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Combination of biomarkers of vascular calcification and sTWEAK to predict cardiovascular events in chronic kidney disease

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ABSTRACT

Background and aims: Vascular calcification (VC) and atherosclerosis are associated with an increased cardiovascular morbimortality in chronic kidney disease (CKD). Osteoprotegerin (OPG) and osteopontin (OPN) are involved in both VC and CKD. Soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) has been related to cardiovascular disease. We hypothesized that OPG, OPN and sTWEAK levels may be associated with a higher prevalence of cardiovascular outcomes in patients with CKD.
Methods: The presence of calcified or non-calcified atherosclerotic plaques was assessed in 1043 stage 3 to 5D CKD patients from The NEFRONA Study. Biochemical measurements and OPG, OPN and sTWEAK serum levels were analyzed. Patients were followed for cardiovascular outcomes (41 ± 16 months).
Results: At recruitment, 26% of CKD patients had VC. The adjusted odds ratios for having VC were 2.22 (1.32−3.75); p=.003 for OPG, and 0.45 (0.24−0.84); p=.01 for sTWEAK concentrations. After follow-up, 95 CV events occurred. In a Cox model, patients with OPG or OPN above and sTWEAK below their optimal cut-off points had an adjusted higher risk of cardiovascular events [HR: 2.10 (1.49−3.90); p=.02; 1.65 (1.02−2.65); p=.04; 2.05 (1.28−3.29), p=.003; respectively]. When CKD patients were grouped according to the number of biomarkers above (OPG and OPN) or below (sTWEAK) their cut-off points, the combination of these biomarkers showed the highest risk for cardiovascular events [HR: 9.46 (3.80 −23.5) $p < .001$]. A composite score of these three biomarkers increased the C-statistic and net reclassification index beyond conventional risk factors and VC.
Conclusions: The combination of OPG, OPN and sTWEAK increased the predictability of cardiovascular outcomes.

1. Introduction

Chronic kidney disease (CKD) is associated with a high incidence of cardiovascular (CV) events and mortality [1]. In fact, CV events and mortality increase progressively as glomerular filtration rate decreases [2,3].

Although traditional risk factors such as hyperlipidemia, hypertension, diabetes and smoking could explain an increased CV risk in CKD populations [4], other factors could also contribute to the increased CV events observed in CKD patients. Therefore, additional diagnostic tools that provide better CV risk assessment in CKD patients are needed. In this context, the addition of vascular calcification (VC) scores to traditional risk factors improves CV risk assessment in CKD patients [5].

Vascular calcification is a progressive accumulation of calcium

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and phosphate salts in the arterial wall and cardiac valves. Data from animal models have identified different factors such as osteopontin (OPN) and osteoprotegerin (OPG) as regulators of calcification in the arterial wall [6,7]. OPG is a cytokine that belongs to the tumor necrosis factor (TNF) receptor superfamily, which has a range of pleiotropic effects on bone metabolism, endocrine function, and the immune system [8]. OPG inhibits osteoclastic bone resorption by binding to the receptor activator of nuclear factor-κB ligand (RANKL), acting as a decoy receptor to competitively inhibit RANKL interaction with its receptor, RANK [9]. Circulating OPG levels have been associated with the presence of VC and all-cause mortality in CKD patients [10]. In addition, elevated serum OPG levels increased the risk of CVD and all-cause mortality in elderly women, with the association being more evident in women with worse renal function [11]. On the other hand, OPN belongs to the small integrin-binding ligand N-linked glycoprotein family [12]. This protein is produced and secreted by different cell types such as macrophages, T cells, renal and vascular smooth muscle cells (VSMCs) as well as osteoblasts and osteoclasts [13]. In CKD, elevated levels of OPN predicted all-cause and cardiovascular mortality, although this effect was absent after adjustment for inflammatory biomarkers [14].

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a pro-inflammatory cytokine of the TNF-superfamily that is present in serum as a soluble circulating form (sTWEAK). Different studies have demonstrated a key role of this cytokine in atherosclerotic plaque development, progression and rupture [15–17]. Recently, it has been demonstrated that TWEAK also participates in VSMC calcification [18]. In addition, loss-of function experiments have shown that TWEAK increases atherosclerotic plaque calcification [15]. Finally, low sTWEAK levels have been associated with cardiovascular outcomes in CKD patients [19,20].

The National Observatory of Atherosclerosis in Nephrology (NEFRONA) Study is a multicenter, observational, prospective study designed to analyze the prevalence of atherosclerosis and its associated risk factors in patients with CKD [21,22]. In this study, we evaluated the association between selected calcification biomarkers, sTWEAK and their combination with cardiovascular outcomes in the NEFRONA population.

2. Materials and methods

2.1. Study population

Participants included 1043 CKD stage 3 to 5D patients from the NEFRONA Study [21,22]. Briefly, the study included males and females without history of CVD (acute myocardial infarction, angina pectoris, hemorrhagic or ischemic stroke, atherosclerosis, and abdominal aortic aneurysm). CKD patients in this sub-study were enrolled within 57 Spanish primary care centers in 32 different regions in Spain. The exclusion criteria for participation in the study were as follows: previous CV events, pregnancy, having received any organ transplantation, active infections, or having a life expectancy of <1 year.

The analysis of the presence of atherosclerotic plaques in the NEFRONA Study was previously described in detail [23]. Briefly, participants underwent 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. 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. The presence of atherosclerotic plaques was defined as an IMT >1.5 mm protruding into the lumen, according to the ASE Consensus Statement and the Mannheim cIMT Consensus [24,25].

2.2. Events

Primary outcomes were CVD events according to the International Classification of Diseases of the World Health Organization, which includes myocardial infarction, unstable angina, transient ischemic attack, cerebrovascular accident, arrhythmia, congestive heart failure, peripheral artery disease or amputation for vascular disease, or aortic aneurysm. CV mortality was defined as death caused by cerebrovascular accident (ischemic or hemorrhagic), myocardial ischemia and infarction, hyperkalemia or arrhythmia, sudden death, hemorrhage due to aneurysm rupture and mesenteric infarct. Non-CV mortality was defined as death caused by neoplasia, accident, infection, non-determined cause, or unknown death. The local Ethics Committee of the Hospital Arnau de Vilanova approved the protocol. The authors adhered to the declaration of Helsinki and all subjects provided informed consent for participation in the study.

2.3. Clinical and biochemical data

Patients were asked to complete a questionnaire at recruitment including clinical history of hypertension, dyslipidemia, diabetes, CV risk factors, and medication use. Biochemical parameters were obtained from a routine fasting blood test. Serum sTWEAK levels were determined using a commercially available ELISA kit (ThermoFisher Scientific), and OPG and OPN levels by MILLIPLEX® MAP kits (EMD Millipore Corporation). The investigators measured all samples in a blinded manner. The minimum detectable levels of OPG, OPN and sTWEAK were 1.9, 37.7, and 10 pg/mL, respectively. Intra-assay coefficients of variation were 5% for OPG, 2% for OPN and 7% for sTWEAK. Inter-assay coefficients of variation were 11% for OPG, 12% for OPN and 9% for sTWEAK.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS 11.0 (SPSS Inc, Chicago, IL) statistical package. Normally distributed variables were expressed as mean ± SD and non-normally distributed variables were expressed as medians (IQR, expressed as the 25th and 75th percentile). Between-group comparisons were assessed for nominal variables with the chi-squared test and Mann-Whitney U test. Spearman rank correlation was used to determine correlations between variables. Multivariable logistic regression analysis was completed to identify the impact of OPG, OPN and sTWEAK levels on the prediction of VC (OPG, OPN and sTWEAK concentrations were log-transformed for the statistical model). A receiver operator characteristic curve analysis was done to determine the OPG, OPN and sTWEAK optimal cut-off points and maximum sensitivity and highest specificity for the prediction of a cardiovascular event. A categorical variable was generated containing the information of the number of biomarkers that were over (or under, in the case of sTWEAK) the cut-off point. Time to event analysis of CV outcomes was done using the Cox proportional hazards model, including adjustment for potential confounding factors (all factors that were statistically significant in the univariate analysis). Data are presented in the form of Hazard ratios (HRs) and 95% confidence intervals (95% CIs). Statistical differences in C-statistics were compared using the method of DeLong et al. [26]; 95% CIs were calculated for each comparison. Kaplan-Meier curves were used to compare time to outcome according to a multimarker score. Net reclassification index was calculated based on Pencina et al. [27]. p value < .05 was considered statistically significant.