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Circulating angiotensin converting enzyme 2 activity as a biomarker of silent atherosclerosis in patients with chronic kidney disease

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ABSTRACT

Background and aims: Circulating Angiotensin Converting Enzyme 2 (ACE2) activity in chronic kidney disease (CKD) patients without previous history of cardiovascular disease (CVD) has been associated with classical risk factors (older age, diabetes and male gender). Furthermore, silent atherosclerosis has been described as a pathological link between CKD and CVD. We analyzed baseline ACE2 activity in non-dialysis CKD stages 3–5 (CKD3-5) patients as a biomarker of renal progression, silent atherosclerosis and CV events after 2 years of follow-up.

Methods: Prospective study of 1458 CKD3-5 subjects without any previous CV event included in the Spanish multicenter NEFRONA study. Association between baseline circulating ACE2 activity and renal parameters, carotid/femoral echography, atheromatous disease, ankle-brachial index, intima-media thickness, need of renal replacement therapy, cardiovascular events and mortality at 24 months of follow-up were analyzed.

Results: Patients with an increase in the number of territories with plaques at 24 months showed significantly higher levels of baseline ACE2 activity as compared to stable patients (29.6 (20.6–47.6)RFU/μL/h *versus* 35.7 (24.5–56), $p < 0.001$). Multivariate linear regression analysis showed that male gender, pathological ankle-brachial index and progressive silent atherosclerosis defined as an increased number of territories with plaques at 24 months were associated with increased baseline ACE2 activity. Male gender, older age, diabetes, smoking and increased baseline circulating ACE2 were independent predictors of atherosclerosis at 24 months of follow-up.

Conclusions: In CKD3-5 patients, higher circulating ACE2 activity at baseline is associated with higher risk for silent atherosclerosis, suggesting that ACE2 may serve as a biomarker to predict CV risk before CVD is established.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD) [1–3]. The risk of death from any cause increases as the estimated glomerular filtration rate (eGFR) declines [2] and patients with end-stage renal disease (ESRD) have a rate of mortality that exceeds the need of renal replacement therapy [4]. Silent atherosclerosis has been described as a pathological link between CKD and CVD and could be a useful marker for predicting adverse CV outcomes in CKD patients [5,6]. In CKD patients, carotid intima-media thickness (cIMT) has been demonstrated to be increased as compared to healthy patients [7,8] and to be an independent predictor of all-cause and CV mortality [9]. In addition, carotid plaque largely increases the risk of CV events [10]. Recent results from the multicenter prospective observational NEFRONA study [11,12], that includes CKD patients and controls without previous CV events,

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have shown that the prevalence of atheromatous plaques is higher among CKD patients, correlating with the severity of CKD. Interestingly, the highest prevalence is observed among dialysis patients [13,14].

The renin-angiotensin system (RAS) plays also a major role in the pathophysiology of CVD and renal injury [15,16]. Within the RAS, angiotensin-converting enzyme 2 (ACE2) [17] acts as a monocarboxypeptidase that cleaves the C-terminal aminoacid of angiotensin II (AngII) to generate the peptide Ang1-7, which counteracts the adverse effects of AngII [18]. ACE2 is an integral cell membrane protein that can undergo cleavage or shedding to release the catalytically active ectodomain into the circulation [19]. Circulating ACE2 activity was at first only detected in subjects with advanced age and with higher prevalence of CVD, diabetes and hypertension [20]. Subsequent studies have confirmed that circulating ACE2 activity can be detected in healthy subjects [21] and that it is increased in heart failure patients, acute myocardial infarction [22,23], diabetes [24], kidney transplant patients [25], and in pre-dialysis CKD patients [26]. We have recently shown that in CKD patients without previous history of CVD, circulating ACE2 activity directly correlates with the classical CV risk factors such as male gender, older age and diabetes [27]. It has also been demonstrated a protective role of ACE2 against atherosclerosis in experimental studies. ACE2 overexpression has been shown to inhibit the development of early atherosclerotic lesions [28,29] and to enhance the stability of atherosclerotic plaques [30] partly due to counterregulation of AngII signaling and inhibition of inflammatory response. In concordance, ACE2 deletion increases the development of atherosclerosis in fat-fed low density lipoprotein receptor ($Ldlr^{-/-}$) mice [31] and plaque accumulation in atherosclerosis-prone apolipoprotein E knockout (*ApoE* KO) mice [32].

We have previously shown that circulating ACE2 activity in CKD patients without previous history of CVD from the NEFRONA study directly correlates with classical CV risk factors namely older age, diabetes and male gender [27]. We now hypothesized that baseline ACE2 activity in CKD stages 3–5 (CKD3-5) patients serves as a biomarker of renal progression, atherosclerosis and CV events after 24 months of follow-up.

2. Patients and methods

2.1. Patients and variables

Study population included 1458 non-dialysis CKD3-5 subjects from the observational and multicenter study (NEFRONA project), recruited from October 2009 to June 2011 [11,12,27] and with 24 months of follow-up. This prospective study included male and female patients without history of CVD (angina pectoris, acute myocardial infarction, ischemic stroke, hemorrhagic stroke, abdominal aortic aneurysm and atherosclerosis), and ages ranged between 18 and 74 years old. Exclusion criteria were pregnancy, VIH infection, any type of organ transplantation, previous history of carotid artery disease, active infections, any hospitalization in the last month, and intercurrent illness that presumes absence of follow-up or survival expectation less than 1 year.

Baseline clinical variables, treatment profile, analytical variables, presence of plaques and ACE2 enzymatic activities have been previously reported [27,33,34] (Supplementary Data). For the prospective study at 24 months, renal parameters (serum creatinine and eGFR), carotid/femoral echography, atheromatous disease (AD), ankle-brachial index (ABI), intima-media thickness (IMT), need of renal replacement therapy (kidney transplantation or dialysis), CV events and mortality were assessed (Fig. 1 and Table S1).

2.2. Renal parameters

Progression of renal disease was evaluated according to doubling of serum creatinine or 50% decrease in eGFR calculated with the modification of diet in renal disease (MDRD)-4 formula at 24 months. Renal replacement therapy was defined as kidney transplantation or dialysis during the 24 months of follow-up. For the assessment of doubling of serum creatinine and the decrease in eGFR, patients that started dialysis or had a kidney transplant during the 24 months of follow-up were excluded. The composite endpoint doubling of serum creatinine and renal replacement therapy was also assessed.

2.3. Silent atherosclerosis

Subclinical atherosclerosis was evaluated as described previously [12]. Participants underwent a carotid and femoral ultrasound to measure IMT, using the Vivid BT09 apparatus (General Electric instrument) equipped with a 6–13 MHz broadband linear array probe. The analysis of the presence of atheromatous plaques was performed by a unique reader in a blinded fashion, using the semi-automatic software EchoPAC Dimension (General Electric Healthcare). To assess the quality of the reading and the intra-observer reliability, a sample of 20 individuals was measured 3–5 times on different days. A kappa coefficient of 1 was obtained, indicating excellent intraobserver reliability.

Plaque presence was evaluated in a total of 10 territories: right common carotid arteries, right carotid bulb, right internal carotid arteries, left common carotid arteries, left carotid bulb, left internal carotid arteries, right common femoral arteries, right superficial femoral arteries, left common femoral arteries, and left superficial femoral arteries. To assess the evolution of plaques from baseline time to 24 months, patients were classified in three groups: no plaque (patients without plaque neither at baseline nor at 24 months); *de novo* plaque (appearance of plaque at 24 months); and baseline plaque (plaques both at baseline and 24 months). Furthermore, a classification according the location of plaques was performed: any plaque (presence of plaque in any territory); carotid plaque (presence of plaque in carotid territories); and femoral plaque (presence of plaque in femoral territories). Patients were classified in three groups according to the number of territories with plaques: 0; 1 to 4; and $\geq$5. The increase in number of territories with plaques from baseline to 24 months was also determined.

Vascular Doppler MD2 Hungleigth was used with an 8 MHz transducer and a sleeve for making manual blood pressure. The determination of blood pressure was performed in the brachial artery in both arms and in both feet. To calculate the ABI, the higher brachial blood pressure or the closest in time to the malleolar measure was used. A pathological ABI was defined as a value $\leq$0.9, diagnostic of a limb ischemia, or $\geq$1.4, diagnostic of arterial incompressibility and stiffness, usually ascribed to vascular wall calcification [12].

AD was initially classified in four groups: AD 0: ABI >0.9 and IMT <80% reference interval; AD 1: ABI 0.7–0.9 and/or IMT $\geq$80% reference interval; AD 2: carotid plaque with stenosis <125 cm/s; and AD3: ABI <0.7 and/or carotid plaque with stenosis $\geq$125 cm/s [11,12,14,35]. For this study, AD was classified in two groups, incipient AD (combining AD 0 and 1) and severe AD (combining AD 2 and 3), as previously reported [36].

2.4. Events

Events were classified as CV events, CV mortality, non-CV mortality, and all-cause mortality (both CV and non-CV). CV