Universitat Autònoma de Barcelona

SUBCLINICAL ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE AND DIABETES

ANA PALANCA

UNIVERSITAT AUTÒNOMA DE BARCELONA

SUBCLINICAL ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE AND DIABETES

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LIST OF ABBREVIATIONS

4D Study The German Diabetes and Dialysis Study

18F-FDG 18F-Fluorodeoxyglucose

25-OH vitamin D 25-hydroxivitamin D

ACC American College of Cardiology

AGEs Advanced glycation end-products

AHA American Heart Association

ARIC Atherosclerosis Risk in Communities

ASCVD Atherosclerotic cardiovascular disease

AWHS Aragon Workers Health Study

CAC Coronary artery calcification

CAFES-CAVES Carotid-Femoral morphology screening and cardiovascular events

CAPS Carotid Atherosclerosis Progression Study

CEUS Contrast-enhanced ultrasound

cIMT Carotid intima-media thickness

CKD Chronic kidney disease

CRIC Chronic Renal Insufficiency Cohort

CT Computed tomography

DALYs Disability-adjusted life years

eGFR Estimated glomerular filtration rate

ESC European Society of Cardiology

EU European Union

FDG Fluorodeoxyglucose

FFA Free fatty acids

FSH Framingham Heart Study

FRS Framingham Risk Score

HbA1c Haemoglobin A1c

HDL High-density lipoprotein

HIV Human immunodeficiency virus

Hs-CRP High-sensitivity C-reactive protein

ICD9-CM International Classification of Diseases, Ninth Revision, Clinical Modification

IDF International Diabetes Federation

IMT Intima-media thickness

INE Instituto Nacional de Estadística

LDL Low-density lipoprotein

MESA Multi-Ethnic Study of Atherosclerosis

MRI Magnetic resonance imaging

NEFRONA National Observatory of Atherosclerosis in Nephrology

PARADIGM Progression of Atherosclerotic Plaque Determined by Computed Tomographic Angiography Imaging

PESA Progression and Early detection of Subclinical Atherosclerosis

PET Positron emission tomography

REGICOR: Registre Gironí del Cor

RNA Ribonucleic *acid*

US United States

WHO World Health Organization

Contents

ABSTRACT

BACKGROUND

Cardiovascular disease is the leading cause of morbidity and mortality worldwide. Individuals with diabetes and chronic kidney disease (CKD) have remarkably high rates of cardiovascular disease risk. Moreover, incremental cardiovascular risk in diabetes is heterogeneous and has been often related to concomitant CKD. On the other hand, typically used risk equations based on traditional cardiovascular risk factors fail to accurately predict cardiovascular risk not only in the general population but also in these subsets of the population. Multi-territorial ultrasonography to assess subclinical atherosclerosis has emerged as a valid tool to refine cardiovascular risk assessment beyond traditional risk factors. The purpose of this thesis was to analyse the prevalence, distribution, and progression of subclinical atherosclerosis, as well as the associated cardiovascular risk factors in a large cohort of CKD subjects with and without diabetes, free from known cardiovascular disease, using multi-territorial ultrasonography. Subsequently, we further evaluated the prognostic value of subclinical atherosclerosis in determining the incidence of first cardiovascular events in this high-risk population.

METHODS

First, we included the data from CKD subjects with and without diabetes and free from previous cardiovascular events from the NEFRONA cohort, that were recruited at baseline, and that attended a follow-up visit 24 months later. Participants underwent a physical examination, fasting blood test, and carotid and femoral ultrasound examinations (including a total of 10 vascular territories: internal, bulb and common carotid, and common and superficial femoral arteries; right and left) at baseline and at 24-month follow-up. Plaque progression was defined as an increase in the number of territories with plaque(s) compared to the baseline examination. Multivariable models were used to assess the contribution of diabetes to the presence and progression of plaques. Other risk factors associated with the prevalence and progression of arterial disease were also evaluated using multivariate model analyses.

We also conducted another study including data from the NEFRONA cohort subjects with and without diabetes that were recruited initially. All of the participants underwent a physical examination, fasting blood test, and carotid and femoral ultrasound examination (including all the 10 vascular territories described above) at baseline and were followed-up for 48 months. During the follow-up period, all cardiovascular events were registered. Bivariate analysis and Fine-Gray competing risk models were used to perform the statistical analysis. Concordance Index (C-statistics) was estimated for the strongest resulting risk models for CKD subjects with diabetes as well as for CKD subjects without diabetes.

RESULTS

With regards to the first study: a total of 419 individuals with diabetes and 1129 without diabetes were included. Individuals with diabetes were older, had a higher body mass index, and had a higher frequency of hypertension and dyslipidaemia. At baseline, the proportion of subjects with plaque at any of the examined territories was higher among diabetic individuals (81.4% *vs* 64.1%, $p < 0.001$). Diabetic subjects more frequently had more than two vascular territories with plaque (64.4% *vs* 48.4%, $p < 0.001$). During a 24-month follow-up period, plaque progression occurred in 72.2% individuals with diabetes whereas, among individuals without diabetes, plaque progression occurred in 55.8%.

Multivariable analysis indicated that plaque at baseline was significantly associated with age, male gender, smoking, and renal replacement therapy in the non-diabetic subjects, while only age and male gender were associated with plaque presence in diabetic subjects. Plaque progression was significantly associated with age, the number of territories with basal plaque, smoking, and renal replacement therapy in both groups.

Regarding the second study, during a mean follow-up time of 48 months, a total of 203 cardiovascular events was registered. One-hundred-seven cardiovascular events

occurred among participants without diabetes (19.58 per 1000 person-years), and 96 cardiovascular events occurred among participants with diabetes (44.44 per 1000 person-years). After competing risk analyses and model selection, those variables that better predicted cardiovascular events in CKD individuals without diabetes were the number of territories with plaque at baseline (HR 1.862, 95% CI [1.432;2.240]), age (HR 1.026, 95% CI [1.003;1.049]) and serum concentrations of 25-OH vitamin D (HR 0.963, 95% CI [0.933;0.094]). Among CKD participants with diabetes, the strongest model predicting incident cardiovascular events had only one variable: the number of territories with a plaque at baseline (HR 1.782, 95% CI [1.393, 2.278]). For both models, the concordance (C) index score was greater than 0.7 at both 24 and 48 months.

CONCLUSIONS

Subclinical atherosclerosis is more prevalent, carries a higher plaque burden, and is more progressive in CKD subjects with diabetes than in subjects without diabetes. In these individuals, diabetes outweighs other described risk factors associated with the presence of subclinical atherosclerosis.

The burden of subclinical atherosclerosis is the strongest predictor of future cardiovascular events in diabetic individuals with CKD. Early detection of the subclinical atherosclerotic burden by multi-territorial vascular ultrasound could improve cardiovascular events prediction in this population.

RESUMEN

INTRODUCCIÓN

La enfermedad cardiovascular es la primera causa de morbilidad y mortalidad en el mundo. Los individuos con diabetes y con enfermedad renal crónica presentan un mayor riesgo de eventos cardiovasculares con respecto a la población general. En la diabetes, el incremento en el riesgo cardiovascular es heterogéneo y éste se ha relacionado frecuentemente con el grado de afectación renal. Por otra parte, los algoritmos para calcular el riesgo cardiovascular basados en factores de riesgo cardiovascular tradicionales no trasladan con suficiente precisión el riesgo futuro de eventos cardiovasculares en este grupo de pacientes que, de base, presenta un alto riesgo de enfermedades cardiovasculares. La evaluación de la aterosclerosis subclínica mediante ecografía multiterritorial representa una herramienta válida para refinar el riesgo cardiovascular más allá de los factores de riesgo tradicionales. El propósito de esta tesis fue analizar utilizando la ecografía multiterritorial la prevalencia, distribución y progresión de la aterosclerosis subclínica, así como factores de riesgo cardiovascular asociados, en una amplia cohorte de pacientes con enfermedad renal crónica con y sin diabetes y sin enfermedad cardiovascular previa conocida. Posteriormente, se evaluó el valor pronóstico de la aterosclerosis subclínica para determinar la incidencia de primeros eventos cardiovasculares en esta población de alto riesgo.

MÉTODOS

En primer lugar, se incluyeron los datos de los sujetos con enfermedad renal crónica, con diabetes y sin diabetes, de la cohorte del estudio NEFRONA que fueron reclutados al inicio del estudio y que asistieron a la visita de control 24 meses más tarde. Todos los participantes se sometieron a una anamnesis completa, un examen físico, un análisis de sangres en ayunas y a un estudio ultrasonográfico a nivel de arterias carotídeas y femorales (incluyendo un total de 10 territorios: arterias carótida interna, bulbo y carótida común y arterias femorales común y superficial; derecha e izquierda) tanto en la visita inicial del estudio como en la visita de seguimiento a los 24 meses.

La progresión de placa se definió como el incremento en el número de territorios con placa comparado con la exploración inicial. Se analizó la contribución de la diabetes a la presencia y progresión de placa mediante análisis de modelos multivariables. Del mismo, también se evaluó la correlación con prevalencia y progresión de placa con otros factores de riesgo asociados.

Por otra parte, se realizó otro análisis que incluyó los datos de todos los sujetos del estudio NEFRONA con y sin diabetes que fueron reclutados inicialmente. Todos los participantes se sometieron a una anamnesis, un examen físico, un análisis de sangre en ayunas y una ecografía de arterias carótidas y femorales (incluyendo los 10 territorios mencionados) al inicio del estudio y fueron seguidos durante 48 meses. Durante el periodo de seguimiento se registró la incidencia de todos los eventos cardiovasculares incidentes. Análisis bivariados y análisis de modelo de riesgos competitivos de Fine-Grey fueron utilizados para realizar el estudio estadística. El índice de concordancia estadístico C se estimó para los modelos de riesgo resultantes con mayor potencia tanto para pacientes renales con diabetes como para pacientes renales sin diabetes.

RESULTADOS

Con respecto al primer estudio, se incluyeron un total de 419 sujetos con diabetes y 1129 sin diabetes. Los sujetos con diabetes tenían más edad, un índice de masa corporal mayor y mayor prevalencia de hipertensión y dislipidemia. Al inicio del estudio, la proporción de individuos con placa en cualquiera de los territorios examinados fue mayor entre los sujetos con diabetes (81.4% versus 64.1%, $p<0.001$). Los sujetos con diabetes también presentaron con mayor frecuencia la afectación con placa de más de dos territorios vasculares (64.4% versus 48.4%, $p< 0.001$). Tras realizar el análisis multivariable, se demostró que la presencia de placa basal se asociaba significativamente con la edad, el género masculino, el hábito tabáquico y la diálisis en los sujetos renales sin diabetes mientras que, en los sujetos renales con diabetes, la presencia

de placa basal se asoció significativamente tan sólo a la edad y al género masculino. En cuanto a la progresión de placa, ésta se asoció de manera significativa a la edad, al número de territorios con placa basal, al hábito tabáquico y a la diálisis en ambos grupos. Durante los 24 meses de seguimiento, se observó progresión de placa en 72.2% de los individuos con diabetes mientras que en los individuos sin diabetes, se onservó progresión de placa en un 55.8%.

En relación al segundo estudio, durante el tiempo medio de seguimiento de la cohorte que fue de 48 meses, se registraron un total de 203 eventos cardiovasculares produciéndose 107 eventos entre los sujetos renales sin diabetes (19.58 por 1000 años-persona) y 96 entre los sujetos renales con diabetes (44.44 por 1000 años-persona). Tras realizar los análisis de riesgo y la selección de los modelos más potentes, las variables que mejor predijeron futuros eventos cardiovasculares en individuos con enfermedad renal crónica y sin diabetes fueron edad y concentraciones séricas de 25-OH vitamina D y número de territorios con placa al inicio del estudio. Entre los participantes con enfermedad renal crónica y diabetes el modelo más robusto en predecir eventos cardiovasculares incidentes contenía tan sólo la variable “número de territorios con placa al inicio del estudio”. Para ambos modelos, el índice estadístico C, estimado a los 24 y a los 48 meses, fue superior a 0.70.

CONCLUSIONES

La aterosclerosis subclínica es más prevalente, conlleva una mayor carga de placa y es más progresiva en individuos con enfermedad renal crónica y diabetes. En estos sujetos, la diabetes supera otros factores de riesgo descritos asociados con la presencia de placa. Así mismo, la carga de aterosclerosis subclínica es el predictor más potente de futuros eventos vasculares en individuos con diabetes y enfermedad renal crónica.

La detección precoz de carga aterosclerótica preclínica mediante ultrasonografía multiterritorial podría mejorar la predicción de eventos cardiovasculares en esta población.

INTRODUCTION

CHAPTER 1. EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

1.1. CARDIOVASCULAR DISEASE IN THE WORLD

Cardiovascular disease is the leading cause of mortality and disability globally, most often resulting from atherosclerosis. According to the World Health Organization (WHO), in 2016, nearly 18 million people died from cardiovascular disease globally[1].

Over a decade ago, the Luxembourg Declaration was adopted across all European countries to strengthen cardiovascular disease prevention plans and to ensure the implementation of effective policies to reduce the burden of cardiovascular disease[2]. Following this, interventions to prevent especially heart disease and stroke resulted in a reduction in the number of people facing disability, reduced quality of life, and death. Despite these efforts, cardiovascular diseases have remained a substantial burden in Europe. According to the 2017 European Cardiovascular Disease Statistics report, in 2015, there were over 6.1 million new cases of cardiovascular disease and almost 49 million people affected in the European Union (EU) alone, for a total cost of €210 billion per year, representing a loss of 26 million disability-adjusted life years (DALYs)[3].

According to the 2017 Spanish Institute of Statistics (Instituto Nacional de Estadística, INE) report, ischaemic heart disease and cerebrovascular disease were the principal causes of death in Spain, accounting for almost 60,000 deaths[4].

The incidence of cardiovascular disease continues to decrease in developed countries as a result of public health strategies leading to risk factor modification and therapeutic advances. However, the number of cardiovascular events remains substantial. Moreover, in low- and middle-income countries, the occurrence of cardiovascular continues to rise steeply[5].

1.2. DIABETES AND CARDIOVASCULAR DISEASE

1.2.1. THE DIABETES EPIDEMIC

Over recent decades, the prevalence of diabetes has been rising globally. In 2014, an estimated number of 422 million adults worldwide were living with diabetes[6]. The latest edition of the International Diabetes Federation (IDF) Diabetes Atlas reports that, today, 463 million people are living with diabetes, which corresponds to 9.3% of adults globally[7]. These estimations have led to the prediction that 700 million people will have developed diabetes worldwide by 2045, with around the same number of individuals developing pre-diabetes[7].

According to population-based studies (international data including 4,372,000 adults), the prevalence of global age-standardised diabetes increased from 4.3% in men and 5.0% in women in 1980 to 9.0% in men and 7.9% in women in 2008[6]. In 2019, an estimated 4.2 million deaths have been attributed to diabetes and its complications globally[7]. Diabetes has caused an annual global health expenditure of 760 billion US dollars, which represents 10% of total expenditure on adults[7].

In the European region, this year, the estimated number of adults with diabetes was 59.3 million, representing 8.9% of the population[7]. In Spain, a national population-based survey, the Di@bet.es study, estimated a prevalence of diabetes of nearly 14% in adults[8].

On the other hand, cardiovascular disease remains the principal cause of death and disability among subjects with diabetes mellitus. It is well established that individuals with diabetes have an increased risk of developing cardiovascular disease and poorer cardiovascular outcomes compared to the general population[9]. These individuals are more likely to die from a cardiovascular event than individuals without diabetes even if other cardiovascular risk factors are properly controlled[10]. In fact, it has been reported that about two-thirds of deaths in individuals with diabetes are attributable to cardiovascular disease, and of these, 40% are due to ischaemic heart disease and 10% to

stroke[11]. Furthermore, atherosclerotic cardiovascular disease typically occurs years earlier in individuals with diabetes compared to individuals without this condition[7]. The excess risk of death from atherosclerotic cardiovascular disease appears to prominently affect younger individuals with diabetes[12].

In diabetes, atherosclerosis is a complex and multi-factorial disease where high concentrations of oxidized LDL, insulin resistance with impaired insulin signalling pathway, hyperinsulinemia and sustained hyperglycaemia contribute to heightened circulating concentrations of free fatty acids (FFA), advanced glycation end-products (AGEs) and oxidative stress, all of which promote endothelial dysfunction and inflammation, i.e. the basis for atherosclerosis development and perpetuation[11].

The impact of diabetes on cardiovascular health is generating a substantial public health challenge that agencies and governments are struggling to tackle[13]. Consequently, reducing atherosclerotic cardiovascular disease burden in diabetes is a major clinical priority that should be addressed to reduce premature death, improve quality of life, and lessen the economic burden of associated disabilities, decreased work productivity, and high costs of medical care.

1.2.2. CARDIOVASCULAR RISK HETEROGENEITY IN DIABETES

In the landmark Framingham Heart Study (FHS), the authors initially demonstrated a higher incidence of cardiovascular disease across all age groups for diabetic individuals compared with those without. An even greater impact was observed among women with diabetes[14]. The increased cardiovascular risk in diabetes could not be fully explained by the associated traditional cardiovascular risk factors. Moreover, the association of diabetes with other cardiovascular risk factors reflects a synergistic effect instead of additional cardiovascular risk[11].

Subsequent data confirmed the relevance of diabetes as a cardinal cardiovascular risk factor across different populations. In a Finnish population-based study, it was

found that individuals with diabetes but without pre-existing cardiovascular disease presented a similar incidence rate of myocardial infarction (20.2% incidence over seven years) as that of individuals with established cardiovascular disease but without diabetes (18.8% incidence over seven years)[10]. Likewise, a prospective study including 3.3 million Danish adults of ≥ 30 years of age found that individuals requiring antidiabetic medications presented a cardiovascular risk comparable to that of non-diabetic adults with a prior myocardial infarction[15]. In concordance with these data, for recent decades, diabetes has been considered an equivalent condition to cardiovascular disease and guidelines have emphasised this heightened cardiovascular risk[16].

That said, some studies have reported that although individuals with diabetes show a two- to four-fold increased risk of cardiovascular risk[14], this risk is not equivalent to that of individuals who have had a cardiovascular event when addressing all the individuals with diabetes[17-19]. A large meta-analysis that included 13 studies involving over 45000 individuals found that subjects with diabetes and without previous myocardial infarction presented a 43% lower risk of experiencing a vascular event when compared to non-diabetic subjects with known cardiovascular disease[20]. A prospective Mediterranean population-based study compared long-term cardiovascular risk among 2260 type 2 diabetes subjects free from pre-existing vascular disease against 2150 first acute myocardial infarction subjects without diabetes[21]. Over a 10-year follow-up period, coronary heart disease incidence and cardiovascular mortality rates were evaluated. The investigators reported that coronary heart disease incidence as well as cardiovascular mortality rates were significantly lower in men and women with diabetes when compared to men and women with cardiovascular disease (HR 0.54 (95% CI 0.45-0.66) and 0.28 (0.21-0.37) and 0.26 (0.19-0.36) and 0.16 (0.10-0.26), respectively).

These data support the concept of heterogeneity in cardiovascular risk within the diabetic population and, therefore, underline the need for better cardiovascular risk strat-

ification to guide appropriate individual measures for cardiovascular disease prevention[22].

1.3. CARDIOVASCULAR DISEASE AND CHRONIC KIDNEY DISEASE

1.3.1. THE GLOBAL BURDEN OF CHRONIC KIDNEY DISEASE AND CARDIOVASCULAR DISEASE

According to the Global Burden of Disease Study, the prevalence of chronic kidney disease (CKD) is rising in almost every country of the world, partly due to ageing populations. In 2015, the total estimated prevalence was 323 million people, which represents a 27% increase since 2005[23,24].

Cardiovascular disease is the major cause of death among individuals with CKD and, among those with end-stage renal disease, cardiovascular disease accounts for more than half of the deaths[23,24]. People with CKD experience more often cardiovascular-related death than progression to end-stage renal disease[25]

Furthermore, individuals with CKD have an extremely elevated prevalence of cardiovascular disease[23]. CKD has been previously described as a risk factor for coronary heart disease[26] and stroke[27]. Compared to the general population, more than two-thirds of individuals over 65-year-old have cardiovascular disease whereas, in individuals without renal involvement, one-third suffer from cardiovascular disease. The reported incremental cardiovascular risk has been described across a wide range of ages, ethnicities, and gender[28].

Among CKD individuals, cardiovascular mortality risk not only has been associated with declining glomerular filtration rate but also to albuminuria. Indeed, the substantial impact of declining renal function and albuminuria on cardiovascular risk has been broadly acknowledged in the literature[29].

In a study including participants in Europe, North America, and Australasia, non-fatal major cardiovascular events were associated with £6133 higher costs for end-stage

renal disease subjects on dialysis and £4350 for other CKD subjects in the year of the event compared with subjects without the renal disease[30].

The excessive risk for cardiovascular disease in renal disease can be partially explained by the fact that CKD subjects often present a higher prevalence of traditional cardiovascular risk factors such as hypertension, diabetes, dyslipidaemia or obesity. However, there are a number of non-traditional risk factors inherently related to renal disease that contribute through different mechanisms to the observed increased rates of vascular events in this population[31]. A multi-center prospective study performed in 2445 CKD subjects, free from previous cardiovascular disease and in 559 non-diabetic participants from the NEFRONA cohort, found that factors predicting incident vascular events in patients not on dialysis were being male, CKD status, lower levels of 25-OH vitamin D, higher levels of cholesterol, higher levels of potassium and subclinical atherosclerotic burden[32].

In fact, CKD is associated with pro-atherosclerotic mechanisms such as oxidative stress, inflammation and endothelial dysfunction[33]. A prospective study enrolling over 600 participants with mild CKD found that renal impairment was independently associated with markers of endothelial dysfunction which contributed to cardiovascular mortality[34]. Along with these findings, data exist regarding the activation of the renin-angiotensin system, malnutrition/low serum albumin, nephrogenic anaemia, increased homocysteine levels, uraemia and alterations of the bone/mineral metabolism, all of which promote cardiovascular disease[35-38].

1.3.2. DIABETES, CHRONIC KIDNEY DISEASE AND CARDIOVASCULAR DISEASE

In adults, the leading cause of CKD is diabetes. CKD cases attributable to diabetes have risen by 27% in the last decade[23]. Also, CKD is estimated to affect 50% of subjects with diabetes globally, and its presence and severity influence disease prognosis[39]. Indeed, a large prospective study in over 4,000 individuals with type 1 diabetes in

Finland found independent associations between the presence and severity of kidney disease and cardiovascular mortality[40].

Moreover, in individuals with diabetes, lower glomerular filtration rates, as well as albuminuria, are independently associated with the incidence of cardiovascular events[29,41-43]. Several studies have reported an increase in the occurrence and severity of coronary heart disease as glomerular filtration rate declines[44,45].

CHAPTER 2. CARDIOVASCULAR RISK ASSESSMENT

Atherosclerosis is the dominant underlying pathology of cardiovascular disease whose major clinical manifestations, after a protracted silent phase, include myocardial infarction and heart failure, cerebrovascular disease and peripheral arterial disease. Therefore, it is fundamental to highlight that the course of atherosclerosis can be modified with an early intervention addressing risk factor exposure; hence the importance of cardiovascular risk assessment in the preclinical stage for primary prevention purposes[46].

2.1. THE FRAMINGHAM HEART STUDY

In the 1930s, as a result of changes observed in the causes of death, the scientific field of cardiovascular epidemiology appeared. In 1948, the longest prospective transgenerational cohort study in the US, the Framingham Heart Study (FHS), was launched in Massachusetts to investigate common risk factors for cardiovascular disease[47,48]. The first cohort included 5,209 healthy individuals aged between 30 and 60 years who underwent recurrent follow-up examinations and surveillance for cardiovascular and non-cardiovascular endpoints. Within a few years into the study, the researchers identified high cholesterol levels and high blood pressure as determinant risk factors for the development of cardiovascular disease[49]. Over the following years, the FHS contributed to the characterisation of classical risk factors of atherosclerotic cardiovascular disease, laying the foundations of successive risk-prediction algorithms[50].

2.2. CARDIOVASCULAR RISK PREDICTION MODELS

Following the FHS findings, and based on the evaluation of traditional cardiovascular risk factors, different multivariate risk algorithms (e.g., the Framingham Risk Score, the SCORE in Europe, and the REGICOR in Spain), have been developed to estimate global risk in asymptomatic individuals from the general population. These prediction algorithms tend to reflect the overall burden of traditional risk factors and provide a global cardiovascular risk estimate, in a quick and easy manner[51].

Current and former international and national guidelines on cardiovascular disease prevention have recommended the use of specific cardiovascular risk algorithms to calculate the risk of future vascular events to guide the most appropriate strategy for primary prevention[52,53]. The WHO has endorsed the use of appropriately validated risk score scales in low- and middle income countries[54]. As a consequence, these scales have been widely incorporated into clinical practice and have been useful in helping physicians tailor individual interventions[55-57].

To determine cardiovascular risk, different countries and national guidelines have developed different specific cardiovascular risk equations to better concile their populations' characteristics[56,58]. Depending on the geographical area, some of these recommended risk models are, for instance, the Framingham Risk Score in Canada[59], the Pooled Cohort Equation (ASCVD (atherosclerotic cardiovascular disease) Risk Estimator) in the US[52,60,61] and the SCORE in European countries[53,62].

2.2.1. The significant gap between conventional scores prediction and actual event rates

Along the years, and despite the relative validity reported on a population level by previous studies, the algorithms mentioned above have failed to fully predict cardiovascular risk at the individual level[46,47,63].

Prior substantial evidence has revealed that high-risk groups contain only a part of those individuals that will experience an incident event. It has also been reported that the majority of cardiovascular events occur among low-to-moderate-risk groups[64-66]. A prospective cohort study conducted in Girona (Spain) including over 3,800 participants, aged between 35 and 74 years and without established cardiovascular disease, examined the validity of 10-year-risk estimate using the REGICOR scale (adapted from the Framingham Risk Score)[67]. It was found that almost two-thirds of all the incident events occurred among individuals in the low- and intermediate-risk groups. More specifically, among women, nearly half of the events occurred in the study-defined low-risk group. These data reflect the significant gap between actual cardiovascular risk and the risk calculated using these conventional cardiovascular risk equations.

2.2.2. Reasons for limited accuracy of conventional score systems

Conventional cardiovascular risk scoring systems harbour significant inherent limitations. Atherosclerosis progression rate can vary greatly between individuals. It is conceivable that this could, in part, be explained by the difference in length and magnitude of an individual's exposure to risk factors, by multiple combinations of risk factors that might act synergistically, by treatment of risk factors in place, by variability in the measurement of risk factors, by genetic susceptibility or even by the presence of non-classical risk factors[55,62,68,69]. Therefore, accurate cardiovascular risk prediction still remains a challenge[70-72].

What is more, extrapolation to populations that are different from the studied cohort will have a tendency to overestimate or underestimate risk in other baseline-risk subgroups. Along these lines, changes in population characteristics over time, because of the time delay between observational studies and application of risk scores, could contribute to further misclassification of individual cardiovascular risk[62,73,74].

Besides, these algorithms rely on limited cross-sectional risk evaluation and do not take into account the age interaction-effect[61]. Younger subjects that would need car-

diovascular prevention interventions earlier on are not flagged-up, whereas, on the other side of the spectrum, older subjects are usually not even included in the algorithm[62,75]. To the same extent, women with a low short-term risk who might experience an event later on in life are often classified as low-risk and, thus, missed[57].

Conventional cardiovascular risk scales appear to show even less accurate predictions when evaluating different subpopulation types, such as individuals with diabetes, renal disease, women, younger subjects, older subjects, individuals with inflammatory diseases or HIV, or specific ethnicities, than when assessing the general population[62,76].

2.3. STRATIFICATION OF CARDIOVASCULAR RISK IN DIABETES AND CHRONIC KIDNEY DISEASE

With regards to diabetes and CKD, it is well known that these individuals present not only with an increased risk of developing cardiovascular disease, but also poorer cardiovascular outcomes compared to the general population[77]. In these individuals, global cardiovascular risk calculation systems are not accurate enough[78].

Over the last decades, several prediction models have been specifically developed in diabetes[79-82]. Also, other prediction models, originally developed in the general population, have been externally validated in diabetes populations[83,84]. However, overall, these risk scores have shown modest discrimination power, as reported in a recent systematic review[85]. With regards to CKD, an inverse relationship between classical cardiovascular risk factors and cardiovascular morbidity and mortality has been described in these subjects. This could explain, at least in part, the less than accurate discrimination ability of risk scores for predicting incident cardiovascular events in this population[86].

Historically, cardiovascular risk prediction scores did not include specific variables related to renal diseases, such as estimated glomerular filtration rate or albuminuria[66,87]. Nevertheless, in the past, various studies failed to demonstrate that adding these

CKD-specific variables translated into a significant increase in accuracy in cardiovascular risk prediction[78,87] . In contrast, a large meta-analysis including data from 637,315 individuals demonstrated that including estimated glomerular filtration rate (eGFR) and albuminuria actually improve discrimination of cardiovascular risk prediction beyond traditional risk factors[29].

In the current European guidelines for prevention and management of cardiovascular disease in diabetes, individuals with an associated decreased estimated glomerular filtration rate and/or albuminuria are considered to be at very high cardiovascular risk, and more aggressive preventive interventions are indicated[13].

2.4. CARDIOVASCULAR RISK ASSESSMENT BEYOND CONVENTIONAL RISK MODELS

For all of the above, strategies to identify with precision individuals who will benefit from appropriate preventive interventions is paramount. An ongoing search for new biomarkers and imaging techniques applicable in the clinical setting is required to enhance and individualise cardiovascular risk prediction, not only in the general population but also in more specific subgroups of the population, such as individuals with diabetes and CKD.

The key to implementing emergent strategies for cardiovascular risk assessment relies upon their inherent utility to add complementary information to refine and individualise risk assessment, as well as their applicability in routine clinical practice[57] .

2.4.1. Non-invasive imaging biomarkers

Development and progression of atherosclerosis have a direct impact on the occurrence of cardiovascular events. Therefore, the use of non-invasive imaging to assess presence and extent of subclinical disease, serving as a surrogate marker for diagnosis and monitoring, has been postulated to enhance cardiovascular risk stratification

in current guidelines[13,60]. Considering their current relevance, non-invasive imaging biomarkers will be described in depth in the next chapter.

2.4.2. Circulating biomarkers

Studied circulating biomarkers are intertwined in atherosclerotic development processes, such as inflammation, oxidative stress, and thrombosis, among others[88-90]. Previous data have demonstrated a significant link between serum concentrations of hs-CRP–and incident vascular events, which is included in the American College of Cardiology/American Heart Association (ACC/AHA) guideline, hsCRP determination for risk re-stratification[52].

However, numerous biomarkers are still in need of further clinical research to determine their validity, clinical significance, and applicability. At the present moment, the addition of circulating biomarkers for cardiovascular risk stratification has been described as having limited clinical value, and in the current European Guideline, it is not routinely recommended[13,53].

2.4.3. Novel avenues: the omics technologies

Advances in RNA-sequencing, mass spectrometry and nuclear magnetic resonance have dramatically improved coverage and quality of the study of emergent biomarkers for cardiovascular disease and risk. The analysis and integration of multiple complementary platforms, such as genomics, transcriptomics, proteomics, metabolomics and lipidomic profiling, are providing substantial data from multiple biological networks, and this vast amount of data is enabling the generation of multi-marker panels for cardiovascular risk prediction, though many of them are yet to be validated[88].

At present, current trends strive for a more global approach that unifies demographic, clinical, pathophysiological, molecular, phenotypic and genotypic information.

CHAPTER 3. SUBCLINICAL ATHEROSCLEROSIS EVALUATION BY NON-INVASIVE IMAGING: ENHANCING CARDIOVASCULAR RISK PREDICTION

As mentioned above, the natural history of atherosclerosis involves a protracted preclinical phase with the disease often detected at an advanced stage or following a cardiovascular event (fatal or non-fatal).

Subclinical atherosclerotic arterial disease has been extensively investigated as a biomarker of cardiovascular disease and has been associated with risk factors, prognosis, and efficient response to interventions.

In addition to traditional risk factors, the measurement of subclinical atherosclerosis using non-invasive imaging facilitates a more individualised cardiovascular risk stratification and provides a means for early detection of preclinical arterial disease at a time when its course can be modified[46,91].

3.1. VASCULAR ULTRASONOGRAPHY BIOMARKERS OF ATHEROSCLEROSIS

Detection and quantification of subclinical atherosclerosis by arterial ultrasonography has been demonstrated to be a valid screening tool for the prediction of future cardiovascular events[92].

Arterial ultrasonography has several advantages compared to other non-invasive techniques, including the absence of radiation and contrast medium (compared to computed tomography, CT), as well as low cost and broad availability (compared to magnetic resonance imaging)[93].

3.1.1. Carotid intima-media thickness assessment

Since its development and validation in the 1980s[94], researchers have focused on carotid intima-media thickness (cIMT) as a surrogate marker for atherosclerosis. cIMT is measured by transcutaneous ultrasound in B-mode as the distance from the media-adventitial interface to the intima-lumen interface of the extracranial carotid tree[63,95].

Intima-media thickness as an indicator of cardiovascular risk

In recent decades, large-scale prospective population-based studies, such as the Rotterdam Study, the Cardiovascular Health Study, the CAPS (Carotid Atherosclerosis Progression Study) or the ARIC (Atherosclerosis Risk in Communities) study have reported that higher cIMT translated into increased risk for coronary heart disease and stroke in the general population[96-100] and across all ages[101]. A systematic review involving over 37,000 individuals gave support to these findings[102]. The authors reported that cIMT was a strong cardiovascular disease predictor and that a cIMT difference of 0.1 mm, corrected for age and gender, was associated with over a 10% increase in the incidence of both myocardial infarction and stroke risk[102].

As a consequence, cIMT has been widely accepted as a useful indicator of the individual vascular status[103,104,]. The 2010 ACC/AHA guidelines outlined that measuring the cIMT was reasonable for refining cardiovascular risk among asymptomatic subjects in the intermediate-risk group[16].

Carotid intima-media thickness utility beyond traditional risk factors

In contrast, further studies found conflicting results regarding cIMT for enhanced reclassification of cardiovascular risk prediction. The Framingham Offspring Study reported no significant improvement in risk classification when adding common carotid IMT values to the Framingham Risk Score (FRS) equation[105]. Bots *et al*. also described a limited utility of common cIMT for cardiovascular events prediction once added to the FRS among individuals with elevated blood pressure[106]. The Tromsø study found that, when excluding the bulb, cIMT did not predict a first myocardial infarction in either sex in the general population[107]. Moreover, a large meta-analysis involving data from 45,828 individuals described only a marginal improvement in cardiovascular risk prediction beyond conventional risk scales when incorporating cIMT values[108]. Other studies have also questioned the clinical relevance of measuring cIMT to re-stratify risk[109-111] since cIMT would respond to a distinct phenotype from athero-

matous plaque[112,113]. As a result of these data, in the 2013 ACC/AHA guidelines for cardiovascular disease prevention, measuring cIMT was no longer recommended to reclassify cardiovascular risk[52].

Carotid intima-media thickness progression and cardiovascular events

When considering the value of cIMT progression over time and cardiovascular events prediction, clinical studies have shown contradictory results. In the MESA (Multi-Ethnic Study of Atherosclerosis) trial, cIMT progression was found to be significantly associated with incident cerebrovascular events[114]. However, a subsequent meta-analysis, including 36,984 participants from the general population with a mean follow-up of seven years, could not demonstrate an association between cIMT progression and cardiovascular risk[115] . Furthermore, a recent systemic review involving 31 cohorts examined the correlation between cIMT rate of change and vascular events among individuals at high-risk, finding no relationship between changes in cIMT and future events[116].

3.1.2. Plaque evaluation

The plaque has been defined as a focal wall thickening at least 50% greater than the surrounding arterial wall or as a focal region with an IMT measurement ≥ 1.5 mm that protrudes into the lumen[117]. However, in the literature, we can find different definitions of plaque that have been published along the years[118].

Plaque presence

More recent data have demonstrated that, despite a degree of heterogeneity in plaque definition and evaluation in previous literature, ultrasound assessment of carotid plaque and its total volume or total area show a significant accuracy for cardiovascular event prediction[119-122].

Carotid plaque appears to be a stronger cardiovascular risk predictor when compared with cIMT alone[123,124]. A meta-analysis of 12 population-based studies involving 54,336

subjects confirmed the correlation between the presence of carotid plaque and cardiovascular events[125]. These authors reported that carotid plaque detected by ultrasonography presented a higher diagnostic accuracy for cardiovascular event prediction than cIMT.

Among asymptomatic individuals with diabetes, detection of carotid plaques has also shown an incremental value over cIMT measurement to inform cardiovascular risk[126,127]. On the other hand, plaque formation in end-stage renal disease subjects has also been found to be an independent predictor of cardiovascular events[128]. Although data on subjects within the NEFRONA cohort in earlier stages of CKD is more limited, our group has demonstrated that presence and extent of subclinical atherosclerosis is a predictor of incident events across all stages of renal disease[32]

Plaque burden

In addition to plaque presence, evidence supports that quantification of an individual atherosclerotic burden is a potent predictor for vascular events[119,123,129,130].

Describing plaque presence or burden does not include details on specific plaque characteristics that might affect progression to adverse vascular events[129]. Previous studies have analysed vulnerable plaques in relation to prediction of cardiovascular events using invasive and non-invasive imaging modalities yielding discordant results[131-133]. Having said that, a recent study assessed atherosclerotic burden as total plaque area, as well as carotid plaque features by B-mode ultrasound among 2,205 middle-aged participants without prior cardiovascular disease from the MESA cohort[129]. The authors observed that plaque characteristics did not predict cardiovascular disease, whereas the total plaque area did. The authors concluded that three-dimensional ultrasonography and magnetic resonance imaging (MRI) could have characterised better plaque features in this specific low-risk population. In any case, it would appear that analysing vulnerable plaques for cardiovascular risk refinement is less effective than assessing plaque burden.

The 2016 European Society of Cardiology (ESC) guideline for cardiovascular disease prevention indicated that cIMT measurement should not be considered for cardiovascular risk re-stratification, whereas plaque detection might be considered as a risk modifier[53]. Along with these recommendations, the 2019 European guideline on diabetes and cardiovascular disease outlined that carotid and/or femoral plaque burden assessment with ultrasound examination should be considered as a risk modifier in asymptomatic individuals with diabetes[13].

3.1.3. Multi-territorial vascular examination

Atherosclerosis is a systemic process. Relevant population-based studies have demonstrated the value of assessing subclinical atherosclerosis not only at the carotid level[134] but also in other additional vascular territories to further enhance cardiovascular risk prediction[135-137].

The CAFES-CAVE (Carotid-Femoral morphology screening and cardiovascular events) study[135], a 10-year follow-up prospective study that included over 10,000 asymptomatic individuals performed, in addition to carotid plaque ultrasound screening, a femoral examination improving the overall cardiovascular risk prediction. Like in the CAFES-CAVE study, Lamina *et al.* also explored, in a random subsample of 1,325 participants from the population-based MONICA Augsburg Survey with a 13 year-period follow-up, the femoral and carotid territories in relation to myocardial infarction and cardiovascular and total mortality. The authors found that the number of plaque-affected arteries in the different vascular beds was a potent predictor for all three outcomes[136].

The PESA (Progression and Early detection of Subclinical Atherosclerosis) study[92] was designed to better understand the development and progression of atherosclerosis in a middle-aged cohort of more than 4,000 healthy individuals by evaluating multiple vascular beds, including carotid and femoral arteries and abdominal aorta using ultrasonography and the coronary arteries performing coronary artery calcification (CAC) score. Interestingly, in this initially low-risk cohort, the authors found a signifi-

cant prevalence of plaque, predominantly within the femoral territory. Also, the AWHS (Aragon Workers Health Study), a longitudinal cohort study of 1,423 healthy Spanish middle-aged men, highlighted the role of screening for subclinical atherosclerosis in femoral arteries in addition to the carotid arteries since the association with risk factors appeared to be stronger within the femoral territory[137].

All these data support the relevance of evaluating distinct vascular beds for a more accurate subclinical atherosclerosis screening.

3.1.4. Novel approaches

It should also be noted that novel sonographic approaches could improve vascular assessment even further and, thus, early prediction of vascular events. Three-dimensional ultrasound using specially-developed software accurately quantifies plaque volume, as described in the BioImage Study and subsequently in the PESA Study[130,138].

On the other hand, contrast-enhanced ultrasound (CEUS) permits more precise measurement of the near cIMT wall, as well as visualisation of early neovascularisation, which has been associated with premature atherosclerosis[139-141].

Although these imaging modalities that have been utilised for years in the investigational field and are yet to be incorporated into clinical practice due to their complexity and costs.

3.2. Coronary artery calcium score

Detection and quantification of coronary artery calcium (CAC) deposition have been, along with arterial sonographic biomarkers, among the more extensively studied imaging techniques for the assessment of atherosclerosis. Most commonly calculated and interpreted through the Agatston score[142], CAC score with computed tomography has enabled non-invasive evaluation of coronary anatomy, determination of presence and extent of coronary atherosclerosis, and an improvement in risk prediction of adverse

cardiovascular events through an easy, quick, and reproducible modality[143,144]. Also, cardiovascular calcification outside the coronary territory (known as extra-coronary calcification) has also been found to improve the individualized risk for coronary heart disease, cardiovascular mortality, and all-cause mortality when added to traditional risk factors[145].

3.2.1. Coronary artery calcium score versus sonographic markers

CAC presence and higher CAC score, as well as plaque presence and higher plaque burden, have been associated with increased cardiovascular risk[118,121,125]. Several cohort studies have shown that CAC presence and CAC score appear to be better at predicting coronary heart disease than vascular sonographic markers in some populations, but not for stroke events[146].

In the MESA study, investigators have reported CAC superiority over IMT for vascular events prediction[147]. A few years later, the MESA investigators that followed up 6,814 individuals without cardiovascular disease for 9.5 years found that, compared to traditional risk factors, CAC and carotid plaque presence significantly improved cardiovascular risk and coronary heart disease prediction, but this was not the case for IMT. Also, only the carotid plaque presence significantly improved stroke prediction[148]. In keeping with these results, another large cohort study found that cardiovascular risk prediction significantly improved with both CAC score and carotid plaque burden when compared against traditional risk factors[119].

Both IMT and CAC have also been compared among 559 participants enrolled in an ageing population-based study, the Cardiovascular Health Study,[149]. After 5 years, IMT at the common carotid artery was found to be more strongly related to cerebrovascular events than CAC. With regards to cardiovascular disease and coronary heart disease, both markers presented similar hazard ratios.

3.2.2. Coronary artery calcium score as a negative marker for cardiovascular risk

On the other hand, it has been demonstrated that low CAC scoring is a strong negative marker for cardiovascular risk and, hence, a valid tool to identify those subjects at very low risk of experiencing future events and to guide clinical decisions[60,150]. A recent study from the BioImage cohort[151] that compared 13 different candidate markers for cardiovascular disease reported that a CAC of zero, as well as CAC ≤10, were the most potent negative risk markers for coronary heart disease.

3.3. MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) enables —without the need for ionising radiation— detection and quantification of subclinical atherosclerosis. MRI provides three-dimensional details on plaque structure, size, and composition, including lipid-rich core, intraplaque haemorrhage and fibrous cap, and also offers accurate information on the volume of vessel disease[152,153]. Evaluation of coronary arteries by MRI remains difficult due to size, tortuosity, and myocardial motion. MRI has most often been utilised in clinical trials as a tool to monitor response to implemented therapies and in prospective longitudinal studies to examine plaque progression and regression[152] .

Having said that, MRI is a complex imaging modality that requires expert skills and has a limited application at the population level for the time being[154].

3.4. POSITRON EMISSION TOMOGRAPHY

Positron emission tomography (PET) utilising the glucose analogue 18F-Fluorodeoxyglucose (18F-FDG) is an emerging imaging modality that provides a non-invasive estimation of inflammatory activity within the arterial wall[155,156]. 18F-FDG is taken up by macrophages, fundamental mediators of atherosclerosis and, as a result, 18F-FDG-PET imaging can detect and localise inflammatory activity and quantify atherosclerotic activity in the arterial wall[157]. Furthermore, fluorodeoxyglucose (FDG) in conjunction

with other imaging techniques, such as computed tomography and MRI, provide incremental benefit[155,156]. Previous studies have shown evidence of a direct relationship between increased arterial FDG uptake and vascular events, mostly in oncology patients[158,159]. It has also been demonstrated that arterial FDG uptake can improve cardiovascular prediction beyond FRS[159].

To sum up, arterial ultrasonography for subclinical atherosclerosis assessment remains a valid tool to evaluate the extent and severity of the disease in distinct vascular regions and among different subsets of populations since it is non-invasive, easy to perform, reproducible, and harmless. Consequently, it could help refine cardiovascular risk in the clinical practice, most notably, among specific groups of the population, such as individuals with diabetes and CKD. Moreover, this imaging modality could also be utilised to monitor the progression or regression of subclinical atherosclerosis assessing the impact of therapeutic interventions on the progression of arterial disease[99].

JUSTIFICATION STATEMENT

As has been described, the impact of cardiovascular disease on mortality and disability in people with diabetes and CKD is devastating. Both conditions experience heightened cardiovascular risks when compared to the general population in terms not only related to an increased prevalence of traditional cardiovascular risk factors but also due to specific alterations inherent to each condition. Furthermore, when they occur together in a given individual, they appear to act synergistically. Nonetheless, we should also bear in mind the existing heterogeneity in terms of cardiovascular risk in these individuals.

For these reasons, accurate risk classification is the foundation to assist more intensified strategies of primary prevention when required. Vascular ultrasonography has emerged as a valid and feasible tool, applicable in the clinical practice and to a broad range of subjects, to assess individualised cardiovascular risk via evaluation of subclinical atherosclerosis in multiple vascular regions and, hence, to respond to this imperious clinical need.

To date, scarce data exist on individuals with both conditions, diabetes and CKD, evaluating their vascular status along with the occurrence of cardiovascular events. There is limited evidence describing the specific prevalence, distribution, and progression of multisite preclinical atherosclerosis and exploring its link to adverse cardiovascular events beyond associated cardiovascular risk factors in this high-risk group of subjects.

The National Observatory of Atherosclerosis in Nephrology (NEFRONA) study is a large, multicentre, prospective cohort study including a large sample of subjects with CKD and without pre-established cardiovascular disease. The NEFRONA cohort represents a unique opportunity to thoroughly evaluate the natural history of the atherosclerotic disease at multiple arterial territories in a specific high-risk subset of the Spanish population.

HYPOTHESIS

The starting hypothesis that we set was that individuals with diabetes and CKD without previous cardiovascular disease would present higher plaque frequency, plaque burden, and preclinical atherosclerotic disease progression compared to renal individuals without diabetes and that the additional atherosclerotic plaque burden that would involve having both conditions would be a marker of heightened risk of cardiovascular disease, thereby contributing to the prediction of incident cardiovascular events in this subset of subjects.

OBJECTIVES

MAIN OBJECTIVE

To examine the prognostic value of multi-territorial vascular ultrasound evaluation in a large cohort of Spanish individuals (the NEFRONA cohort) free from known clinical cardiovascular disease with CKD and with and without diabetes

SECONDARY OBJECTIVES

1. To investigate the impact of diabetes on subclinical atherosclerotic vascular disease in a cohort of individuals with CKD and without known clinical cardiovascular disease.

1.1. To analyse the prevalence and distribution of subclinical atherosclerotic lesions in individuals with CKD and diabetes versus individuals with CKD and without diabetes.

1.2. To analyse atherosclerotic plaque progression in individuals with CKD and diabetes versus individuals with CKD and without diabetes.

1.3. To analyse and compare factors associated with presence and progression of atherosclerotic lesions in both study groups.

2. To investigate the impact of diabetes on cardiovascular events in a cohort of individuals with CKD and without previous vascular events.

2.1. To analyse the incidence of cardiovascular events in individuals with CKD and diabetes versus individuals with CKD and without diabetes.

2.2. To analyse and compare factors associated with events in both study groups.

2.3. To examine the impact of subclinical atherosclerosis and diabetes for the prediction of incident cardiovascular events in individuals with CKD

PUBLISHED PAPERS

FIRST PAPER

TITLE

PREVALENCE AND PROGRESSION OF SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND DIABETES

AUTHORS

Palanca A, Castelblanco E, Perpiñán H, Betriu À, Soldevila B, Valdivielso JM, Bermúdez M, Duran X, Fernández E, Puig-Domingo M, Groop PH, Alonso N, Mauricio D.

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SECOND PAPER

TITLE

SUBCLINICAL ATHEROSCLEROSIS BURDEN PREDICTS CARDIOVASCULAR EVENTS IN INDIVIDUALS WITH DIABETES AND CHRONIC KIDNEY DISEASE

AUTHORS

Palanca A, Castelblanco E, Betriu À, Perpiñán H, Soldevila B, Valdivielso JM, Bermúdez-Lopez M, Puig-Jové C, Puig-Domingo M, Groop PH, Fernández E, Alonso N, Mauricio D.

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Cardiovascular Diabetology

ORIGINAL INVESTIGATION

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Subclinical atherosclerosis burden predicts cardiovascular events in individuals with diabetes and chronic kidney disease

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Abstract

Background: Individuals with diabetes have remarkably high rates of cardiovascular morbidity and mortality. However, the incremental cardiovascular risk in diabetes is heterogeneous and has often been related to renal involvement. The purpose of this study was to analyse the prognostic value of subclinical atherosclerosis in determining the incidence of first cardiovascular events (CVEs) in individuals with diabetes and chronic kidney disease (CKD) compared to CKD individuals without diabetes.

Methods: We included data from individuals with CKD with and without diabetes, free from pre-existing cardiovascular disease, from the NEFRONA cohort. Participants underwent baseline carotid and femoral ultrasound and were followed up for 4 years. All CVEs during follow-up were registered. Bivariate analysis and Fine–Gray competing risk models were used to perform the statistical analysis.

Results: During the mean follow-up time of 48 months, a total of 203 CVE was registered. 107 CVE occurred among participants without diabetes (19.58 per 1000 person-years) and 96 CVE occurred among participants with diabetes (44.44 per 1000 person-years). Following the competing risk analysis, the variables predicting CVEs in CKD individuals without diabetes were the number of territories with plaque at baseline (HR 1.862, 95% CI [1.432;2.240]), age (HR 1.026, 95% CI [1.003;1.049]) and serum concentrations of 25-OH vitamin D (HR 0.963, 95% CI [0.933;0.094]). The only variable predicting CVEs among CKD participants with diabetes was the number of territories with plaque at baseline (HR 1.782, 95% CI [1.393, 2.278]). For both models, concordance (C) index yielded was over 0.7.

Conclusions: The burden of subclinical atherosclerosis is the strongest predictor of future CVEs in diabetic individuals with CKD. Early detection of subclinical atherosclerotic burden by multiterritorial vascular ultrasound could improve CVE prediction in this population.

Keywords: Chronic kidney disease, Diabetes, Multiterritorial arterial ultrasound, Subclinical atherosclerosis, Cardiovascular events

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Background

It is well established that individuals with diabetes have an increased risk of developing cardiovascular disease (CVD) as well as poorer cardiovascular outcomes compared to the general population [1]. For the last two decades, diabetes has been considered an equivalent condition to CVD [2], and this heightened cardiovascular risk has been emphasized in former clinical guidelines [3, 4]. That said, some studies have reported that although individuals with diabetes show a two to fourfold increased risk of CVD, this risk is not equivalent to that of individuals who have had a cardiovascular event (CVE) [5–8]. These data support the concept of heterogeneity in cardiovascular risk within the diabetic population and therefore underline the need for cardiovascular risk stratification [9, 10].

On the other hand, quantification of the burden of atherosclerotic plaque assessed by noninvasive ultrasonography is a strong predictor for CVEs and, hence, is considered a valid tool for cardiovascular risk stratification [11, 12]. In addition, several studies support the value of measuring subclinical atherosclerosis (SA) in multiple arterial territories for an improved CVE predictive value and, thus, a more accurate cardiovascular risk stratification [13, 14]. In addition, the substantial impact of declining renal function and albuminuria on cardiovascular risk has been broadly acknowledged in the literature [15]. In fact, it has been reported that in individuals with diabetes, the presence of chronic kidney disease (CKD) is associated with an increased risk of CVD, explaining in part their heightened burden of CVD [16].

To date, there is limited evidence describing the specific prevalence of SA in individuals with both diabetes and CKD. Recently, our group reported that SA is more prevalent, carries a higher plaque burden and is more rapidly progressive in individuals with CKD and diabetes [17]. In addition, our group also reported that renal individuals show a higher prevalence of plaques [18, 19] and a higher incidence of future CVEs across all stages of CKD compared with controls [20].

Therefore, we examined the prognostic value of multiterritorial vascular evaluation and the extent of SA in predicting the incidence of new CVEs in individuals with renal disease and diabetes, using data from the National Observatory of Atherosclerosis in Nephrology (NEFRONA) study [21]. We hypothesized that the additional atherosclerotic plaque burden that involves having both conditions would be a marker of an increased risk of CVEs and contribute to the prediction of the incidence of CVEs among CKD individuals with diabetes in our cohort.

Thus, the aim of our study was to analyse the incidence of CVEs and its association with baseline subclinical atherosclerotic status in CKD individuals with and without diabetes.

Methods

Design and study population

The present study included 1747 individuals with CKD without diabetes and 698 individuals with CKD with diabetes from the NEFRONA cohort. The design, objective and methods of the NEFRONA study have been published in detail previously [21]. Briefly, the NEFRONA study is a multicentre, prospective observational study. We aimed to evaluate the prevalence and evolution of subclinical atheromatosis in CKD individuals, as well as the contribution of vascular imaging for a more precise cardiovascular risk assessment.

Between October 2010 and June 2012, 2445 CKD individuals with and without diabetes who were free from previous cardiovascular disease and were 18 to 75 years of age were recruited from 81 Spanish hospitals and dialysis clinics [19]. Participants with known CVD or who had undergone any carotid artery intervention were excluded. Other exclusion criteria were pregnancy, life expectancy of less than 12 months, any active infection or previous organ transplantation. Participants underwent baseline carotid and femoral ultrasound examinations. During a 4-year follow-up period, all cardiovascular events, noncardiovascular deaths and kidney transplantations were registered.

The Ethical Committees of all involved Spanish nephrology centres approved this study with the final approval by the Ethics committee board of the Hospital Universitario Arnau de Vilanova (Lleida, Spain). The investigation has been conducted according to the principles expressed in the Declaration of Helsinki, and all the included participants provided written informed consents.

Clinical data and laboratory examinations

Information on the participant's medical history, cardiovascular risk factors and drug use was collected at baseline. Dyslipidaemia was therefore defined as a recorded clinical diagnosis or current use of lipid-lowering medication. A detailed history was taken. A physical examination including standard vital tests and anthropometric measures, such as height, weight and waist–hip ratio, was performed [21]. A routine fasting blood test was carried out within 3 months of the vascular ultrasound examination, and biochemical parameters were obtained. The estimated glomerular filtration rate was determined using the Modification of Diet in Renal Disease Study formula (MDRD-4). High-sensitive C reactive protein (hsCRP) plasma concentrations were measured with an immunoturbidimetric method (Roche/Hitachi modular analytics). Serum concentrations of 25-OH vitamin

D were determined by an ELISA (IDS, UK) as described previously [20].

Diagnosis of diabetes mellitus

The criteria used to make the diagnosis of diabetes and, thus, to include a individual in the diabetes group have been reported previously [17]. The criteria used were as follows: a previous diagnosis of diabetes recorded in the individual's medical history, a fasting plasma glucose ≥ 126 mg/dl or HbA1c ≥ 6.5% determined by laboratory testing or a current prescription of any anti-diabetic drug.

Carotid and femoral imaging

B-mode ultrasound and colour Doppler examinations of carotid and femoral sites were performed using the Vivid BT09 apparatus (GE Healthcare, Waukesha, WI) equipped with a 6–13 MHz broadband linear array probe previously explained [21]. The presence of atheromatous plaque was assessed in ten vascular territories: internal, bulb and common carotid, and common and superficial femoral arteries. Atheromatous plaque was defined as intima media thickness (cIMT) > 1.5 mm protruding into the lumen, according to the ASE Consensus Statement [22] and the Mannheim cIMT Consensus [23]. The ultrasound examination was performed in a blinded fashion by three itinerant teams belonging to the UDETMA (Unit for Detection and Treatment of Atherothrombotic Diseases, Hospital Universitari Arnau de Vilanova, Lleida, Spain) using semi-automatic EchoPAC Dimension software (GE Healthcare) according to a standardized protocol. To evaluate intraobserver plaque assessment reliability, a sample of 20 randomly chosen subjects was assessed 3 to 5 times on different days, obtaining a kappa coefficient of 1 and, therefore, indicating excellent intraobserver reliability [20].

Follow-up period and cardiovascular events

Participants were followed-up for 4 years. During the follow-up period, data on fatal and nonfatal cardiovascular events were recorded by the referring physician as reported previously [20]. The CVE were defined according to the International Classification of Diseases, Ninth Revision, Clinical Modification [ICD9-CM], which includes unstable angina, myocardial infarction, transient ischaemic attack, cerebrovascular accident, congestive heart failure, arrhythmia, peripheral arterial disease or amputation due to peripheral arterial disease, and aortic aneurism [21]. Cardiovascular mortality causes included myocardial infarction, arrhythmia, congestive heart failure, stroke, abdominal aortic aneurism, mesenteric infarction, and sudden death. CVEs and death were accurately recorded. Noncardiovascular death and kidney transplants were also recorded during follow-up.

Statistical analysis

Data for quantitative variables are presented as a median and interquartile range, and qualitative variables are presented as a number and percentage. Participant characteristics were compared between nondiabetic and diabetic participants using the Mann–Whitney U test and Pearson's Chi-square test for non-normal quantitative and categorical variables, respectively.

The association between potential risk factors and CVEs was investigated using bivariate analyses (Cox proportional hazards model). Significant variables in bivariate analyses and potential confounding factors were used to develop appropriate multiple models for estimating CVE risk under competitive risk conditions.

The Fine–Gray competing risk regression model was used to estimate the contribution of baseline patient characteristics to the cumulative incidence of CVEs. Noncardiovascular death and kidney transplantation were both considered competing events. Different models were fitted for CKD participants without diabetes as well as for CKD participants with diabetes. The model in each case was selected using the Bayesian information criteria [24]. A concordance index (C-index) was used to measure the discriminative power of the Fine–Gray models at 24 and 48 months. A statistical significance level of 0.05 was used. The statistical analysis was carried out with R statistical software version 3.3.1.

Results

Baseline patient characteristics and study endpoints

The participants included 1747 CKD individuals without diabetes and 698 CKD individuals with diabetes. The baseline characteristics of the participants are described in Table 1. The median follow-up time was 48 months in both groups.

During the mean follow-up time of 48 months, a total of 203 CVE was registered, of which 107 occurred among participants without diabetes and 96 among participants with diabetes. Corresponding CVE rates were 19.58 versus 44.44 per 1000 person-years, respectively (Additional file 1: Figure S1). We also observed that among diabetes participants, CVE rates increased with declining renal function: CVE rates were 30.90 per 1000 person-years in CKD-3 versus 41.14 per 1000 person-years in CKD-4/5 and 100.67 per 1000 person-years in individuals on renal replacement therapy (RRT) ($p\text{-}trend < 0.001$). This tendency was milder among renal participants without diabetes: CVE rates were 15.04 per 1000 person-years in CKD-3 versus 18.19 per 1000 person-years in

Table 1 Population baseline characteristics

	CKD without diabetes N = 1747	CKD with diabetes N = 698	*p*-value
Gender, male	1047 (59.9%)	461 (66.0%)	0.006
CKD stage			< 0.001
CKD-3	662 (37.9%)	288 (41.3%)	
CKD-4/5	551 (31.5%)	256 (36.7%)	
RTT	534 (30.6%)	154 (22.1%)	
Age [years]	59.0 [48.0;67.0]	65.0 [56.0;70.0]	< 0.001
Current smoker	967 (55.4%)	404 (57.9%)	0.275
Hypertension	1554 (89.0%)	673 (96.4%)	< 0.001
25-OH vitamin D [ng/ml]	15.4 [11.4;19.6]	13.8 [10.4;18.5]	< 0.001
eGFR [ml/min per 1.73 m^2]	31.6 [20.9;44.3]	31.7 [21.5;44.2]	0.812
Glucose [mg/dl]	92.0 [85.0;101]	133 [108;164]	< 0.001
Total cholesterol [mg/dl]	177 [153;205]	171 [143;197]	< 0.001
HDL cholesterol [mg/dl]	48.0 [39.0;59.0]	44.0 [36.0;53.9]	< 0.001
LDL cholesterol [mg/dl]	103 [82.0;123]	92.0 [71.4;113]	< 0.001
non-HDL cholesterol [mg/dl]	128 [105;153]	122 [100;148]	0.011
Triglycerides [mg/dl]	118 [89.0;162]	147 [103;205]	< 0.001
hsCRP [mg/dl]	1.86 [0.90;4.17]	2.58 [1.16;5.84]	< 0.001
Antidiabetic treatment			< 0.001
Diet	–	166 (23.8%)	
Oral hypoglycemic drugs	–	164 (23.5%)	
Insulin treatment	–	368 (52.7%)	
HbA1c [g/dl]	5.40 [5.10;5.70]	6.80 [6.10;7.80]	< 0.001
Albumin/creatinine ratio mg/g	89.1 [11.2;384]	149 [18.6;601]	0.001
Pulse pressure [mmHg]	56.0 [47.0;69.0]	68.0 [54.0;81.0]	< 0.001
Antihypertensive treatment	1506 (86.2%)	650 (93.1%)	< 0.001
Lipid-lowering drugs	975 (55.8%)	465 (66.6%)	< 0.001
Number of plaques	1.00 [0.00;3.00]	3.00 [1.00;5.00]	< 0.001
Presence of any plaque	1138 (65.1%)	570 (81.7%)	< 0.001
Presence of carotid plaques	901 (51.6%)	502 (71.9%)	< 0.001
Carotid plaque [only]	291 (16.7%)	165 (23.6%)	< 0.001
Presence of femoral plaques	847 (48.5%)	405 (58.0%)	< 0.001
Femoral plaque [only]	237 (13.6%)	68 (9.74%)	0.012
> 2 territories with plaque	853 (48.8%)	465 (66.6%)	< 0.001
Carotid and femoral plaques	610 (34.9%)	337 (48.3%)	< 0.001
Follow-up time [months]	48.4 [27.1;52.0]	48.3 [25.7;52.0]	0.675

CKD chronic kidney disease, *RRT* renal replacement therapy, *eGFR* estimated glomerular filtration rate determined by the Modification of Diet in Renal Disease Study formula (MDRD-4), *HDL* high density lipoprotein, *LDL* low density lipoprotein, *hsCRP* high sensitivity C-reactive protein

CKD-4/5 versus 31.72 per 1000 person-years for RRT (*p-trend* < 0.001). (Additional file 1: Figure S1).

Among women without diabetes, the CVE rate during follow-up was 16.04 per 1000 person-years and in men without diabetes, it was 22.05 per 1000 person-years. Among women with diabetes, the CVE rate was 32.17 per 1000 person-years, whereas among men with diabetes, it was 50.92 per 1000 person-years. Women with diabetes presented with more CVEs than men without diabetes (32.17 versus 22.05 per 1000 person-years, respectively; $p = 0.0019$), and this was observed consistently across all stages of CKD. In line with this result, stage 3 CKD women with diabetes experienced a slightly higher rate of incident CVEs compared to men with diabetes and stage 3 CKD (31.52 versus 30.67 per 1000 person-years, respectively) (Additional file 1: Figure S1).

Factors associated with CVE

Among CKD participants without diabetes, the bivariate analysis showed that age (HR 1.04, 95% CI [1.02;1.06]),

RRT (HR 2.23, 95% CI [1.40;3.55]), current smoker (HR 1.60, 95% CI [1.08;2.39]), serum concentration of HDL-cholesterol (0.98, 95% CI [0.96;0.99]), serum concentration of hsCRP (HR 1.01, 95% CI [1.00;1.03]) and pulse pressure values (1.02, 95% CI [1.01;1.03]) were associated with the occurrence of CVEs during the follow-up period (Additional file 1: Table S1). Among CKD participants with diabetes, factors associated with a CVE were RRT (HR 3.58, 95% CI [2.16;5.93]) and insulin treatment (HR 1.87, 95% CI [1.06;3.30]). Additionally, in this group of CKD participants with diabetes, being a female was associated with fewer CVEs (0.63, 95% CI [0.40;1.00]) as well as having better renal function (HR 0.98, 95% CI [0.96;1.00]) (Additional file 1: Table S1). In both groups, it was found that serum concentrations of 25-OH vitamin D were inversely associated with the incidence of CVE, participants without diabetes HR 0.96, 95% CI [0.93;0.99], and participants with diabetes HR 0.95, 95% CI [0.92;0.98] (Additional file 1: Table S1).

In both the nondiabetic and diabetic study groups, the bivariate analysis showed that the presence of plaque (HR 5.83, 95% CI [2.95;11.54] and HR 3.39, 95% CI [1.48;7.75], respectively) and the number of territories with basal plaque (HR 1.26, 95% CI [1.18;1.35] and HR 1.18, 95% CI [1.09;1.27], respectively) as well as having more than two vascular territories affected with plaque at baseline (HR 3.26, 95% CI [2.09;5.07] and HR 3.35, 95% CI [1.87;6.01], respectively) were associated with suffering from a CVE during follow-up (Table 2). To the same extent, the presence of atherosclerotic plaques in both vascular sites, carotid and femoral, was also found to be positively associated with incident CVEs in both groups (HR 2.57, 95% CI [1.75;3.78] and HR 2.09, 95% CI [1.37;3.17], respectively) (Table 2).

Following the competing risk model analysis, the variables predicting CVE in CKD participants without diabetes were the number of territories with plaque at baseline (HR 1.862, 95% CI [1.432;2.240]), age (HR 1.026, 95% CI [1.003;1.049]) and serum concentrations of 25-OH vitamin D (HR 0.963, 95% CI [0.933;0.094]) (Table 3). On the other hand, among CKD participants with diabetes, the only variable that predicted CVEs was the number

Table 2 Bivariate unadjusted analysis of the plaque at baseline according to the incidence of cardiovascular events

	CKD without diabetes				CKD with diabetes			
	No CVE N = 1640	CVE N = 107	HR [95% CI]	*p* value*	No CVE N = 602	CVE N = 96	HR [95% CI]	*p* value*
Territories with plaque	1.0 [0.0;3.0]	3.0 [2.0;5.0]	1.26 [1.18;1.35]	< 0.001	2.0 [1.0;5.0]	4.0 [2.0;6.0]	1.18 [1.09;1.27]	< 0.001
Presence of plaque	1040 (63.4%)	98 (91.6%)	5.83 [2.95;11.54]	< 0.001	480 (79.7%)	90 (93.8%)	3.39 [1.48;7.75]	0.004
Presence of carotid plaque	815 (49.7%)	86 (80.4%)	3.80 [2.36;6.12]	< 0.001	418 (69.4%)	84 (87.5%)	2.86 [1.56;5.24]	0.001
Carotid plaque [only]	267 (16.3%)	24 (22.4%)	1.41 [0.90;2.22]	0.137	143 (23.8%)	22 (22.9%)	0.92 [0.57;1.49]	0.748
Presence of femoral plaque	773 (47.1%)	74 (69.2%)	2.42 [1.60;3.64]	< 0.001	337 (56.0%)	68 (70.8%)	1.81 [1.17;2.82]	0.008
Femoral plaque [only]	225 (13.7%)	12 (11.2%)	0.83 [0.45;1.51]	0.538	62 (10.3%)	6 (6.25%)	0.56 [0.25;1.29]	0.173
> 2 territories with plaque	772 (47.1%)	81 (75.7%)	3.26 [2.09;5.07]	< 0.001	382 (63.5%)	83 (86.5%)	3.35 [1.87;6.01]	< 0.001
Carotid and femoral plaque	548 (33.4%)	62 (57.9%)	2.57 [1.75;3.78]	< 0.001	275 (45.7%)	62 (64.6%)	2.09 [1.37;3.17]	0.001

CVE fatal and non-fatal cardiovascular event

**p* values correspond to HR. Chi-squared test for trend in proportions p < 0.005

Table 3 Fine and Gray multiple regression to model incidence of cardiovascular events

	CKD without diabetes		CKD with diabetes	
	HR [95% CI]	*p*-value	HR [95% CI]	*p*-value
Age	1.026 [1.003;1.049]	0.024	–	–
Baseline PL [sq]	1.862 [1.432;2.420]	< 0.001	1.782 [1.393;2.278]	< 0.001
25-OH vitamin D	0.963 [0.933;0.994]	< 0.001	–	–
C-index (24 months)	78.88		71.82	
C-index (48 months)	75.63		71.49	

The variables introduced to build the model were: gender, age in years, smoking, CKD stage, HDL cholesterol, 25-OH vitamin D and the square root of number of territories with plaque(s) at baseline, and glycated haemoglobin, oral treatment, and insulin treatment only for DM patients

CKD chronic kidney disease, *Baseline PL [sq]* the square root of number of territories with plaque

of territories with plaque at baseline (HR 1.782, 95% CI [1.393;2.278]) (Table 3). Estimated C-index values over 0.7 were found for the selected model in each group (Table 3).

Discussion

In the present study, we found that in CKD participants with diabetes and without known CVD, an increased burden of basal atherosclerotic plaque translates into an increased risk of incident CVEs. We also demonstrated that the number of vascular territories affected with plaque is the strongest associated factor for future CVEs in CKD individuals with diabetes, whereas in nondiabetic CKD individuals, other factors influenced the occurrence of CVE, such as age and serum concentrations of 25-OH vitamin D. To the best of our knowledge, this is an original work showing that in individuals with CKD and diabetes, multiterritorial vascular ultrasonography could help in future CVE prediction.

Atherosclerosis burden and CVE

Atherosclerosis is a diffuse condition. Historically, necropsy examinations have revealed extensive atherosclerotic lesions in subjects experiencing fatal CVEs [25], thus linking CVD and atherosclerosis burden. Data from the literature have shown that the detection of preclinical atherosclerosis improved cardiovascular risk prediction beyond traditional cardiovascular risk factors [26, 27]. In addition, the quantification of plaque burden improved risk prediction even further [27–29]. Proxy measures of atherosclerosis burden, including noninvasive techniques, such as coronary artery calcification (CAC) score and vascular ultrasonography, have been the object of multiple investigations as a tool to predict incident CVEs. In individuals without established CVD, previous studies have reported that atherosclerosis burden, measured either by CAC score or plaque burden at carotid or femoral sites, is a strong and independent predictor of CVEs [30, 31]. A high atherosclerotic burden has also been associated with increased cardiovascular and total mortality risk [32–34], even in very elderly people [35]. Additionally, data from prior studies including renal participant cohorts have also demonstrated the relationship between atherosclerosis burden and CVD. A study from our group published in 2017 [20] found that the number of arterial territories affected with atherosclerotic plaque predicted the occurrence of CVEs in a cohort of asymptomatic renal individuals, including all-stages of CKD. Likewise, in a work that followed 226 individuals on haemodialysis for 5 years with carotid ultrasonography [36], plaque number was found to be an independent marker of fatal CVEs.

Concerning individuals with diabetes, the atherosclerotic burden measured by the CAC score has been shown to predict CVEs in asymptomatic subjects [7], while the presence of carotid and/or lower limb atherosclerosis assessed by ultrasonography has been shown to be associated with the prevalence of CVD [37], with the risk being greater in those subjects with concomitant carotid and femoral atherosclerosis.

Other factors associated with CVE

The analysis of associated factors with the incidence of CVEs in our cohort of individuals has shown that age and serum 25-OH vitamin D concentrations were predictive of CVEs only in CKD individuals without diabetes, while the number of territories with plaque at baseline was predictive of CVEs both in individuals with and without diabetes.

Age is a well-known factor for CVD [38]. In our study, age was independently associated with CVEs in CKD individuals without diabetes but not in those with diabetes, inferring that individuals with diabetes present with a higher incidence of CVEs at younger ages, as has been reported in the literature [39]. Likewise, the association between 25-OH vitamin D serum concentrations and the incidence of CVE found in the present study in individuals without diabetes is in keeping with studies that appear to link vitamin D deficiency to CVD [40].

In the current analysis, there were no other risk factors that improved the assessment of CVE risk once added to the number of territories with plaque. Strikingly, the only factor predicting CVEs in this group with diabetes was atherosclerotic burden.

In fact, in our study, sex was not a risk factor for the incidence of CVEs in individuals with or without diabetes. In the general population, it is known that nondiabetic women have fewer CVEs than nondiabetic men of the same age. However, this advantage appears to be lost in the presence of diabetes [41–45]. The present study is in agreement with these findings showing that the loss of the protective cardiovascular sex effect is also observed in women with diabetes and CKD. This statement is reinforced by the fact that the incidence of CVEs in women from our cohort with diabetes was higher than that in men without diabetes, as well as by the data that the incidence of CVEs was similar in women with diabetes in stage 3 CKD compared to males with diabetes with similar kidney function.

Regarding cardiovascular risk prediction in patients with diabetes, Colom et al. have recently described that in patients with type 1 diabetes without previous cardiovascular disease alterations in the composition of HDL are associated with subclinical coronary artery disease as well as with an increased volume of epicardial adipose

tissue suggesting that HDL composition may be a link between coronary atherosclerosis and the accumulation of this type of adipose tissue in these subjects [46]. Also in subjects with type 1 diabetes free from CV disease, our group has recently described that presence of advanced stages of diabetic retinopathy are associated with presence and burden of subclinical carotid atherosclerosis [47]. On the other hand, in healthy normotensive and normoglycaemic subjects classical risk factors, such as age and blood pressure, have been found to be associated with subclinical vascular damage while no association has been found with plasma biomarkers involved in the inflammatory process of atherosclerosis [48].

Finally, as we have demonstrated in the present work, quantification of atherosclerotic lesions by vascular ultrasonography is a feasible and reliable tool for CVE prediction. It should also be noted that new three-dimensional [28] and contrast-enhanced ultrasonography [49] could improve vascular assessment even further and thus contribute to refining the early prediction of CVEs.

Limitations

A limitation of the current work relates to the baseline clinical characteristics relevant to diabetes that were not available due to the inherent nature of the NEFRONA study that was initially designed to investigate renal disease and cardiovascular risk. In addition, the endpoints registered during the observation period entailed the discontinuation of the follow-up after a first CVE, after nonCV death or after renal transplantation. However, this has been considered and mitigated with the competing risk analysis.

Conclusions

CVD screening in asymptomatic individuals with diabetes remains controversial [50]. Moreover, CVD risk stratification remains an important challenge in the CKD population. However, our results shed some light on this matter. We have demonstrated that multiterritorial ultrasonography is a valid and strong noninvasive tool to help predict CVEs among diabetic individuals with CKD. In this subgroup of high-risk individuals, quantifying SA by the combined application of carotid and lower extremity vascular ultrasonography could improve CVE prediction and may help identify those individuals with higher CV risk. The strategies implemented in routine practice to efficiently quantify subclinical arterial lesions in diabetic individuals with CKD might be of clinical benefit and should be considered with a special emphasis on younger women with diabetes.

Additional file

Additional file 1: Figure S1. Cardiovascular event incidence rates per 1000 person-years according to chronic kidney disease stage, diabetes status and gender. **Table S1.** Bivariate analysis of baseline characteristics in the NEFRONA cohort by incidence of cardiovascular events.

Abbreviations

CKD: chronic kidney disease; CVEs: cardiovascular events; SA: subclinical atherosclerosis; CVD: cardiovascular disease; cIMT: carotid intima media thickness; RRT: renal replacement therapy; CAC: coronary artery calcification; MI: myocardial infarction.

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Authors' contributions

AP and EC contributed to the study design, conduct of the study, data analysis, and the writing of the manuscript. JMV, MB-L and AB contributed to the data collection and conduct of the study. HP and XD contributed to the data analyses and writing of the manuscript. EF, PHG and MPD contributed to data interpretation and discussion. NA and DM contributed to the study design and coordination, conduct of the study, data analysis, and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version for publication. DM and NA are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

Primary material is held by the authors.

Ethics approval and consent to participate

The Ethical Committees of all involved Spanish nephrology centres approved this study with the final approval by the Ethics committee board of the Hospital Universitario Arnau de Vilanova (Lleida, Spain). The investigation has been conducted according to the principles expressed in the Declaration of Helsinki, and all the included participants provided written informed consents.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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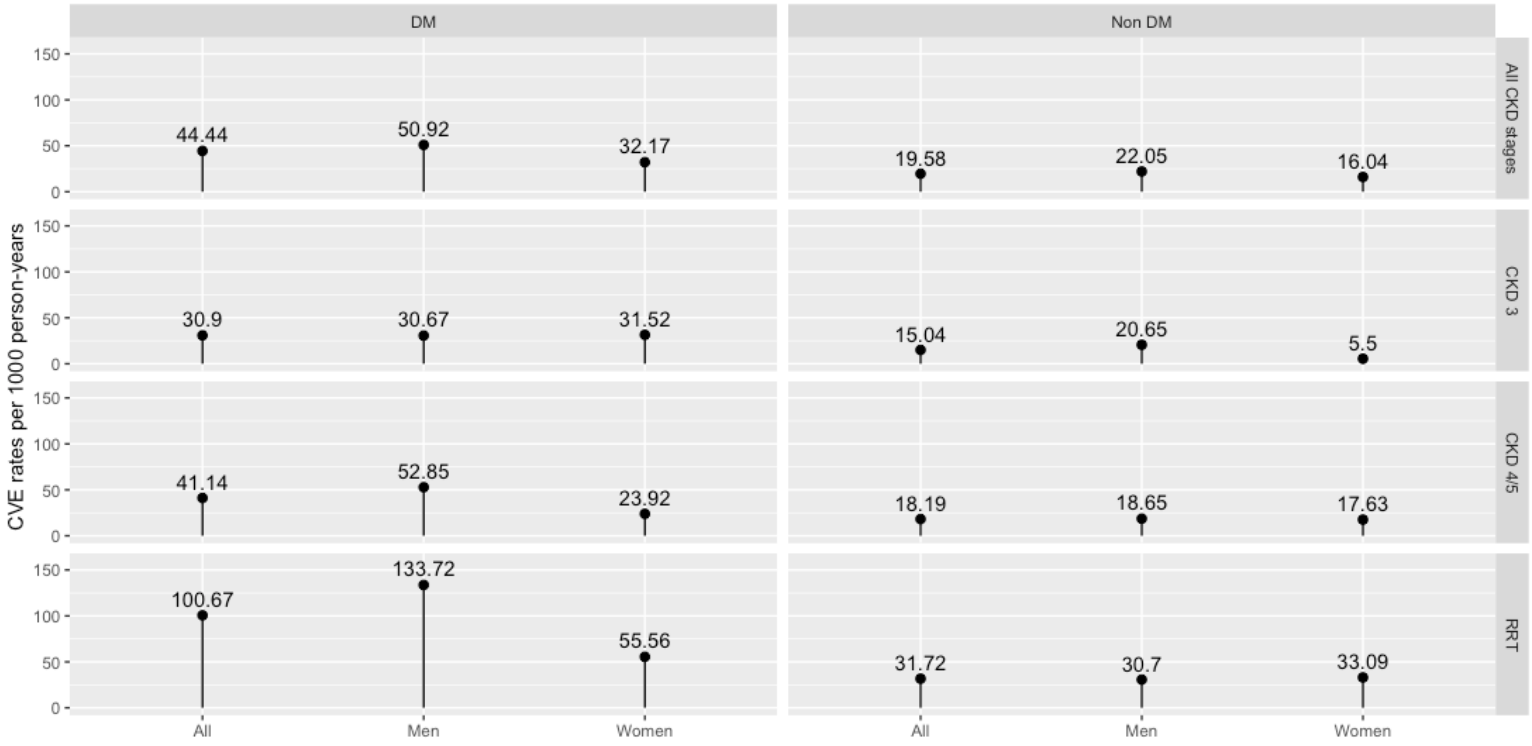
Publisher's Note

Supplementary materials

Figure S1. Cardiovascular event incidence rates per 1,000 person-years according to chronic kidney disease stage, diabetes status and gender.



CVE: fatal and non-fatal cardiovascular event; CKD: chronic kidney disease; RRT: renal replacement therapy

Table S1. Bivariate analysis of baseline characteristics in the NEFRONA cohort by incidence of cardiovascular events

	CKD without diabetes				CKD with diabetes			
	No event N=1640	**Event N=107**	**HR [95%CI]**	***p* value**	**No CVE N=602**	**CVE N=96**	**HR [95%CI]**	***P* value**
Gender:								
Male	976 (59.5%)	71 (66.4%)	Ref.	Ref.	389 (64.6%)	72 (75.0%)	Ref.	Ref.
Female	664 (40.5%)	36 (33.6%)	0.72 [0.48;1.08]	0.115	213 (35.4%)	24 (25.0%)	0.63 [0.40;1.00]	0.050
CKD stage:								
CKD-3	625 (38.1%)	37 (34.6%)	Ref.	Ref.	256 (42.5%)	32 (33.3%)	Ref.	Ref.
CKD-4/5	517 (31.5%)	34 (31.8%)	1.22 [0.76;1.94]	0.412	222 (36.9%)	34 (35.4%)	1.35 [0.83;2.19]	0.220
RTT	498 (30.4%)	36 (33.6%)	2.23 [1.40;3.55]	0.001	124 (20.6%)	30 (31.2%)	3.58 [2.16;5.93]	<0.001
Age [years]	59.0 [47.0;67.0]	65.0 [58.5;70.0]	1.04 [1.02;1.06]	<0.001	64.5 [56.0;70.0]	65.0 [58.0;70.0]	1.01 [0.99;1.03]	0.414
Current smoker	897 (54.7%)	70 (65.4%)	1.60 [1.08;2.39]	0.020	342 (56.8%)	62 (64.6%)	1.36 [0.90;2.07]	0.148
Arterial hypertension	1455 (88.7%)	99 (92.5%)	1.47 [0.71;3.01]	0.297	579 (96.2%)	94 (97.9%)	1.43 [0.35;5.80]	0.617
25-OH Vitamin D [ng/ml]	15.5 [11.6;19.7]	13.9 [10.3;18.0]	0.96 [0.93;0.99]	0.007	14.0 [10.6;18.9]	12.5 [9.19;16.7]	0.95 [0.92;0.98]	0.006
eGFR [mL/min per 1.73 m2]	31.7 [20.8;44.4]	30.9 [21.1;43.2]	0.99 [0.97;1.01]	0.302	32.2 [22.1;44.5]	28.0 [18.5;41.0]	0.98 [0.96;1.00]	0.038
Glucose [mg/dl]	92.0 [85.0;101]	96.0 [86.0;105]	1.01 [0.99;1.02]	0.314	132 [108;161]	147 [108;170]	1.00 [1.00;1.00]	0.346
Total cholesterol [mg/dl]	178 [154;205]	174 [145;210]	1.00 [0.99;1.00]	0.752	172 [144;197]	166 [139;198]	1.00 [0.99;1.01]	0.952
HDL cholesterol [mg/dl]	48.0 [39.0;59.0]	45.0 [39.0;53.0]	0.98 [0.96;0.99]	0.002	44.0 [37.0;53.9]	43.0 [32.0;52.0]	0.99 [0.98;1.01]	0.291
LDL cholesterol [mg/dl]	103 [82.0;123]	101 [73.0;126]	1.00 [0.99;1.00]	0.437	92.5 [72.0;112]	92.0 [71.0;118]	1.00 [1.00;1.01]	0.154
non-HDL cholesterol [mg/dl]	128 [105;152]	132 [103;158]	1.00 [1.00;1.01]	0.555	122 [100;147]	126 [96.0;155]	1.00 [1.00;1.01]	0.579
Triglycerides [mg/dl]	118 [88.0;162]	134 [107;175]	1.00 [1.00;1.00]	0.060	147 [104;206]	148 [91.8;192]	1.00 [1.00;1.00]	0.492
hsCRP [mg/dl]	1.82 [0.90;4.00]	2.52 [1.24;7.32]	1.01 [1.00;1.03]	0.010	2.48 [1.12;5.44]	3.10 [1.44;6.84]	1.01 [0.99;1.04]	0.248
HbA1c [g/dl]	5.40 [5.05;5.70]	5.35 [5.10;5.73]	0.71 [0.38;1.35]	0.299	6.80 [6.10;7.80]	6.90 [6.30;8.10]	1.12 [0.96;1.31]	0.143
Antidiabetic treatment								
Diet					151 (25.1%)	15 (15.6%)	Ref.	Ref.
Oral hypoglycemic drugs					144 (23.9%)	20 (20.8%)	1.17 [0.60;2.28]	0.649
Insulin treatment					307 (51.0%)	61 (63.5%)	1.87 [1.06;3.30]	0.029
Albumin/creatinine ratio mg/g	86.2 [10.5;381]	177 [22.4;451]	1.00 [1.00;1.00]	0.179	139 [18.0;565]	279 [29.3;1309]	1.00 [1.00;1.00]	0.045
Pulse pressure [mmHg]	56.0 [47.0;68.0]	61.0 [48.5;75.0]	1.02 [1.01;1.03]	0.002	66.5 [54.0;80.0]	72.0 [55.8;83.2]	1.01 [1.00;1.02]	0.155

CVE: fatal and non-fatal cardiovascular event; CKD: chronic kidney disease; RRT: renal replacement therapy; eGFR: estimated glomerular filtration rate determined by the Modification of Diet in Renal Disease Study formula (MDRD-4); HDL: high density lipoprotein; LDL: low density lipoprotein; hsCRP: high sensitivity C-reactive protein.

SUMMARY OF THE RESULTS

The present work was conducted within the frame of the aforementioned NEFRONA cohort. The NEFRONA study included 2,445 subjects across all five stages of renal disease as well as individuals on renal replacement therapy. All participants, recruited from 81 Spanish hospitals between October 2010 and June 2012, were free from pre-existing clinical cardiovascular disease at baseline.

The first study was designed to examine the prevalence, distribution, and 2-year progression of subclinical atherosclerosis in renal subjects with and without diabetes from the NEFRONA cohort. Of the original NEFRONA cohort, the analysis was restricted to participants who attended both baseline and a 24-month follow-up visit when another ultrasound examination was performed. Overall, 1,548 subjects, 419 with and 1,129 without diabetes, were included in the plaque progression analysis.

The second study was designed to investigate the incidence of cardiovascular events and associated factors in the studied cohort. We included 698 individuals with CKD with diabetes and 1,747 individuals with CKD without diabetes from the NEFRONA cohort. The median follow-up time was 48 months in both groups. During the follow-up period, data on fatal and non-fatal cardiovascular events were recorded.

With regards to baseline characteristics of the participants, subjects with diabetes were older and had higher body mass index, waist-to-hip ratio, triglycerides, serum hsCRP concentrations, and lower concentrations of high- and low-density lipoprotein cholesterol compared to the patient without diabetes. A higher proportion of subjects with hypertension and dyslipidaemia were observed in the group with diabetes compared with the non-diabetes group. Nearly 19.2% of the non-diabetic subjects were on renal replacement therapy vs 11.7% of those with diabetes.

At baseline, the proportion of individuals with plaque at any of the examined vascular sites was higher among subjects with diabetes than in subjects without diabetes (81.4% vs 64.1%, $p < 0.001$). When examining plaque burden, the proportion of patients with atherosclerotic plaques at both sites, carotid and femoral, was also higher

in the diabetes group compared to the group without diabetes (46.1% vs 34.2%, p < 0.001). Furthermore, the prevalence of patients presenting plaques at either the carotid or the femoral tree was also significantly higher among patients with diabetes (81.4% vs 64.1%, p < 0.001). The percentage of diabetic subjects with more than two vascular territories with plaque was also greater (64.4% vs 48.4%, p < 0.001) than in non-diabetic subjects. Moreover, we found that the pattern of these findings was also consistent when examining data by gender. Interestingly, women with diabetes presented a similar prevalence of plaque at any vascular site as men without diabetes.

With regards to plaque distribution at baseline, we observed that the number of subjects with carotid plaques who did not have plaques at the femoral sites was significantly greater in the diabetes group (22.9% vs. 16.7%, p< 0.006). To the same extent, the number of subjects with carotid plaques at any site (with or without the presence of femoral plaques) was higher in the diabetes group (22.9% vs. 16.7%, p< 0.006). Moreover, the proportion of individuals with femoral plaques at any site (with or without the presence of carotid plaques) was also significantly higher among the group with diabetes (58.5% vs. 47.5%, p < 0.001). However, no significant differences were found in the percentage of patients with femoral plaques but without carotid plaques between the two groups.

Considering plaque progression, the proportion of subjects with atherosclerosis progression was also found to be greater in individuals with CKD and diabetes compared to CKD individuals without diabetes (72.2% vs 55.7%). Interestingly, when estimating the probability of plaque progression for any given number of territories with basal plaque, it was found that individuals with diabetes were more likely to experience plaque progression across all the ranges of the spectrum compared to individuals without diabetes: whether they had no basal plaque or whether they had substantial plaque burden at baseline.

Following multivariate analysis, we found that factors associated with the presence of atheromatous disease differed between subjects with and without diabetes. Among

non-diabetic individuals, plaque prevalence was associated with age, male gender, smoking habit and renal replacement therapy. Notably, the incremental risk of plaque presence associated with ageing was faster in men than in women. In subjects with diabetes, age and male gender were positively associated with the presence of plaque. Interestingly, the increased risk with ageing that was observed in subjects with diabetes tend to lessen at older ages. Additionally, 25-OH vitamin D serum concentrations were inversely associated with the presence of plaque in both CKD subjects with and without diabetes. Conversely, factors associated with atheromatous disease progression were almost the same for CKD individuals with or without diabetes. Among individuals without diabetes, plaque progression at 24 months was positively associated with age, number of territories with basal plaque, smoking habit, renal replacement therapy and pulse pressure. Plaque progression in individuals with diabetes was associated with the same variables as in individuals without diabetes with the exception of pulse pressure. In both groups, a statistically significant interaction between ageing and number of territories with plaque at baseline was observed, meaning that disease progression over time was depended on the number of territories affected at baseline.

With respect to the incidence of cardiovascular event during the 4-year follow-up period, we observed that in asymptomatic CKD participants with diabetes, an increased burden of baseline atherosclerotic plaque translated into an increased risk of incident cardiovascular events when compared to CKD participants without diabetes. In fact, 203 cardiovascular events were registered, of which 107 occurred among participants without diabetes and 96 among participants with diabetes with corresponding cardiovascular event rates were 19.58 and 44.44 per 1000 person-years, respectively. Moreover, we observed that in participants with diabetes, cardiovascular event rates significantly increased along with declining renal function with cardiovascular event rates ranging from 30.90 per 1000 person-years in stage 3 CKD up to 100.67 per 1000 person-years in individuals on renal replacement therapy ($p\text{-trend} < 0.001$). This tendency was milder among renal participants without diabetes but remained significant ($p\text{-trend} < 0.001$). Considering the event rates by sex, women with diabetes expe-

rienced more cardiovascular events than men without diabetes. We observed that in women without diabetes the cardiovascular event rate during follow-up was 16.04 per 1000 person-years and in men without diabetes, it was 22.05 per 1000 person-years. Among women with diabetes, the cardiovascular event rate was 32.17 per 1000 person-years, whereas among men with diabetes, it was 50.92 per 1000 person-years. This finding was consistently found across all stages of CKD.

On the other hand, following the competing risk model analysis, we found that the number of vascular territories affected with plaque appeared to be the strongest associated factor for future cardiovascular events in CKD individuals with diabetes, whereas, in non-diabetic CKD individuals, other factors were influencing the occurrence of vascular events, such as age and serum concentrations of 25-OH vitamin D.

With regards to the selected models, the diabetes group model only included number of territories with plaque at baseline as a variable whereas in the non-diabetes group model, number of territories with basal plaque, age and serum concentrations of 25-OH vitamin D were the included variables.

Finally, the concordance index (C-index) that was estimated to evaluate the predictive value of the selected models yielded values over 0.7 for the selected model in each group, CKD individuals with diabetes and without.

JOINT DISCUSSION

In the present work, we evaluated, in the first instance, the presence, distribution, and progression of subclinical atherosclerosis by multi-territorial vascular ultrasonography in a large cohort of CKD subjects with and without diabetes and free from pre-existing cardiovascular disease. We found that renal subjects with diabetes showed a higher frequency and greater burden of atherosclerotic plaques, defined as the number of vascular territories affected by plaque, in various vascular territories compared to non-diabetic individuals. Atherosclerosis progression was also more rapidly progressive in individuals with diabetes. Additionally, we found that factors associated with the presence of atherosclerotic disease differed between individuals with and without diabetes, whereas factors associated with progression of atherosclerosis were similar for both groups, except for pulse pressure. Overall, diabetes outweighed other risk factors associated with atherosclerosis in CKD. Subsequently, we complemented our initial results with information on incident clinical vascular events following a 4-year-period. We found that in participants with diabetes, an increased burden of baseline atherosclerotic plaque translated into an increased risk of incident cardiovascular events. We also demonstrated that burden was the most potent associated factor for future events in CKD individuals with diabetes, whereas, in non-diabetic individuals other factors influenced the occurrence of vascular events, such as age and serum concentrations of 25-OH vitamin D.

To the best of our knowledge, this is an original work showing that in individuals with CKD and diabetes, multi-territorial vascular ultrasonography could help in the prediction of future vascular events.

Numerous studies have reported that an increased prevalence and burden of atherosclerotic lesions are linked with an increased risk in the occurrence of cardiovascular events and, therefore, that detection of preclinical atherosclerosis could improve cardiovascular risk assessment[121,138,145,160]. Historically, post-mortem studies have linked fatal vascular events to extensive atherosclerotic lesions[161]. At a later stage, solid data have come to demonstrate that preclinical detection of atherosclerosis improved pre-

diction of cardiovascular risk beyond traditional risk factors[121]. Moreover, it has been shown that plaque burden quantification improved cardiovascular risk prediction even further[138,145,160]. An array of proxy measures of atherosclerotic burden can be found in the literature. Those relating to non-invasive imaging, such as coronary artery calcification score and arterial ultrasonography examination, have been extensively studied.

Among the general population, previous studies reported that atherosclerosis burden (measured either by coronary artery calcification score or plaque burden) in asymptomatic individuals was a strong independent predictor of cardiovascular events. In the MESA study, including 6779 individuals without established cardiovascular disease, Gibson *et al*. assessed coronary artery calcification score to predict cerebrovascular events[162]. After 9.5 years, these authors reported that coronary atherosclerotic extent was an independent factor for incident cerebrovascular events. Another work based on the prospective cohort Rotterdam study investigated the association between carotid plaque burden by ultrasonography and the risk of stroke in 4,217 asymptomatic subjects[163]. The authors found that plaque burden, a marker for generalised atherosclerosis, was associated with the risk of cerebrovascular events. Along with these findings, data from the Tromsø Study, a Norwegian population-based prospective study that recruited over 6000 men and women, demonstrated that the extent of carotid atherosclerosis, defined as carotid plaque area, was a strong risk factor not only for myocardial infarction[107], but also for ischemic stroke[123]. A high atherosclerotic burden has also been associated with increased cardiovascular and total mortality risk[136], even in older people[164]. A prospective study performed in 1,325 participants from Southern Germany[136] assessed the presence of carotid and femoral plaque in relation to myocardial infarction and cardiovascular mortality during a 13-year follow-up. The investigators found that the number of plaque-affected sites was a potent predictor not only for myocardial infarction but also for cardiovascular mortality. More recently, in a smaller prospective study cohort of 342 individuals over 85 years old with no prior cardiovascular disease, Hirata *et al.* evaluated the relationship between carotid plaque burden

and cardiovascular mortality in oldest subjects[164]. They concluded that a high atherosclerotic burden was associated with increased cardiovascular mortality.

In diabetes, an increase in the prevalence and development of preclinical atherosclerosis has been described in the literature. In a cross-sectional case-control study evaluating 106 subjects with new-onset type 2 diabetes and 99 subjects without diabetes, matched by age, gender, and cardiovascular risk factors, the authors observed that the frequency of substantial preclinical atherosclerotic burden (defined as the presence of 3 or more atherosclerotic plaques at any carotid site) was significantly higher in subjects with diabetes than in controls (35% vs 16% $p < 0.01$, respectively)[165]. Another work, including data from 1,945 Framingham Offspring Study participants, examined the prevalence of subclinical cardiovascular disease and the incidence of cardiovascular disease associated with metabolic syndrome and diabetes. A higher prevalence of subclinical vascular disease was described among subjects with diabetes when compared to subjects with metabolic syndrome or with subjects with no diabetes or metabolic syndrome. At follow-up (mean 7.2 years), the group of subjects with diabetes and preclinical vascular disease showed the highest risk for over cardiovascular disease[166]. Moreover, in a previous work investigating atherosclerotic burden by coronary artery calcium scoring and vascular events in diabetes, it was observed that those subjects with more diffuse disease presented a greater risk of events[19]. In line with these findings, a study by Li *et al.* showed that concomitant carotid and femoral atherosclerosis assessed by vascular ultrasonography further increased cardiovascular risk in type 2 diabetes subjects[167].

When compared to the general population, subjects with CKD also present a higher prevalence of preclinical atherosclerotic lesions, even at earlier stages of the disease[168-170]. Additional data from previous studies, including renal cohorts, have also demonstrated the association between atherosclerotic burden and cardiovascular disease. In a sub-analysis from the Chronic Renal Insufficiency Cohort (CRIC), it was found that coronary artery calcification score appeared to be the best predictor

for prevalent cardiovascular disease, whereas the Framingham Risk Score was the weakest discriminator[168]. It is noteworthy that the differences between coronary artery calcification score, plaque presence, and cIMT measurements did not reach statistical significance, perhaps due to a lack of power of this ancillary study. A study from our group found that the number of arterial territories affected with atherosclerotic plaque predicted the occurrence of vascular events in a cohort of subjects with CKD, including all stages of the condition[32]. Likewise, in a study that followed 226 participants on haemodialysis for 5 years, ultrasound assessment of plaque number was found to be an independent marker of fatal events and, as suggested by the authors, a useful predictor of long-term prognosis in this subgroup of subjects[171]. Interestingly, our group found a 2-fold higher risk of subclinical atherosclerosis in patients with diabetic nephropathy compared with kidney disease from other causes[172].

With regards to plaque distribution, the presence of atherosclerotic plaques in the carotid vascular bed has been linked to a substantial risk of future vascular events. Similarly, the detection of concomitant plaques in the carotid and the femoral trees is associated with an even higher risk[130], as it has been shown in several studies that explored arterial lesions at different vascular territories in the general population[92,135-137]. Also, in the PESA study and in the Aragon Workers Health cohorts, which included middle-aged subjects, investigators observed that plaques were more common in the femoral vascular bed than in the carotid bed[92,137]. On the other hand, in a post-mortem study that included 100 autopsies investigating the relationship between plaque burden and coronary death, the authors found that femoral plaque, but not carotid plaque, was significantly associated with coronary death[173]. Another study, including 391 asymptomatic middle-aged men, showed that subjects with femoral plaques at baseline presented a 3-fold increased risk of experiencing a vascular event during a 6.6-year follow-up period[174]. In contrast, in our study, we found that carotid plaques were more prevalent than femoral plaques in diabetic participants. No differences in plaque distribution were observed in non-diabetic subjects. Notably, these findings were consistent also when stratified by gender. These data could indicate that factors

playing a role in femoral plaque formation might be different from those related to carotid plaque formation.

Considering vascular disease progression, Spence *et al.* demonstrated that, in subjects with vascular disease, individuals that experienced plaque progression exhibited twice the risk of those events compared to individuals with no plaque progression[175]. In a subsequent study, Wannarong *et al.* reported that in subjects with known atherosclerotic lesions, plaque progression remained a significant predictor of events after adjusting for coronary risk factors[122]. Additionally, atherosclerotic plaque build-up appears to be more progressive in diabetic subjects when compared to the general population, according to previous research[176,177]. A systematic review evaluated data from 2,237 participants enrolled in randomised controlled trials of atherosclerosis progression comparing subjects with diabetes with those without diabetes. On multivariate analysis, a more rapid progression of atherosclerotic burden was found among diabetic individuals[177]. A more recent work evaluating data from 1602 participants included in the Progression of Atherosclerotic Plaque Determined by Computed Tomographic Angiography Imaging (PARADIGM) trial showed that plaque progression was more prevalent in individuals with diabetes. The investigators also observed that diabetes was associated with greater plaque progression[176]. Another recent retrospective study assessed coronary plaque progression and its link to cardiovascular events in asymptomatic subjects with diabetes, finding that plaque progression was significantly associated with subsequent vascular events[178]. Prior observational studies have investigated the progression of subclinical atherosclerosis in CKD subjects[32,179,180]. Benedetto *et al.,* in a study enrolling 135 participants with end-stage renal disease, reported that, over a 15-month follow-up and after adjusting for baseline plaque burden and other confounders, the rate of formation of new plaques was an independent predictor of incident vascular events[128]. Previous work from our group elicited data demonstrating that, in all stage CKD individuals, the progression of preclinical atherosclerosis was closely linked to basal plaque prevalence and CKD progression[179].

However, fewer data exist regarding the specific prevalence, burden, and progression of preclinical arterial lesions when both conditions, diabetes and CKD, are present. In our study, we found that individuals with renal disease and diabetes presented not only a greater prevalence and burden of plaques but also had a more advanced and progressive disease than non-diabetic individuals with CKD.

Previous research has correlated traditional cardiovascular risk factors with the presence and progression of preclinical atherosclerosis in the general population[92,181]. In concordance with these data, our study found that in individuals with CKD without diabetes, plaque presence was significantly associated with traditional risk factors, such as age, male gender, and smoking, and dialysis therapy. On the other hand, in individuals with renal disease and diabetes, only age and male gender were independent predictors of plaque presence, suggesting that diabetes is a potent risk condition for the atherosclerotic disease that outweighs predominant risk factors, such as smoking and dialysis. Regarding atherosclerosis progression, the present analysis showed that significantly associated factors were the same for both conditions, including age, smoking, dialysis, and basal plaque status, except for pulse pressure, which was not associated with plaque progression in diabetes.

In the present work, we observed that those individuals with renal disease and diabetes experienced more vascular events than individuals with renal disease and without diabetes during the follow-up period corresponding to a cardiovascular event rate of 44.44 and 19.58 per 1000 person-years, respectively. The analysis of factors associated with the incidence of cardiovascular events in our cohort has shown that atherosclerosis burden, defined as the number of territories with plaque at baseline, was predictive of cardiovascular events in both individuals with and without diabetes. Besides, age and serum 25-OH vitamin D concentrations were predictive of vascular events only in individuals with kidney disease without diabetes. Large-scale epidemiological studies have illustrated the relationship between age and vascular events[182-184]. In our study, age was independently associated with cardiovascular events in individuals

without diabetes but not in those with diabetes. As previously reported in the literature, this finding supports the proposition that individuals with diabetes suffer from vascular events at younger ages. On the other hand, some studies have shown that 25-OH vitamin D deficiency is a significant risk factor for cardiovascular disease and mortality in the general population, in CKD and in type 2 diabetes[186-189]. A work by Drechsler *et al.* provided data on long-term cardiovascular events in haemodialysis individuals with diabetes and its relationship with 25-OH vitamin D deficiency. In their study, serum concentrations of 25-OH vitamin D were measured in 1,108 diabetic participants on renal replacement therapy from The German Diabetes and Dialysis Study (4D study) who were then followed-up for a mean period of 4 years[189]. The authors found that severe 25-OH vitamin D deficiency was strongly associated with an increase in vascular events, mortality, and sudden death. In keeping with these findings, we found 25-OH vitamin D status to be one of the strongest factors associated with incident vascular events in CKD participants without diabetes. In contrast, this was not the case for CKD participants with diabetes from our cohort.

In our analysis, there were no other risk factors that improved the assessment of the risk of cardiovascular events once added to the number of territories with plaque. Also, the most potent factor predicting vascular events in individuals with diabetes was atherosclerotic burden alone.

Gender is noteworthy when discussing our findings. In the general population, it has been reported that non-diabetic women have fewer events than non-diabetic men of the same age. However, this advantage appears to be lost in the presence of diabetes[190-194]. Our study agrees with these data, supporting the proposition that the loss of the protective cardiovascular sex effect is also observed in women with diabetes and renal disease. This statement is reinforced by the fact that the incidence of vascular events in women with diabetes from our cohort was higher than that of men without diabetes and similar in diabetic women with stage 3 CKD compared to men with diabetes with similar kidney function.

To conclude, in the present work, we have demonstrated that quantification of atherosclerotic lesions by multi-territorial vascular ultrasonography is a feasible and reliable tool for cardiovascular event prediction. It should also be noted that recently developed three-dimensional[138] and contrast-enhanced ultrasonography[195] could further improve vascular assessment.

LIMITATIONS

A limitation of the current work relates to the absence of precise phenotyping of the type of diabetes. Initially, the NEFRONA study was designed to investigate renal disease and cardiovascular risk. Therefore, data on clinical characteristics relevant to diabetes at baseline are missing.

Another limitation with regards to the first study relates to the substantial number of drop-outs due to death, cardiovascular disease, or kidney transplantation. Additionally, the length of the follow-up was limited to 24 months, and a more prolonged period of follow-up could have provided more data on plaque progression.

Also, the endpoints that were registered during the observation period entailed the discontinuation of the follow-up after a first cardiovascular event, after non-cardiovascular death, or after renal transplantation. However, this has been dealt with through the competing risk analysis. Nevertheless, it would have been interesting to have follow-up data on those subjects that underwent kidney transplantation.

CONCLUSIONS

- Subjects with CKD and diabetes show a higher prevalence and burden of subclinical atherosclerosis in different vascular territories and experience a more progressive vascular disease than subjects with CKD and without diabetes;
- In CKD subjects with diabetes, diabetes is such a strong risk factor for atherosclerotic disease that it outweighs other risk factors associated with atherosclerosis prevalence;
- The incidence of cardiovascular events is greater in subjects with CKD and diabetes than in those with non-diabetic CKD, across all stages of CKD; for both groups of subjects, cardiovascular event rates significantly increase along with declining renal function; however, in diabetic subjects, the pattern appears to be stronger;
- In our cohort, women with diabetes experience more cardiovascular events than men without diabetes, and this appears to be consistent across all stages of CKD;
- In CKD subjects with diabetes, the most potent variable independently associated with future cardiovascular events is the number of territories with a plaque at baseline, and in CKD subjects without diabetes, the number of territories with plaque at baseline is one of the most potent variables linked to cardiovascular events;
- Multi-territorial ultrasonography is a valid non-invasive tool to help predict cardiovascular events among individuals with CKD, with and without diabetes.

FUTURE PERSPECTIVES

To better understand the characteristics, evolution, and outcomes of preclinical atherosclerosis in individuals with diabetes and chronic kidney disease (CKD), large-scale prospective population-based studies are required. It would be of benefit to design cohort studies enrolling participants not only at a national level but also in other European countries, from different socio-economic backgrounds, and including a wide age range and a prolonged follow-up period. For these purposes, ongoing national and European registries, such the Swedish National Diabetes Register that was launched in 1996 to monitor diabetes quality of care in Sweden, would be a useful tool and a visionary move from governments.

It would also be interesting to translate the results from this thesis into routine practice. In fact, implementing strategies, such as multi-territorial ultrasound examination could:

– Efficiently detect and quantify subclinical arterial lesions and progression in diabetic individuals with CKD with a special emphasis on younger women.
– Identify subjects without vascular lesions and, therefore, at lower cardiovascular risk among these, a priori, high-risk subsets of the population.
– Monitor the response to therapeutic interventions in terms of progression and regression of plaques as well as in terms of incident cardiovascular events.

Further studies would then be required to evaluate the impact and clinical benefit of these measures in terms of disability, mortality, quality of life, and cost-effectiveness. These studies should be aimed at reducing mortality and morbidity, increasing the subject's quality of life, and maximising cost-effectiveness.

Another point to highlight would be the concept of negative risk markers for cardiovascular disease. The absence of atherosclerotic lesions identified by multisite ultrasound examination could have potential to be used as a negative risk marker to downgrade and refine an individual's cardiovascular risk in these very high- and high-risk populations. Therefore, this should be further explored in ad-hoc clinical trials and cohort studies.

Recently developed three-dimensional ultrasonography —an accurate and reproducible potential tool to predict cardiovascular risk through an examination of an individual's arterial lesions— remains investigational. Time and further research at the population level are needed to transfer the benefits of this technique into routine practice.

Finally, there is a need to continue supporting research on identification and validation of emerging biomarkers from across multiple platforms for a global approach on cardiovascular risk assessment to complete and complement current practice.

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