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Traditional and non-traditional risk factors for incident peripheral arterial disease among patients with chronic kidney disease

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ABSTRACT

Background. The risk of peripheral arterial disease (PAD) is higher in patients with chronic kidney disease (CKD) compared with those without. However, reasons for this increased risk are not fully understood.

Methods. We studied risk factors for incident PAD among 3169 participants in the Chronic Renal Insufficiency Cohort (CRIC) Study. Patients with CKD aged 21–74 years were recruited between 2003 and 2008 and followed for a median of 6.3 years. Incident PAD was defined as a new onset ankle-brachial index (ABI) of <0.9 or confirmed clinical PAD.

Results. In a multivariate-adjusted model, older age, female sex, non-Hispanic Black, current smoking, diabetes, higher pulse pressure, lower high-density lipoprotein cholesterol, higher low-density lipoprotein cholesterol, and lower estimated glomerular filtration rate were significantly associated with the increased risk of incident PAD. After adjustment for these traditional risk factors as well as use of medications and CRIC Study clinic sites, the following baseline novel risk factors were significantly associated with risk of incident PAD [hazard ratio and 95% confidence interval (CI) for a one standard deviation (SD) higher level]: log[C-reactive protein (CRP)] (1.16, 1.06–1.25, $P < 0.001$), white blood cell count (1.09, 1.01–1.18, $P = 0.03$), fibrinogen (1.15, 1.06–1.26, $P = 0.002$), log(myeloperoxidase) (1.12, 1.03–1.23, $P = 0.01$), uric acid (0.88, 0.80–0.97, $P = 0.01$), glycated hemoglobin (1.16, 1.05–1.27, $P = 0.003$), log(homeostatic model assessment-insulin resistance) (1.21, 1.10–1.32, $P < 0.001$) and alkaline phosphatase (1.15, 1.07–1.24, $P < 0.001$).

Conclusions. Among patients with CKD, inflammation, prothrombotic state, oxidative stress, glycated hemoglobin, insulin resistance and alkaline phosphatase are associated with an increased risk of PAD, independent of traditional risk factors.

Keywords: chronic kidney disease, novel risk factors, peripheral arterial disease, traditional risk factors

INTRODUCTION

The risk of peripheral arterial disease (PAD) is higher in patients with chronic kidney disease (CKD) compared with those without [1–3]. In the Atherosclerosis Risk in Communities (ARIC) Study, the age-, gender- and race-adjusted risk for PAD was 82% higher for those with CKD compared with those with normal kidney function [1]. In addition, several studies

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have demonstrated a relationship between PAD and an elevated risk for cardiovascular disease (CVD) and premature death in patients with CKD and in the general population [3–7]. However, risk factors for the excess risk of PAD among patients with CKD are not well studied. Identifying novel risk factors for the development of PAD would be key to furthering our understanding of the etiology of PAD in CKD and helping to establish new strategies for the prevention of PAD among patients with CKD.

Traditional risk factors such as older age, cigarette smoking, physical inactivity, hypertension, diabetes and dyslipidemia play an important etiopathogenetic role in the development of PAD among patients with CKD [8], but these traditional risk factors do not completely explain the excess risk of PAD [9–11]. After adjustment for traditional risk factors, patients with CKD still had higher risk for PAD [11]. In a recent cross-sectional analysis, we documented that several novel risk factors, including biomarkers of inflammation, prothrombotic state, oxidative stress, insulin resistance and cystatin C, were associated with an increased prevalence of PAD in patients with CKD independent of traditional risk factors [11]. However, there have been no published studies that have prospectively evaluated the association between novel risk factors and incident PAD among patients with CKD. The Chronic Renal Insufficiency Cohort (CRIC) Study is a large prospective cohort study of patients with varying degrees of CKD aimed at investigating risk factors for the progression of CKD and development of CVD [12]. In this analysis, we examine the prospective relationship of novel risk factors for the development of PAD among CKD patients.

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MATERIALS AND METHODS

The CRIC Study population is comprised of men and women, aged 21–74 years old, of diverse racial and ethnic backgrounds who had CKD determined by age-based estimated glomerular filtration rate (eGFR) [12]. Recruitment was carried out by identifying participants through searches of laboratory databases, medical records and referrals from health care providers. A total of 3939 CRIC participants were enrolled through seven clinical centers in the USA between May 2003 and August 2008. Patients with cirrhosis, HIV infection, polycystic kidney disease or renal cell carcinoma; those on dialysis or on immunosuppressant drugs; or recipients of a kidney transplant were excluded. The current analysis is based on a total of 3169 CRIC participants, after the additional exclusion of those with an ankle-brachial index (ABI) <0.9 or a history of revascularization at baseline ($n = 770$).

This study was approved by the Institutional Review Boards at each of the participating clinical centers and the scientific and data coordinating center. All study participants provided written informed consent prior to enrollment.

All study data were collected by trained research staff during clinical visits, using data collection procedures and equipment that had been standardized across study sites. Demographic characteristics, behavioral risk factors and medical history, including information relating to participants prior history of PAD (claudication, amputation or angioplasty and procedures to open up blood vessels in arm or legs), were obtained via baseline questionnaires. These questionnaires also assessed smoking and drinking habits; current cigarette smoking was defined as currently smoking and having smoked at least 100 cigarettes in a lifetime, and alcohol drinking was defined as consuming at least one beverage containing alcohol each week over the previous year. Anthropometric measures included body weight and height (each reported as the average of two separate measurements); body mass index (BMI), calculated as weight in kilograms divided by height in meters squared; and waist circumference, which was measured using a Gulick II tape at the uppermost lateral border of the iliac crest and repeated until two measures agreed within 1 cm. Three seated blood pressure (BP) measurements were obtained using an aneroid sphygmomanometer after at least 5 min of quiet rest by trained and certified staff following a standard protocol [13]. Pulse pressure was calculated by subtracting diastolic BP from systolic BP. Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg and/or current use of antihypertensive medication.

ABI was measured per a standard protocol at the baseline and annual follow-up visits among all study participants to detect incident PAD. Following 5 min of supine rest, systolic BP was measured using a continuous-wave Doppler ultrasound probe in both arms with the appropriate-sized arm cuff and in the posterior tibial and dorsalis pedis artery in each leg. The leg-specific ABI was calculated by dividing the higher systolic BP in the posterior tibial or dorsalis pedis by the higher of the right or left brachial systolic BPs.

Study participants were queried for medical history, including hospitalization and medical procedures, at annual clinical visits and 6-month telephone interviews. PAD information was abstracted from hospital records by a trained research nurse. Clinical PAD events were defined as occlusive artery disease resulting in amputation, peripheral surgical or percutaneous revascularization procedures, any arterial angioplasty, or any artery-artery bypass graft. An incident PAD event was defined as a new onset ABI of <0.9 or confirmed clinical PAD during follow-up. There were 516 cases of incident PAD defined by ABI <0.9 and 73 cases defined by clinical PAD events. The median duration of follow-up was 6.3 years through March 2012. We conducted a sensitivity analysis in which an incident PAD event was defined as a new onset ABI of <0.9 or >1.4 or confirmed clinical PAD during follow-up ($n = 832$). In this analysis, study participants with an ABI >1.4 at the baseline examination ($n = 84$) were excluded. Traditional and non-traditional risk factors were selected according to current knowledge and potential biological mechanisms for PAD.

The CRIC Study collected measurements of blood glucose, cholesterol, triglycerides, glycated hemoglobin (HbA1c), phosphate, calcium, alkaline phosphatase, total parathyroid hormone (PTH), uric acid, hemoglobin, albumin and white blood cells according to standard laboratory methods. Serum high sensitive C-reactive protein (hsCRP), homocysteine and cystatin C were measured by particle enhanced immunonephelometry; fibrinogen was measured using an immunochemical

reaction method; serum myeloperoxidase was measured with a chemiluminescent microparticle immunoassay (Abbott Diag. Architect ci8200); and urinary albumin was quantified by radioimmunoassay. Diabetes was defined per the following criteria: a fasting glucose ≥126 mg/dL or a random glucose ≥200 mg/dL, and/or use of insulin or other anti-diabetic medication. eGFR was calculated using the estimating equation, which takes into account serum creatinine and cystatin C [14]. Insulin resistance was evaluated using a homeostasis model assessment (HOMA), which was computed by multiplying fasting serum insulin (μU/mL) by fasting plasma glucose (mmol/L)/22.5 [15]. Finally, albumin corrected calcium levels were determined as follows: serum calcium (mg/dL) + [0.8 × (4 − serum albumin (g/dL)] [16]. The CRIC Central Clinical Laboratory at the University of Pennsylvania handled all laboratory measurements.

Statistical analysis

Continuous data are presented as means ([standard deviation (SD)] and categorical data are expressed as percentages by incident PAD status for baseline characteristics. ANOVA and χ^2 tests were used to ascertain statistical significance for continuous and categorical variables, respectively. Cox proportional hazard models were used to examine the prospective association between baseline risk factors and the incidence of PAD. For the multivariable analysis of traditional risk factors, the backward elimination method was used, and only covariates that were significant ($P < 0.05$) were retained in the final model. Age, sex, race/ethnicity, cigarette smoking, alcohol intake, hypertension, diabetes, physical activity [total metabolic equivalent (MET)/week], BMI, waist circumference, systolic BP, pulse pressure, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, eGFR and CRIC clinic center were included in the initial model. For each novel risk factor, we fitted two models separately. In the first model, we adjusted for age, gender, race and clinic center. For the second model, we included all variables retained in the final traditional risk factor model (including age, gender, race/ethnicity, cigarette smoking, diabetes, HDL cholesterol, LDL cholesterol, pulse pressure, eGFR, and CRIC study clinic center) in addition to the use of angiotensin converting enzyme inhibitor or angiotensin receptor blocker, aspirin, statins and β-blocker. For those novel risk factors that were not normally distributed, including HOMA-insulin resistance, hsCRP, total PTH, myeloperoxidase and urinary albumin, the log-transformation was performed. Hazard ratios and 95% confidence intervals (CIs) of PAD associated with categorical variables or one SD increase in continuous variables are presented. In addition, the associations of PAD with HbA1c and HOMA-insulin resistance were also analyzed by diabetes status in multivariable models. All analyses were conducted using SAS v9.1 (Cary, NC, USA), and statistical significance was defined as a two-sided $P < 0.05$.

RESULTS

Table 1 displays baseline characteristics of CRIC Study participants by incident PAD status. During 6.3 years of follow-up, 589 (18.6%) of 3169 CRIC participants developed incident PAD. Of them, participants with incident PAD were older, more likely to be female and black, and less likely to be high school graduates compared with their counterparts without incident PAD. They were less physically active, less likely to drink alcohol, and more likely to smoke cigarettes. In addition, they were more likely to have a history of hypertension and diabetes, and use β-blockers, aspirin and statins. BMI, waist circumference, systolic BP, pulse pressure, LDL cholesterol, fasting glucose and albuminuria were significantly higher while eGFR was lower in patients with incident PAD compared with those without. Furthermore, hsCRP, white blood cell count, fibrinogen, myeloperoxidase, homocysteine, HbA1c, HOMA-insulin resistance, phosphate, total PTH, and alkaline phosphatase were significantly higher while hemoglobin was lower in patients with incident PAD. Baseline ABI was lower and decreased during follow-up in patients with incident PAD.

Hazard ratios and 95% CIs of PAD associated with statistically significant traditional risk factors from a multivariable model are shown in Table 2. Increased risk of incident PAD was found to be positively and significantly associated with older age, female sex, non-Hispanic black, current cigarette smoking, diabetes, LDL cholesterol and pulse pressure, but inversely and significantly associated with HDL cholesterol and eGFR.

Age-, gender-, race- and clinic site-adjusted and multivariate-adjusted hazard ratios of PAD associated with a one SD difference in novel risk factors in individual adjusted models are shown in Table 3. After adjusting for demographic characteristics, hsCRP, white blood cell count, fibrinogen, myeloperoxidase, HbA1c, HOMA-insulin resistance, phosphate, total PTH, alkaline phosphatase and albuminuria were positively and significantly associated with higher risk of PAD, while calcium was inversely and significantly associated with higher risk of PAD. Further adjustment for multiple traditional risk factors listed in Table 2 produced a model wherein hsCRP, white blood cell count, fibrinogen, myeloperoxidase, HbA1c, HOMA-insulin resistance and alkaline phosphatase remained positively and significantly associated with higher risk of PAD, while uric acid became inversely and significantly associated with higher risk of PAD.

In the subgroup analysis by diabetes status, hazard ratios (95% CI) associated with HbA1c were 1.14 (1.03–1.27; P = 0.01) in participants with diabetes and 1.29 (0.88–1.88; P = 0.20) in those without diabetes (P-value for interaction = 0.92); hazard ratios (95% CI) associated with HOMA-insulin resistance were 1.23 (1.11–1.36; P = 0.0001) in participants with diabetes and 1.10 (0.91–1.33; P = 0.32) in those without diabetes (P-value for interaction = 0.17).

Sensitivity analyses

Using a new onset ABI of <0.9 or >1.4 or confirmed clinical PAD as the outcome and excluding patients with baseline ABI >1.4 from analyses, results are very similar (Supplementary Tables S1 and S2). For example, multiple-adjusted hazard ratios (95% CI) were 1.19 (1.02, 1.39) for non-Hispanic blacks, 1.65 (1.36, 2.00) for current smoking, 1.78 (1.52, 2.08) for diabetes, 1.09 (1.01, 1.16) for a one SD higher in waist circumference, 1.23 (1.15, 1.33) for a one SD higher in pulse pressure and

Table 1. Baseline characteristics and changes in ankle-brachial index during follow-up among study participants by incident peripheral artery disease

Variables	Incident PAD		P-value
	Yes ($n = 589$)	No ($n = 2580$)	
Age, mean (SD), years	59.3 (10.3)	56.7 (11.3)	<0.0001
Female, *n* (%)	332 (56.4)	1091 (42.3)	<0.0001
Race/ethnicity, *n* (%)			
Hispanic	64 (10.9)	327 (12.7)	<0.0001
Non-Hispanic black	311 (52.8)	960 (37.2)	
Non-Hispanic white	196 (33.3)	1176 (45.6)	
Other	18 (3.1)	117 (4.5)	
High school education, *n* (%)	446 (75.7)	2119 (82.2)	0.0003
Physical activity, mean (SD), MET/week	189.1 (147.0)	210 (150.4)	0.002
Current cigarette smoking, *n* (%)	117 (19.9)	253 (9.8)	<0.0001
Alcohol drinking, *n* (%)	91 (15.4)	592 (22.9)	<0.0001
Hypertension, *n* (%)	538 (91.3)	2136 (82.8)	<0.0001
Diabetes mellitus, *n* (%)	343 (58.2)	1063 (41.2)	<0.0001
Use of ACEI or ARB, *n* (%)	408 (69.9)	1703 (66.5)	0.12
Use of β-blockers, *n* (%)	321 (55.0)	1139 (44.5)	<0.0001
Use of aspirin, *n* (%)	259 (44.3)	986 (38.5)	0.009
Use of statins, *n* (%)	353 (60.4)	1262 (49.3)	<0.0001
BMI, mean (SD), kg/m^2	33.0 (8.1)	31.6 (7.5)	0.0001
Waist circumference, mean (SD), cm	107.7 (18.1)	104.8 (17.3)	0.0003
Systolic BP, mean (SD), mmHg	131.0 (22.6)	126.3 (21.5)	<0.0001
Pulse pressure, mean (SD), mmHg	59.6 (19.3)	53.8 (18.0)	<0.0001
HDL cholesterol, mean (SD), mg/dL	47.5 (13.8)	48.2 (16.2)	0.32
LDL cholesterol, mean (SD), mg/dL	106.5 (39.3)	102.9 (34.9)	0.03
Fasting glucose, mean (SD), mg/dL	124.7 (62.2)	109.5 (44.5)	<0.0001
eGFR, mean (SD), mL/min/1.73 m^2	42.2 (14.7)	47.6 (17.6)	<0.0001
Proteinuria, median (IQR), g/24 h	0.09 (0.01, 0.69)	0.06 (0.01, 0.50)	0.004
hsC-reactive protein, median (IQR), mg/L	3.38 (1.26, 7.97)	2.19 (0.96, 5.50)	<0.0001
White blood cell count, mean (SD), thousand/μL	6.76 (2.21)	6.39 (1.90)	<0.0001
Fibrinogen, mean (SD), mg/dL	4.42 (1.25)	4.01 (1.14)	<0.0001
Myeloperoxidase, median (IQR), pmol/L	120.1 (85.1, 169.4)	103.5 (76.7, 149.0)	<0.0001
Uric acid, mean (SD), mg/dL	7.29 (1.83)	7.31 (1.91)	0.81
Homocysteine, mean (SD), μmol/L	15.3 (5.9)	14.6 (5.9)	0.02
Hemoglobin, mean (SD), g/dL	12.3 (1.6)	12.8 (1.8)	<0.0001
Glycated hemoglobin, mean (SD), %	6.98 (1.78)	6.45 (1.49)	<0.0001
HOMA-insulin resistance, median (IQR)	4.98 (2.78, 9.05)	3.80 (2.41, 6.42)	<0.0001
Calcium, mean (SD), mg/dL	9.16 (0.53)	9.19 (0.49)	0.2
Phosphate, mean (SD), mg/dL	3.79 (0.63)	3.68 (0.66)	0.0003
Calcium phosphate product, mean (SD)	34.7 (6.0)	33.8 (6.3)	0.003
Total parathyroid hormone, median (IQR), pg/mL	60.0 (39.0, 96.0)	49.9 (32.8, 83.0)	<0.0001
Alkaline phosphatase, mean (SD), U/L	99.2 (42.5)	89.0 (32.1)	<0.0001
Baseline ABI, mean (SD)	1.05 (0.14)	1.12 (0.15)	<0.0001
Change in ABI[a], mean (SD)	−0.20 (0.19)	0.06 (0.24)	<0.0001

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; IQR, interquartile range.
[a]Change in ABI is calculated as the last measure prior to clinical PAD event or last measure if it is <0.9 during the follow-up.

0.79 (0.73, 0.86) for a one SD higher in eGFR. Likewise, multiple-adjusted hazard ratios (95% CI) associated with a one SD higher in novel risk factors were 1.09 (1.02, 1.17) for log (CRP), 1.08 (1.00, 1.15) for white blood cell count, 1.16 (1.08, 1.25) for fibrinogen, 1.09 (1.01, 1.18) for log (myeloperoxidase), 0.92 (0.85, 1.00) for uric acid, 1.09 (1.01, 1.18) for glycated hemoglobin, 1.13 (1.04, 1.22) for log(HOMA-insulin resistance), 1.09 (1.02, 1.16) for alkaline phosphatase and 1.12 (1.04, 1.21) for log (albuminuria).

DISCUSSION

The present study indicates that there is a significant prospective association between several novel CVD risk factors and the incidence of PAD among patients with CKD. Per our knowledge, this is the first study to report a significant and positive association of hsCRP, white blood cell count, fibrinogen, myeloperoxidase, HbA1c, HOMA-insulin resistance and alkaline phosphatase with the incidence of PAD and an inverse association of uric acid with the incidence of PAD among a large cohort of pre-dialysis CKD patients, independent of traditional CVD risk factors.

These findings have important clinical and public health implications because CKD patients have an increased risk of PAD [1, 2]. The underlying reasons for excess risk of PAD in CKD patients are unclear. Our study found that traditional CVD risk factors such as age, non-Hispanic black, cigarette smoking, history of diabetes, higher LDL cholesterol, lower HDL

Table 2. Multivariable-adjusted hazard ratios (95% confidence intervals) of peripheral artery disease associated with traditional cardiovascular risk factors

Variables	Hazard ratios (95% CIs)			
	Age-, gender-, race-, and clinic site-adjusted	P-value	Multivariate-adjusted[a]	P-value
Age (11 years[b])			1.26 (1.14, 1.40)	<0.001
Female sex			1.55 (1.30, 1.86)	<0.001
Race/ethnicity				
Non-Hispanic white			1.00 (ref)	
Non-Hispanic black			1.47 (1.22, 1.78)	<0.001
Hispanic			0.93 (0.66, 1.32)	0.70
Other			0.87 (0.52, 1.43)	0.58
Less than high school education	1.17 (0.94, 1.44)	0.16		
Current smoking	2.22 (1.80, 2.74)	<0.001	2.30 (1.86, 2.84)	<0.001
Alcohol drinking	0.81 (0.64, 1.02)	0.08		
Physical activity (150 MET/week[b])	0.89 (0.81, 0.98)	0.02		
Hypertension	1.48 (1.10, 1.99)	0.01		
Diabetes mellitus	1.69 (1.42, 2.00)	<0.001	1.56 (1.30, 1.87)	<0.001
BMI (8 kg/m^{2b})	1.08 (1.00, 1.17)	0.07		
Waist circumference (18 cm[b])	1.14 (1.05, 1.23)	0.002		
Systolic BP (22 mmHg[b])	1.13 (1.04, 1.23)	0.004		
Pulse pressure (18 mm Hg[b])	1.21 (1.11, 1.32)	<0.001	1.11 (1.01, 1.21)	0.03
HDL cholesterol (16 mg/dL[b])	0.84 (0.76, 0.92)	<0.001	0.86 (0.78, 0.95)	0.002
LDL cholesterol (36 mg/dL[b])	1.09 (1.00, 1.18)	0.05	1.16 (1.07, 1.26)	<0.001
eGFR (17 mL/min/1.73 m^{2b})	0.80 (0.72, 0.88)	<0.001	0.87 (0.78, 0.96)	0.007

[a]Backward elimination method was used to select final model and only traditional risk factors that were significant ($P < 0.05$) were retained. Age, sex, race/ethnicity and CRIC clinic site were included.
[b]One SD.

cholesterol and declined eGFR were significantly associated with the incidence of PAD. These findings are consistent with the results from cross-sectional and prospective cohort studies in the general population [1, 17–19]. Our study suggests that the prevention and treatment of traditional CVD risk factors may help to prevent excess PAD in CKD patients.

Our study found that pulse pressure was more strongly associated with incident PAD than systolic BP. It is possible that pulse pressure may reflect peripheral arterial stiffness better than systolic BP does. Our study also found that women were at higher risk for incident PAD than men among patients with CKD. Sex differences in the incidence of PAD are less clear than those in other CVDs [19]. Several cohort studies in the general populations have reported conflicting findings—women might have higher, the same or lower incidence of PAD than men [19]. In general, PAD diagnosed based on ABI might be higher in women than men because, on average, ABI was lower in women [20].

Several prospective cohort studies have examined the association between inflammatory biomarkers and risk of PAD in the general population [21–23]. In a nested case–control study, Ridker *et al.* reported that CRP and fibrinogen were associated with incident PAD in US male physicians [21]. In the Edinburgh Artery Study, Tzoulaki *et al.* also reported that CRP and fibrinogen were associated with incident PAD [22]. In the ARIC Study, fibrinogen and white blood cell count were associated with PAD incidence in persons with diabetes [23]. Our study assessed the association of a panel of inflammatory biomarkers including CRP, white blood cell count, fibrinogen and myeloperoxidase with the incidence of PAD in CKD patients. Our study results indicate that the inflammatory pathway may play an important role in the development of PAD in CKD patients, independent of traditional risk factors. In addition, fibrinogen is a biomarker for prothrombotic state and thrombosis [24]. Our study also suggests that prothrombotic state may play a role in the development of PAD in CKD patients.

Myeloperoxidase, an enzyme secreted by activated neutrophils, participates in various inflammatory processes involved in atherosclerosis [25, 26]. Myeloperoxidase has also been considered as a biomarker of oxidative stress and plays a key role in promoting atherosclerosis via oxidative stress by modification of LDL, production of other bioactive molecules, consumption of nitric oxide leading to endothelial dysfunction, and generation of numerous oxidative reactants and diffusible free radical species [27–29]. Ali *et al.* reported a cross-sectional association between myeloperoxidase and lower ABI and prevalent PAD in 1324 African-Americans and 1237 non-Hispanic white individuals [30]. Our study is the first to report a significant association between baseline level of myeloperoxidase and incidence of PAD, and this finding indicates that oxidative stress may contribute to the risk of developing PAD among CKD patients.

A few prospective studies have examined the association between insulin resistance or HbA1c and risk of PAD [31, 32]. HOMA-insulin resistance was associated with newly onset abnormal ABI or clinical PAD incidence in the Cardiovascular Health Study [31]. HbA1c was significantly associated with clinical PAD (hospitalization, amputation or revascularization) but not low ABI after adjusting for CVD risk factors in diabetes patients from the ARIC Study [32]. In our study, both HOMA-insulin resistance and HbA1c were significantly associated with the risk of PAD independent of CVD risk factors, including diabetes. However, such associations were stronger among patients with diabetes compared with those without. The results from our study and others support the notion that higher levels of HbA1c and insulin resistance are associated with an increased risk of PAD in CKD patients, particularly

Table 3. Multivariable-adjusted hazard ratios (95% CIs) of PAD associated with a one SD difference in novel risk factors

Variables (SD)	Age-, gender-, race-, and clinic site-adjusted		Multivariable-adjusted[a]	
	Hazard ratios (95% CI)	P-value	Hazard ratios (95% CI)	P-value
Log(hsCRP), 0.86 mg/L	1.19 (1.10, 1.29)	<0.001	1.16 (1.06, 1.25)	<0.001
White blood cell count, 2.0 thousand/μL	1.20 (1.12, 1.29)	<0.001	1.09 (1.01, 1.18)	0.03
Fibrinogen, 1.2 mg/dL	1.30 (1.20, 1.41)	<0.001	1.15 (1.06, 1.26)	0.002
Log(myeloperoxidase), 0.81 pmol/L	1.15 (1.05, 1.26)	0.003	1.12 (1.03, 1.23)	0.01
Uric acid, 1.9 mg/dL	0.96 (0.88, 1.05)	0.33	0.88 (0.80, 0.97)	0.01
Homocysteine, 5.9 μmol/L	1.03 (0.95, 1.11)	0.55	0.95 (0.86, 1.05)	0.34
Hemoglobin, 1.6 g/dL	0.92 (0.83, 1.01)	0.07	1.00 (0.90, 1.11)	0.99
HbA1c, 1.6%	1.27 (1.19, 1.37)	<0.001	1.16 (1.05, 1.27)	0.003
Log(HOMA-insulin resistance), 0.66	1.29 (1.19, 1.40)	<0.001	1.21 (1.10, 1.32)	<0.001
Calcium, 0.50 mg/dL	0.90 (0.82, 0.98)	0.01	0.94 (0.86, 1.02)	0.16
Phosphate, 0.66 mg/dL	1.11 (1.03, 1.21)	0.01	1.00 (0.91, 1.09)	0.96
Calcium phosphate product, 6.3	1.07 (0.98, 1.16)	0.11	0.98 (0.89, 1.07)	0.59
Log(total PTH), 0.70 pg/mL	1.11 (1.02, 1.21)	0.02	1.00 (0.91, 1.11)	0.92
Alkaline phosphatase, 34.5 U/L	1.20 (1.13, 1.29)	<0.001	1.15 (1.07, 1.24)	<0.001
Log(albuminuria), 0.50 g/24 h	1.22 (1.12, 1.32)	<0.001	1.03 (0.94, 1.14)	0.49

[a]Adjusted for age, gender, race/ethnicity, current cigarette smoking, diabetes, HDL cholesterol, LDL cholesterol, pulse pressure, eGFR, use of angiotensin converting enzyme inhibitor or angiotensin receptor blocker, aspirin, statins and β-blocker, and CRIC Study clinic site.

among CKD patients who have diabetes. More strict control of HbA1c and insulin resistance may reduce risk of PAD in the CKD patients with diabetes.

Several cross-sectional studies have reported that serum uric acid, a biomarker related to inflammation and metabolic syndrome, was increased among patients with PAD [11, 33]. Our investigation is the first cohort study that identifies an inverse association between serum uric acid and incidence of PAD among CKD patients. Future studies are warranted to confirm or refute this finding.

Serum alkaline phosphatase may promote vascular calcification in patients with CKD [34]. A clinical study among 137 hemodialysis patients reported that serum alkaline phosphatase was significantly associated with coronary artery calcification [35]. In addition, an analysis of data form the United States National Health and Nutrition Examination Survey identified a cross-sectional association between serum alkaline phosphatase and prevalent PAD [36]. Our study is the first one to document that alkaline phosphatase was associated with increased incidence of PAD, independent of CVD risk factors. Future studies are warranted to examine this association in patients with CKD and in the general population.

Our study identified several modifiable traditional risk factors for PAD among CKD patients, which include cigarette smoking, diabetes, dyslipidemia, elevated pulse pressure and declined eGFR. The control of these risk factors should play an important role in the prevention of PAD. In addition, our study identified several biomarkers in inflammation, glycemic metabolism and vascular calcification pathways that were associated with increased risk of PAD. These results warrant future clinical trials to test novel treatment strategies in these pathways aimed at the prevention of PAD in CKD patients.

There are several strengths of our study. This is the first large prospective cohort study to examine novel risk factors for PAD among a diverse group of patients with CKD. Multiple biomarkers in several important pathways for atherosclerotic diseases were measured at the baseline examination. Numerous important traditional risk factors for PAD were collected and adjusted in the multivariable models. Moreover, ABI was carefully measured annually among all study participants during the follow-up period. Therefore, our study should provide a valid and reliable assessment of associations between these novel risk factors and incidence of PAD. However, our study cannot establish the causal relationship between the novel risk factors and incident PAD due to its observational study design. Furthermore, repeated measures of the novel risk factors were not obtained so we cannot examine the effect of changes in risk factors and incident PAD.

In conclusion, these data indicate that inflammation, prothrombotic state, oxidative stress, glycated hemoglobin, insulin resistance and alkaline phosphatase are associated with an increased risk of PAD, independent of traditional risk factors, among patients with CKD. Clinical trials are warranted to investigate the effect of modifying these novel risk factors on the prevention and treatment of PAD in CKD patients.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

The authors have no financial relationships to disclose. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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