.001 |
| Total cholesterol (mg/dL) | 176.6 (38.4)[b] | 175.7 (39.9)[c] | 148.3 (36.7) | <0.0001 |
| HDL cholesterol (mg/dL) | 58.7 (44.1)[a,b] | 50.2 (43.5) | 48.0 (16.8) | <0.0001 |
| LDL cholesterol (mg/dL) | 105.9 (105.6)[b] | 98.6 (50.0)[c] | 73.8 (30.7) | <0.0001 |
| Total calcium (mg/dL) | 9.5 (0.4)[b] | 9.5 (0.5)[c] | 9.4 (3.5) | <0.0001 |
| Potassium (mEq/L) | 4.6 (3.2)[a,b] | 4.7 (0.5)[c] | 5.0 (0.6) | <0.0001 |
| Sodium (mEq/L) | 141.4 (2.3)[b] | 141.5 (2.5)[c] | 139.7 (9.4) | 0.001 |
| ACR (mg/g) in urine 24h | 98 (364)[a,b] | 405 (982)[c] | 963 (1.287) | <0.0001 |
| eGFR CKD-EPI, lm/min/1.73 $m^2$ | 93.8 (17.0)[a,b] | 42.9 (9.9)[c] | 17.4 (6.3) | <0.0001 |
| Endothelin | 1.65 (0.71)[a,b] | 1.82 (0.71)[c] | 2.39 (1.08) | <0.0001 |
| Systolic blood pressure. (mmHg) | 137.2 (23.1)[a,b] | 143.5 (25.2) | 145.0 (25.5) | <0.0001 |
| Diastolic blood pressure. (mmHg) | 81.5 (12.7) [a,b] | 77.7 (15.1) | 75.2 (13.5) | <0.0001 |
| Pulse pressure, (mmHg) | 55.3 (17.6) [a,b] | 65.8 (21.4) | 69.6 (23.6) | <0.0001 |
| Hypertension (%) | | | | 0.024 |
| No | 42 (22.7) [a,b] | 25 (13.8) | 45 (13.8) | |
| Yes | 143 (77.3) | 156 (86.2) | 281 (86.2) | |
| Diabetes mellitus (%) | | | | <0.0001 |
| No | 155 (83.8) [a,b] | 96 (53.0) | 173 (53.1) | |
| Yes | 30 (16.2) | 85 (47.0) | 153 (46.9) | |
| Smoking (%) | | | | 0.027 |
| Non smoker | 89 (48.6)[a] | 60 (34.9)[c] | 143 (44.0) | |
| Ever smoker | 94 (51.4) | 112 (65.1) | 182 (56.0) | |

BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio.
[∫] p-value for the difference between all groups.
[a] significant differences with the CKD3 group.
[b] significant differences with the CKD4–5 group.
[c] significant differences with the CKD4–5 group.

### 3.2. Association of endothelin-1 levels with cardiovascular risk in CKD patients

A total of 683 participants were followed for a mean of $48.6 \pm 27.4$ months to record the occurrence of CVE. During this period, 69 events (10.1 %) were documented. Participants who experienced CVE had significantly higher age ($p = 0.002$), glucose levels ($p < 0.0001$), all types of cholesterol ($p < 0.05$), and urinary albumin-to-creatinine ratio (UACR) values ($p = 0.004$). Regarding cardiac biomarkers, patients with CVE showed higher levels of both troponin and NT-proBNP compared to those without events ($p < 0.01$). These and other features of individuals with and without CVE are listed in Table 3.

Survival analyses were carried out in the three groups based on the cluster stratification of ET-1 concentrations. Elevated ET-1 levels were significantly associated with reduced CV event-free survival ($59.44 \pm 26.39$, $43.10 \pm 87$ and $35.55 \pm 23.90$, for clusters 1, 2 and 3, respectively, $p = 0.002$, Fig. 2). After controlling for other CV risk factors, namely age, sex, BMI, hypertension, diabetes, smoking status, and history of CVE, this association remained significant ($p = 0.034$). HR values for individuals in cluster 3 (highest ET-1 levels) and cluster 2 (intermediate ET-1 levels) compared to those in cluster 1 (lowest ET-1 levels) were 2.50 (1.09–5.73), $p = 0.03$ and 2.24 (1.12–4.45), $p = 0.022$, respectively.

### 3.3. Association of endothelin-1 levels with all-cause mortality in CKD patients

Follow-up showed that ET-1 circulating concentrations were significantly associated with overall survival, $61.82 \pm 25.71$, $45.59 \pm 26.08$ and $36.59 \pm 24.97$), for clusters 1, 2 and 3, respectively, $p < 0.001$, Fig. 3. HR values for clusters 2 and 3 were 2.00 (1.17–3.43), $p = 0.012$ and 3.32 (1.82–6.05), $p < 0.001$. However, when age was added to the Cox regression model containing additional risk factors (age, sex, BMI, hypertension, diabetes, smoking status, and history of CVE), the differences between clusters lost statistical significance ($p = 0.237$).

A random forest model including the ET-1 cluster and relevant covariates (age, sex, BMI, diabetes, hypertension, smoking and CV history) was implemented for both CV event-free survival and all-cause mortality. C-indices for both models were respectively 0.835 and 0.837. Fig. 4**A-B** shows the ranking of variable importance for each model, where ET-1 ranked fourth for CV risk and third for all-cause mortality. The estimation of the number of events according to the ET-1 cluster is depicted in Fig. 4**C-D**.

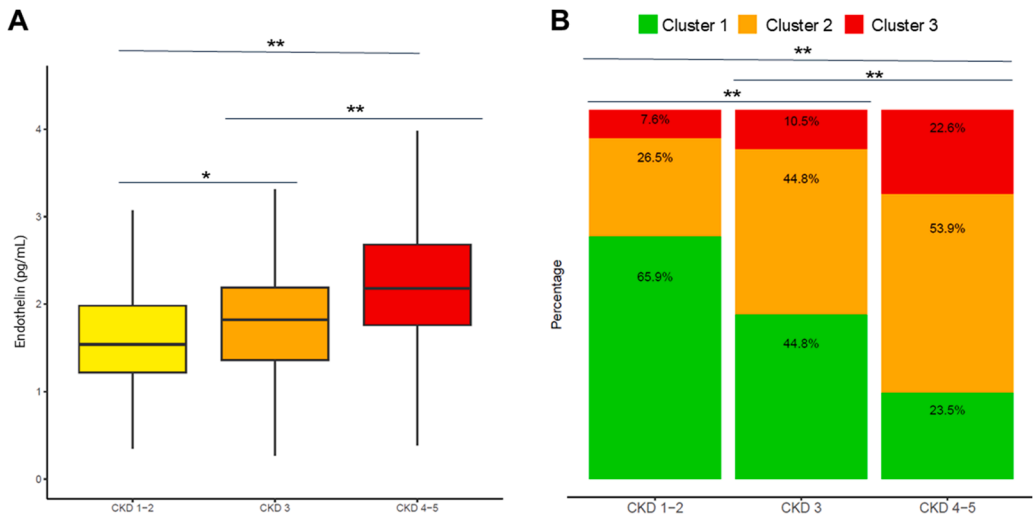


**Fig. 1.** A, plasma concentrations of endothelin-1 (pg/mL) stratified by CKD stage; B, distribution of endothelin-1 clusters across CKD stages. *$p < 0.01$; **$p < 0.001$.

**Table 2**
Linear mixed-effects model for the association of clusters of endothelin-1 concentrations with the decline of estimated glomerular filtration rate over time.

| | Value | Std. Error | t-value | p-value |
|---|---|---|---|---|
| Intercept | 77.09 | 9.20 | 8.37 | <0.0001 |
| Time (years) | −1.77 | 0.15 | −11.55 | <0.0001 |
| Cluster 2: 2.188 [1.740–2.830] pg/mL | −12.64 | 3.33 | −3.79 | 0.0002 |
| Cluster 3: 3.482 [2.850–5.860] pg/mL | −11.71 | 5.49 | −2.13 | 0.034 |
| Diabetes | −17.94 | 3.40 | −5.27 | <0.0001 |
| Hypertension | −1.43 | 4.05 | −0.35 | 0.724 |
| Body-mass index | 0.04 | 0.32 | 0.13 | 0.898 |
| Smoking status (ever/never) | −6.97 | 3.10 | −2.25 | 0.025 |

## 4. Discussion

CKD represents a significant and growing global health challenge characterized by a progressive decline in renal function frequently associated with a substantial burden of CV morbidity and mortality [13]. Effective CKD management depends on early risk identification and intervention, but limited understanding of its mechanisms highlights the need for research on reliable biomarkers.

Our results showed that ET-1 levels solidly correlated with the severity of CKD, as concentrations were significantly higher in patients with more advanced stages of the disease, which agrees with previous studies carried out in undersized cohorts [14,15]. In contrast, Sagi et al., also in a small group of patients, did not find significant differences between CKD stages [16]. The ET-1-CKD link is supported by several physiopathological mechanisms. Indeed, the activation of ETA by ET-1 has been shown to directly target the structure and function of podocytes, inducing proteinuria [17]. In addition, it has been suggested that ET-1 induces mesangial cell proliferation and fibrosis within the glomeruli [18] and exacerbates the damage to the endothelial glycocalyx, also increasing albuminuria [19]. Furthermore, systemic overexpression of ET-1 has been linked to inflammation in the kidney by mediating the effects of angiotensin II [18] and to impaired control over sodium and fluid homeostasis [20]. Moreover, in clinical trials, ETA-selective ERAs have been shown to reduce albuminuria and slow kidney function decline in CKD patients. However, their clinical use has been limited due to concerns about fluid retention and heart failure, and today they are only approved for the treatment of primary pulmonary hypertension and, recently, for IgA nephropathy [21].

Interestingly, our results also show that basal ET-1 circulating concentrations could also be indicative of CKD progression, as patients with higher values had a greater eGFR decline over time. To date, this association had only been indirectly studied by clinical trials designed to examine the effects of ERAs. For instance, in the recent DUET and PROTECT trials, sparsentan, a dual ET-1-angiotesin receptor antagonist, was associated with a slower annual rate of eGFR decline [22,23], although its benefits did not seem to improve those observed for irbesartan, which only targets angiotensin [24]. Also indirectly, a recent prospective study in a large cohort showed that higher concentrations of

**Table 3**
Demographic and clinical features of participants that did or did not experience cardiovascular events in the study. BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio.

| | No CVE ($n = 614$) | CVE ($n = 69$) | p-value |
|---|---|---|---|
| Males (%) | 390 (63.5) | 48 (69.6) | 0.315 |
| Age (Years) | 64.7 (14.0) | 70.4 (8.9) | 0.002 |
| Weight (Kg) | 82.2 (46.3) | 79.7 (13.8) | 0.791 |
| BMI | 29.1 (5.1) | 29.4 (4.8) | 0.541 |
| Glucose (mg/dL) | 111.8 (37.5) | 131.5 (48.7) | <0.0001 |
| Total cholesterol (mg/dL) | 163.9 (39.5) | 155.6 (47.5) | 0.023 |
| HDL cholesterol (mg/dL) | 52.1 (35.3) | 46.0 (15.1) | 0.039 |
| LDL cholesterol (mg/dL) | 89.4 (68.4) | 81.4 (42.7) | 0.027 |
| Total calcium (mg/dL) | 9.4 (0.5) | 10.2 (7.4) | 0.404 |
| Potassium (mEq/L) | 4.8 (2.0) | 4.9 (0.7) | 0.016 |
| Sodium (mEq/L) | 140.4 (7.6) | 140.8 (2.9) | 0.963 |
| ACR (mg/g) in urine 24 h | 578 (1.041) | 1.068 (1.607) | 0.004 |
| Endothelin | 2.01 (0.95) | 2.35 (1.1) | 0.008 |
| Troponin | 45.8 (44.5) | 81.0 (143.8) | 0.007 |
| NT_proBNP | 2.674 (5.861) | 5.606 (8.460) | 0.0004 |
| Hypertension (%) | | | 0.415 |
| No | 103 (16.8) | 9 (13.0) | |
| Yes | 511 (83.2) | 60 (87.0) | |
| History of CV event | | | 0.0001 |
| No | 393 (76.0) | 32 (52.5) | |
| Yes | 124 (24.0) | 29 (47.5) | |
| Diabetes mellitus (%) | | | 0.0003 |
| No | 388 (63.2) | 28 (40.6) | |
| Yes | 226 (36.8) | 41 (59.4) | |
| Smoking (%) | | | 0.644 |
| Non smoker / Former | 492 (81.5) | 53 (79.1) | |
| Smoker | 112 (18.5) | 14 (20.9) | |
| Systolic blood pressure. mmHg | 141.8 (23.5) | 151.9 (34.9) | 0.023 |
| Diastolic blood pressure. mmHg | 77.4 (13.1) | 77.7 (20.9) | 0.428 |
| Pulse pressure. mmHg | 64.3 (22.1) | 74.2 (23.9) | 0.001 |
| CKDstage_1_2_ctrles | | | 0.001 |
| CKD 1–2 | 174 (28.3) | 7 (10.1) | |
| CKD 3 | 154 (25.1) | 25 (36.2) | |
| CKD 4–5 | 286 (46.6) | 37 (53.6) | |

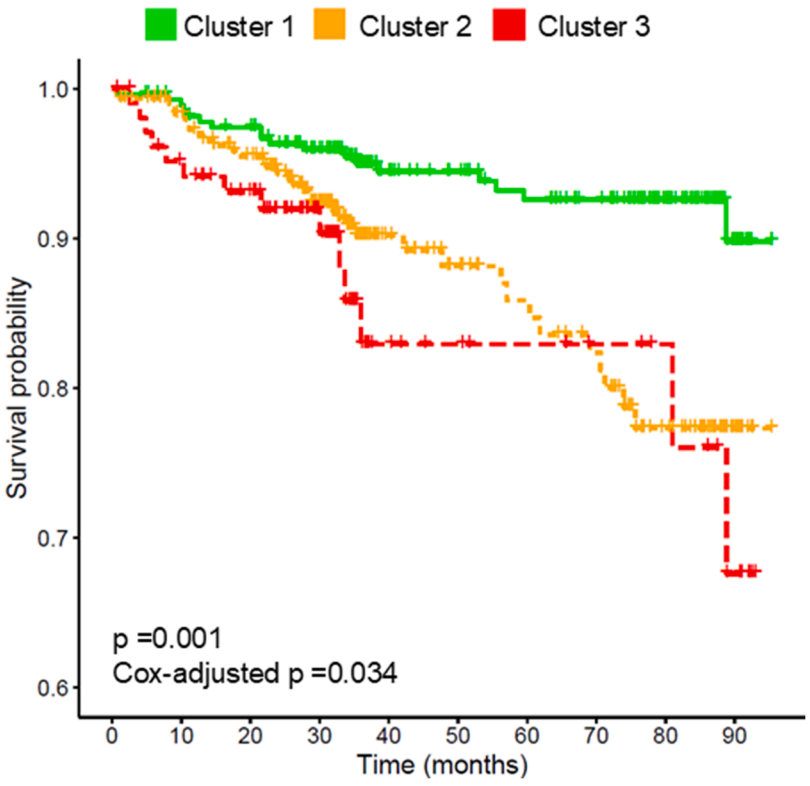


**Fig. 2.** Cardiovascular event-free survival in the three groups yielded by the cluster analysis based on endothelin-1 concentrations.

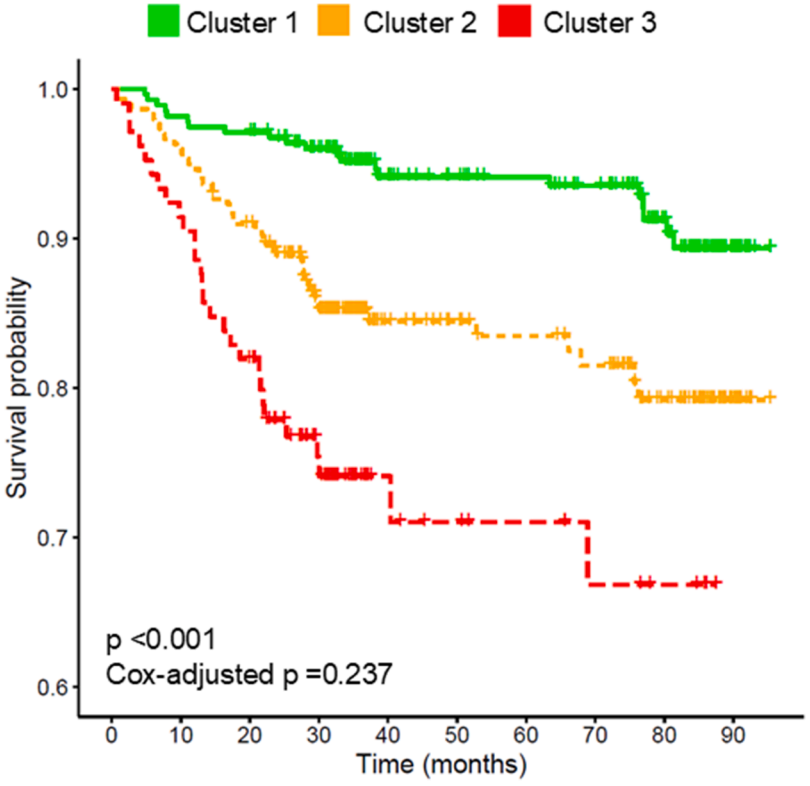


**Fig. 3.** Global survival in the three groups yielded by the cluster analysis based on endothelin-1 concentrations.

several endothelial inflammatory mediators induced by ET-1 were associated with a greater eGFR decrease [25]. Now, our data adds to this incidental evidence to support the notion that ET-1 concentrations might be useful to monitor the progression of renal damage.

Individuals with CKD face a higher likelihood of succumbing to CV diseases than progressing to kidney failure [2]. In this regard, ET-1 has

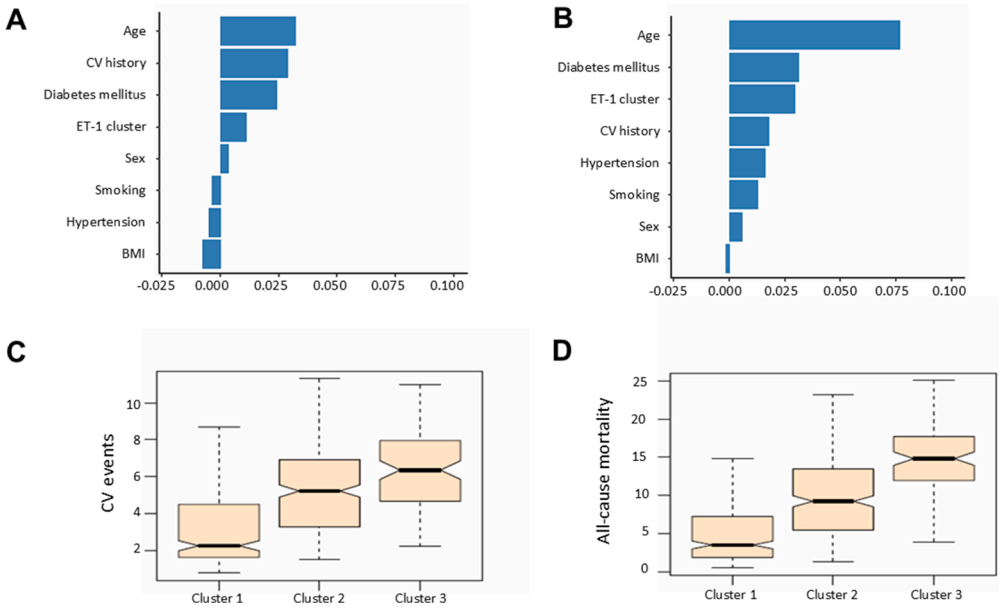


**Fig. 4.** Permutation-based importance ranking for the variables included in the model for CV risk (A) and all-cause mortality (B). Number of CV events (C) and deaths (D) according to the endothelin-1 cluster estimated by the average value of the covariates included in the models.

been related to CV dysregulation since it was discovered [26,27]. Our findings show that elevated circulating levels of ET-1 were independently associated with a significantly reduced CV event-free survival (over 2 years less in patients within cluster 3 compared to those in cluster 1), suggesting a potential prognostic role of ET-1 in CV risk stratification. Accordingly, studies on patients with CV disease, without renal involvement, also have reported elevated ET-1 concentrations [11, 28–30]. Indeed, ET-1 has been recently proposed as a useful biomarker for some of these disorders [29,31]. In the CKD setting, however, the information on ET-1 impact on CVE is very scarce. In a small group of CKD patients, Peng et al. reported that ET-1 levels exhibited a significant correlation with markers of cardiac complications [15], which supports our observations in this large CKD cohort. Persistent ET-1 elevation in CKD supports its role as a central mediator in cardiorenal syndrome pathophysiology. Although causality cannot be confirmed, evidence suggests that ET-1 may contribute to the heightened CV risk in CKD patients through mechanisms such as the exacerbation of atherosclerosis [32], hypertension [21] or by promoting myocardial fibrosis and remodeling [15]. It should be noted that, regarding the results obtained for all-cause mortality, the association lost significance when age was included in the regression model, which suggests that caution should be exerted when extrapolating these results. Notwithstanding, an explanation for the effect of age on the model could be that we included a wide array of patients: from individuals with CKD stages 1–2, usually younger, to those with advanced renal dysfunction (stages 4–5), who were significantly older. This led to age having a disproportionate weight in the regression model, which may have overshadowed the impact of ET-1 levels.

Finally, our findings showed that a predicting CV risk model containing the ET-1 cluster displayed a very good discriminating ability of over 80 %. There seems to be no previous reports in the CKD setting, but some similar models have been tested in other populations. For instance, serum ET-1 levels have recently demonstrated promising prognostic value in CV risk models for patients with myocardial infarction [33] or heart failure [11]. Additionally, CT-proET-1, has also been utilized in models assessing coronary calcification and cardiac vasculopathies following heart transplantation [34]. Our findings open the door to the possibility of utilizing ET-1 as a CV biomarker in CKD as well. In the same manner, our observation that ET-1 played a relevant role in mortality risk in CKD, third variable in importance after age and diabetes, has also been confirmed in other patient populations. Thus, recent studies have shown that ET-1 and its precursor CT-proET-1 have prognostic value for short-term mortality in acute heart failure [11], ischemic stroke [35], and coronary artery disease [36].

This study presents several limitations. First, our CKD cohort included all stages of the disease, which allowed determining more precisely the role of ET-1 in the evolution of CKD, but, on the other hand, it also implied a higher heterogeneity of the population, particularly regarding age which might affect some of the analyses. Second, a longer follow-up could likely have resulted in a higher incidence of CVE and mortality, which would have increased the consistency of the models. Third, this marker requires further in-depth investigation before it can be considered for routine use in clinical practice. Finally, all our patients were Caucasians, and therefore the extrapolation of our results to other ethnicities might be limited.

Former studies had suggested that ET-1 could play a significant role in CKD; however, this is the first study to demonstrate, in a large and well-characterized cohort of renal patients, that baseline ET-1 levels not only increase with CKD severity, but are also independently associated with a higher incidence of CVE and faster decline in kidney function over time. Moreover, through advanced predictive models, we were able to show that ET-1 levels solidly correlate with the incidence of CV complications in CKD, which is the main cause of death in this population. Pending confirmation in prospective studies, these results, taken together, support the idea that ET-1 could be a very useful biomarker in CKD, both for the monitorization of renal function and CV comorbidities.

## Author contributions

L G-R, data curation and writing of original draft; M-A and EL formal analysis; S M-Z, data curation; CC, methodology; BC, AA and ZV, investigation; FB, supervision, investigation; NRR, conceptualization, investigation; GG, conceptualization, funding acquisition, supervision, writing (review and editing).

## Funding sources

This research was funded by Instituto de Salud Carlos III with grants PI22/00181 and RICORS2040 RD24/0004/0012. Co-financed by the

European Union, the Extremadura Research Chair in Cardiovascular Risk Reduction (CIRRCE) and Fundación CB. The work was also financed at 85 % by the European Union, European Regional Development Fund, and the Regional Government of Extremadura. Managing Authority: Ministry of Finance (Grant GR24027). Funding sources did not have any involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

## Data statement

The data that support the findings of this study are available on reasonable request from the corresponding author (GG).

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

## Declaration of competing interest

The authors declare they have no conflict of interest.

## Acknowledgments

We would like to thank the FRESENIUS clinic for their collaboration in this study, as well as the nursing staff at the different hospitals, whose assistance was key to the completion of this work.

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