## Title

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## Journal

American Journal of Kidney Diseases, 65(1)

## ISSN

0272-6386

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## Publication Date

2015

## DOI

10.1053/j.ajkd.2014.05.014

Peer reviewed

Published in final edited form as:
*Am J Kidney Dis*. 2015 January ; 65(1): 33–40. doi:10.1053/j.ajkd.2014.05.014.

# Association of Albumin-Creatinine Ratio and Cystatin C With Change in Ankle-Brachial Index: The Multi-Ethnic Study of Atherosclerosis (MESA)

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## Abstract



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N SECTION: Because the Editor-in-Chief and Deputy Editor recused themselves from consideration of this manuscript, the peer-review and decision-making processes were handled entirely by a Co-Editor (Laura Dember, MD) who served as Acting Editor-in-Chief. Details of the journal's procedures for potential editor conflicts are given in the Information for Authors & Editorial Policies.

*Financial Disclosure:* The authors declare that they have no other relevant financial interests. *Contributions:* Research idea and study design: PSG, MJS; data analysis/interpretation: RK, PSG, MJS; statistical analysis: RK; critical review of results: JI, MGS, HK, DS, MHC, CTS; supervision or mentorship: MJS, JI, MGS. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. PSG and MJS take responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Supplementary Material
Table S1: Baseline characteristics stratified by ACR.
Table S2: Baseline characteristics stratified by cystatin C.
Table S3: Association of eGFR with progression to low ABI.
Table S4: Association of eGFR with progression to high ABI.
*Note:* The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

**Descriptive Text for Online Delivery of** Supplementary Material
Supplementary Table S1 (PDF)
Baseline characteristics stratified by ACR.
Supplementary Table S2 (PDF)
Baseline characteristics stratified by cystatin C.
Supplementary Table S3 (PDF)
Association of eGFR with progression to low ABI.
Supplementary Table S4 (PDF)
Association of eGFR with progression to high ABI.

**Background—**Low ankle-brachial index (ABI) is a reflection of atherosclerotic disease, and high ABI is an indicator of calcified vessels. The associations of albuminuria and cystatin C with incidence of either low or high ABI are unknown.

**Study Design—**Prospective longitudinal cohort study.

**Setting & Participants—**The Multi-Ethnic Study of Atherosclerosis (MESA) enrolled community-dwelling adults (N=6,814) aged 45–84 years who were free of clinical cardiovascular disease (CVD) at baseline.

**Predictors—**Baseline albumin-creatinine ratio (ACR) and serum cystatin C levels.

**Outcomes—**Development of low (< 0.90), and high (> 1.40) ABI using multinomial regression among persons with ABI 0.90–1.40 at baseline.

**Results—**During 9.8 years of follow up, 221 and 89 participants progressed to low and high ABI, respectively. Baseline ACR and cystatin C were higher among progressors compared to non-progressors. In multivariable analyses, doubling of ACR was associated with increased risk of progression to low (OR, 1.08; 95% CI, 0.99–1.20) and high (OR, 1.16; 95% CI, 1.01–1.32) ABI. Compared to the lowest quintile, the highest quintile of ACR had a significantly increased risk of progression to low (OR, 1.79; 95% CI, 1.03–3.12) and high (OR, 2.76; 95% CI, 1.32–5.77) ABI. Higher cystatin C levels were associated with progression to low (OR per 1-SD greater, 1.12; 95% CI, 1.00–1.26) but not high (OR per 1-SD greater, 1.01; 95% CI, 0.81–1.25) ABI, but the highest quintile of cystatin C was not independently associated with either outcome.

**Limitations—**Single measure of albuminuria and low number of progressors to high ABI.

**Conclusions—**In adults free of clinical CVD, albuminuria was a strong, independent risk factor for the development of both high and low ABI, important and different measures of peripheral artery disease.

## Keywords

Cystatin C; albuminuria; albumin-creatinine ratio (ACR); peripheral artery disease (PAD); ankle-brachial index (ABI); chronic kidney disease (CKD); cardiovascular disease (CVD); atherosclerotic disease

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The ankle-brachial index (ABI) is a simple and inexpensive tool to diagnose peripheral artery disease (PAD).[1] An ABI value < 0.90 has high sensitivity and specificity for PAD by angiography[2] and persons with lower values are at higher risk of adverse PAD and cardiovascular disease (CVD) events.[3] Values at the high end of the spectrum are also associated with increased morbidity and CVD mortality.[4–8] Elevated ABI is more common in persons with either diabetes or advanced kidney disease and is thought to reflect a higher prevalence of medial arterial calcification (MAC) in these populations.[9–11]

Cross sectional analyses of the Multi-Ethnic Study of Atherosclerosis (MESA) and the National Health and Nutrition Examination Survey (NHANES) have demonstrated that albuminuria is associated with increased risk of PAD.[12,13] Furthermore, higher levels of albuminuria have also been associated with coronary artery calcification,[14,15] although the longitudinal association with either peripheral vascular disease development or stiffening

and increasing of the ABI over time is unknown. One study reported an association between serum cystatin C with clinical PAD events using data from the Cardiovascular Health Study (CHS),[16] and in a recent cross sectional analysis from CHS, we demonstrated that lower estimated glomerular filtration rate (eGFR) was associated with both low and high ABI values.[11] Given the cross sectional associations of worse kidney function and albuminuria with both high and low ABI, in the present study we evaluated the association of urine albumin-creatinine ratio (ACR) and cystatin C with progression to abnormal ABI in a community-living population. We hypothesized that higher ACR and cystatin C would each be associated with progression to both a high and low ABI.

## METHODS

### Study Design and Participants

Detailed descriptions of the MESA study design and objectives have been previously published,[17] but, in brief, it is a population-based investigation of the prevalence, correlates, and progression of subclinical CVD. The study cohort comprised 6814 men and women aged 45–84 years, recruited from six US communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St. Paul, MN) from July 2000 through June 2002. Subjects enrolled in MESA were free of clinical CVD at baseline. Institutional review board approval was obtained at all MESA sites.

### Exposure Variables

Urinary albumin concentration was determined by nephelometry, using the Array 360 CE Protein Analyzer (Beckman Instruments Inc); the lowest detectable level was 0.2 mg/dL. Urinary creatinine was measured by the Jaffé rate method using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics Inc); the range was 0.05–16.5 mg/dL, with a coefficient of variation range of 2.5%–2.9%. Spot urine ACR was calculated. Cystatin C was measured using a BN II nephelometer (Siemens; www.medical.siemens.com) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Siemens) on fasting plasma specimens stored at −70°C. The intra-assay coefficient of variation for cystatin C ranged from 2.0% to 2.8%.

### Outcomes

At examinations 1, 3 (3.2 years after examination 1), and 5 (9.5 years after examination 1), systolic blood pressure measurements for calculation of the ABI were obtained using a hand-held Doppler instrument with a 5-mHz probe (Nicolet Vascular, Golden, Colorado). In brief, systolic blood pressures were measured in the bilateral brachial, dorsalis pedis, and posterior tibial arteries. Brachial artery pressures were averaged to obtain the ABI denominator. When the two brachial artery pressures differed by 10 mmHg or more, the highest brachial artery pressure was used as the denominator.[18] For each lower extremity, the ABI numerator used was the highest pressure (dorsalis pedis or posterior tibial) from that leg. The lower of the right and left ABI values was used as the index ABI for that participant. Change in ABI was defined as the difference between ABI at examinations 1 and 5, or the difference between examinations 1 and 3 if examination 5 was not available (9). Progression to low ABI was defined as an ABI transition from 0.90 – 1.40 at baseline to

< 0.90. Progression to high ABI was defined as transition from ABI 0.90 – 1.40 to ABI > 1.40.

## Covariates

All participants completed self-administered questionnaires and standardized interviews by trained research staff to collect information for demographic characteristics, medical history, and smoking status. Trained and certified clinic staff obtained blood pressure and anthropometric measurements during each visit. Hypertension was defined as self-reported treatment for hypertension or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg. Diabetes was defined as fasting glucose level ≥126 mg/dL or use of oral hypoglycemic medications or insulin. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and high-sensitivity C-reactive protein (hsCRP) were measured using blood samples obtained after a 12-hour fast. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[19] A BN II nephelometer was used to measure hsCRP.

## Statistical Analyses

We evaluated the distribution of demographic characteristics, traditional cardiovascular risk factors, anthropometry measures and laboratory values by quintiles of ACR and cystatin C level. Continuous data were presented as mean ± standard deviation (SD), and categorical variables, as proportions. Because ACR values were not normally distributed, ACR was log transformed to the base 2 and analyses were reported per doubling of ACR. Differences across ABI progression groups were assessed using Chi-square tests for categorical variables and ANOVA for continuous variables. The longitudinal association of cystatin C level with development of both high and low ABI was modeled as a continuous variable per 1-SD increase using multinomial ordinal logistic regression with a baseline category logit model. We then investigated the longitudinal associations of quintiles of ACR and cystatin C with ABI outcomes. Covariates were selected based on their biologically plausible potential to confound the association between ACR, cystatin C level, and incident low and high ABI. Analyses were adjusted for demographic factors, cardiovascular risk factors including diabetes, hypertension, smoking, BMI, LDL cholesterol, HDL cholesterol, hsCRP, antiplatelet agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and statin use and were further adjusted for cystatin C level in ACR analyses and for ACR in cystatin C analyses. Finally we adjusted for the baseline ABI of participants in order to control for regression to the mean. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported. We evaluated subgroups based on the presence or absence of chronic kidney disease defined by either eGFR < 60 ml/min/1.73m$^2$ using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) cystatin C equation[20] or ACR >30 mg/g, given that prior studies had shown increased adverse kidney, cardiovascular and mortality outcomes in persons with chronic kidney disease. [21,22,23]

In sensitivity analysis, we evaluated the association of eGFR defined using the CKD-EPI cystatin C equation and change in ABI both in continuous models and across quintiles of eGFR.[20]

We also tested for interactions between ACR and diabetes for the ABI endpoints given that albuminuria is differentially associated with PAD in persons with and without diabetes in MESA.[12] We also evaluated for interactions between cystatin C and diabetes status.

## RESULTS

### Study Population

Of the 6814 participants in MESA, we excluded participants who at baseline were missing ABI measurements (n=79), had ABI < 0.90 (n=252) or >1.40 (n=44); were missing cystatin C (n=53), urine albumin (n=26), or urine creatinine (n=6) measurements; or were missing ABI data at any follow-up examinations (n=697). We also excluded those who met the inclusion criteria but had an ABI <0.9 at examination 3 and >0.9 at examination 5 (n=38); or an ABI > 1.40 at examination 3 but <1.40 at examination 5 (n=28), as these individuals had inconsistent outcomes. Follow-up ABI measures at examination 5 were used for 5482 participants and at examination 3 in 108 participants. Thus, the final sample for analysis comprised 5,591 individuals.

Overall, the mean age of participants included in this analysis was 61 ± 10 (SD) years, 47% were men, 40% were white, 26% were African-American, 22% were Hispanic and 12% were Chinese. Hypertension was present in 42% of the population and diabetes in 11%. Median ACR was 5.1 (interquartile range, 3.2–10.0) mg/g and mean cystatin C was 0.88 ± 0.2 mg/L (Tables S1 and S2, available as online supplementary material). Participants who progressed to a low or high ABI during follow-up were older than those who remained within the reference ABI category (Table 1). Diabetes, hypertension and smoking were more prevalent among those progressing to low ABI than among non-progressors. Baseline ACR and cystatin C were higher among progressors compared to non-progressors. The mean ABI was lower in those who progressed to ABI < 0.90 and higher in those who progressed to ABI > 1.40.

### Progression to Low ABI

Over a median follow up of 9.8 years, 221 of 5591 participants (4%) progressed to an ABI < 0.90. The incidence of progression to low ABI increased linearly from 1.7% in the lowest quintile of ACR to 6.5% in those in the highest quintile. A similar trend was seen across quintiles of cystatin C starting at 2.5% in the lowest quintile to 7.9% in those in the highest quintile (Figure 1).

Each 2-fold higher ACR was associated with progression to ABI < 0.90 in demographic-, CVD risk factor–, and kidney function–adjusted analysis. This association was no longer statistically significant after adjustment for baseline ABI. In demographic-adjusted analysis, compared to the lowest quintile of ACR (<3 mg/g), the highest quintile (>14 mg/g) demonstrated a more than 2-fold greater risk of progression to low ABI, and although attenuated, this association remained strong and significant even after adjusting for other confounding variables, cystatin C level, and ABI value at baseline (Table 2). Compared to those with ACR ≤ 30 mg/g, participants with ACR ≥ 30 mg/g were not at significantly increased risk of progression to low ABI. When modeled as a continuous variable, each 1-

SD greater cystatin C level was associated with increased risk of progression to low ABI. This was attenuated after multivariable adjustment but remained statistically significant. Higher quintiles of cystatin C were not significantly associated with low ABI after multivariable adjustment.

Our results were similar when modeled with kidney function using eGFR as a continuous variable and quintiles (Table S3) The risk of progression to low ABI was also not greater among those with eGFR < 60 ml/min/1.73 $m^2$ compared to persons with eGFR ≥ 60 ml/min/1.73 $m^2$.

### Progression to High ABI

Over a median of 9.8 years, 89 of 5591 participants (1.6%) progressed to an ABI > 1.40. The incidence of progression to high ABI was greater in the highest quintile (2.4%) of ACR compared to the lowest (1.2%) although a linear trend was not observed. Similarly the incidence of progression to high ABI was greatest in the highest quintile of cystatin C at 2.2% compared to lowest quintile of 1% (Figure 1).

Each 2-fold greater ACR was associated with greater risk of progression to ABI > 1.40 across the series of adjusted models. After adjusting for confounding variables, cystatin C and baseline ABI values, the highest ACR quintile had a more than 2.5 times the risk of high ABI compared with the lowest quintile. Similarly, an ACR >30 mg/g was independently associated with a doubling in risk of developing high ABI (Table 3). Cystatin C levels were not associated with incident high ABI in the linear variable–, quintile-, or eGFR <60 ml/min/1.73 $m^2$–dichotomized analyses.

In addition, no association of progression to high ABI was seen when eGFR was modeled as a continuous or categorical variable (Table S4).

The presence of diabetes did not modify the association between ACR and progression to low ABI (p=0.6) or high ABI (p=0.3), and between cystatin C and progression to low ABI (p=0.4) or high ABI (p=0.7).

## DISCUSSION

In this large multiethnic cohort, we found an association between elevated ACR levels and incident low and high ABI, independent of cardiovascular risk factors and cystatin C. Cystatin C levels were associated with an increased risk of progression to low ABI in continuous models only, not across quintiles, and had no association with development of high ABI. Our study therefore suggests that albuminuria is the dominant marker of kidney function which is associated with progression of ABI to both abnormally low and high values. To our knowledge, this is the first study to prospectively evaluate the relationship between albuminuria and cystatin C with longitudinal changes in ABI.

Prior studies have reported associations between albuminuria and ABI in cross sectional analyses. In MESA, Wattanakit and colleagues noted that after adjusting for cardiovascular risk factors, the presence of albuminuria was associated with a 65% greater odds of PAD (defined as ABI < 0.90 or > 1.40) in persons with diabetes.[12] Using data from NHANES

1999–2004, Wu et.al noted that albuminuria (ACR > 30 mg/g) was associated with PAD (defined as ABI < 0.90 or > 1.40) in non-diabetic individuals (OR, 1.87; 95% CI, 1.34–3.95; p<0.001).[13] In the Strong Heart Study, albuminuria was strongly associated with both low and high ABI at baseline.[5,24] Data from this cohort also demonstrated that the association of high ABI and mortality was similar to that seen with a low ABI.[5] More recently, in a study of 185 persons with type 1 diabetes, levels of albuminuria were significantly higher among persons with medial arterial calcification or a high ABI (>1.30) compared to persons without these abnormalities.[25] Our results provide a longitudinal view of these previously published cross-sectional findings. We demonstrate that albuminuria is associated with incident subclinical vascular disease independent of other cardiovascular risk factors, and that the association is similar irrespective of diabetic status. While the exact reason for the lack of graded increase in risk with increasing levels of albuminuria is unclear, it is possible that albuminuria may be non-linearly associated with progression to both a low and high ABI.

Albuminuria is a marker of generalized endothelial dysfunction,[26,27] which is a risk factor for atherosclerosis and PAD. The ABI is a simple, inexpensive, and noninvasive measure of subclinical CVD and atherosclerosis used clinically to confirm suspicion of occlusive PAD.[1] Medial arterial calcification leads to abnormal elevations of the ABI and may result in either a false normal ABI value or, in extreme cases, high ABI measurements. The incidence of MAC has also been reported to be high among those with diabetes and albuminuria particularly in the setting of concomitant peripheral neuropathy.[28] Peripheral neuropathy leading to autonomic denervation of arterial smooth muscle walls has been postulated as a risk factor for development of MAC[29,30] and has also been associated with other micro-vascular complications such as proteinuria and retinopathy.[31] Numerous studies have also reported that albuminuria is associated with arterial stiffness,[32,33] and calcifications in the arterial tree.[14,15,34] It is thus plausible that MAC and resulting arterial stiffness may be consequences of complex interactions between areas of vascular endothelial damage, neuropathy, albuminuria and other vascular risk factors. Although studies have shown that atherosclerosis and MAC are disparate entities,[35] they share a number of risk factors including male sex, older age, longer duration of diabetes and lower eGFR.[30,36,37] The presence of albuminuria preceding development of low and high ABI abnormalities provides a novel temporal insight, suggesting that albuminuria may be an early marker of vascular dysfunction. This may identify persons at risk of both peripheral atherosclerosis and vascular stiffening at an earlier stage.

Previous studies have demonstrated an association between cystatin C and prevalent PAD in cross sectional analyses. Using data from NHANES 1999–2002, Selvin et.al found a graded independent association between cystatin with PAD, defined as ABI < 0.90.[38] Similarly, in a cohort of 1,609 Chinese patients with type 2 diabetes, the odds of PAD (defined as ABI < 0.90) increased with serum cystatin C levels > 1.2 mg/L.[39] To our knowledge, only one study has prospectively evaluated the association between serum cystatin C and risk for incident PAD events. O'Hare and colleagues evaluated the association of cystatin C with clinical PAD events in 4,025 community-dwelling older adults (aged 65 years or older) from the CHS.[16] They noted a 2.5-fold higher risk exclusively among persons in the highest quartile of cystatin C compared to those in the lowest quartile of cystatin C. There are a number of differences between our study and that from the CHS, which may explain

differences in the observed results. First, the CHS population is an older population with a greater burden of pre-existing CVD compared to the MESA cohort, which was free of CVD at baseline. Second, the CHS study used clinical PAD (revascularization and amputation) as an endpoint rather than change in ABI as in our study. It is possible that cystatin C is a better predictor of clinical cardiovascular events in those at higher risk and in the elderly[40] than progression of subclinical disease in younger and lower risk individuals. Third, the CHS did not adjust for level of albuminuria. The lack of association of cystatin C to subclinical PAD in comparison to albuminuria may also suggest that albuminuria is a better marker of generalized vascular disease burden

Our study has several limitations. First, there are relatively few individuals who reach the ABI boundaries, particularly the high ABI cutoff; therefore it is possible we may be limited by statistical power. Second, we only had one measure of albuminuria. Transient albuminuria may therefore have resulted in a misclassification bias, although this would bias the results towards the null. Finally, the reduction in our final sample size due to missing data at baseline and follow up could bias our results. We avoided the use of multiple imputations since we believe the data are not missing at random. Participants with more comorbidities were less likely to appear at subsequent MESA visits. For not-missing-at-random data, multiple imputations can produce optimal inferences. However, this requires that the missing data mechanism be modeled as part of the inference. The main problem is that the data contain no information to determine the correct model for the missing data mechanism and thus inference may be sensitive to model choice. Our study also has numerous strengths. To our knowledge, this is the first study to prospectively evaluate the relationship between albuminuria and cystatin C with longitudinal changes in ABI. The MESA cohort is a large community-dwelling cohort and its results are generalizable given the broad age range and diverse race and ethnic mixture of the population. The assessment of risk factors and clinical outcomes of MESA is also extremely robust.

In summary, in a multi-ethnic cohort free of clinical cardiovascular disease at baseline, the presence of albuminuria was a strong independent risk factor for the development of both high and low ABI. Cystatin C levels were weakly associated only in continuous models with increased risk of progression to low ABI, and not associated with progression to high ABI. Further studies are needed to confirm these findings and to evaluate if earlier treatment of vascular disease risk factors like albuminuria may prevent progression to abnormal ABI values and subsequent adverse outcomes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The authors thank the other investigators, the staff, and the participants of MESA for their valuable contributions. A full list of participating MESA investigators and institutions can be found at www.mesa-nhlbi.org.

*Support:* This research was supported by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the National Heart, Lung and Blood Institute, by grants UL1-TR-000040 and UL1-

RR-025005 from the National Center for Resaearch Resources and K24 DK078204 from the National Institute of Diabetes and Digestive and Kidney Diseases. The funding agencies had no role in the study design; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication.

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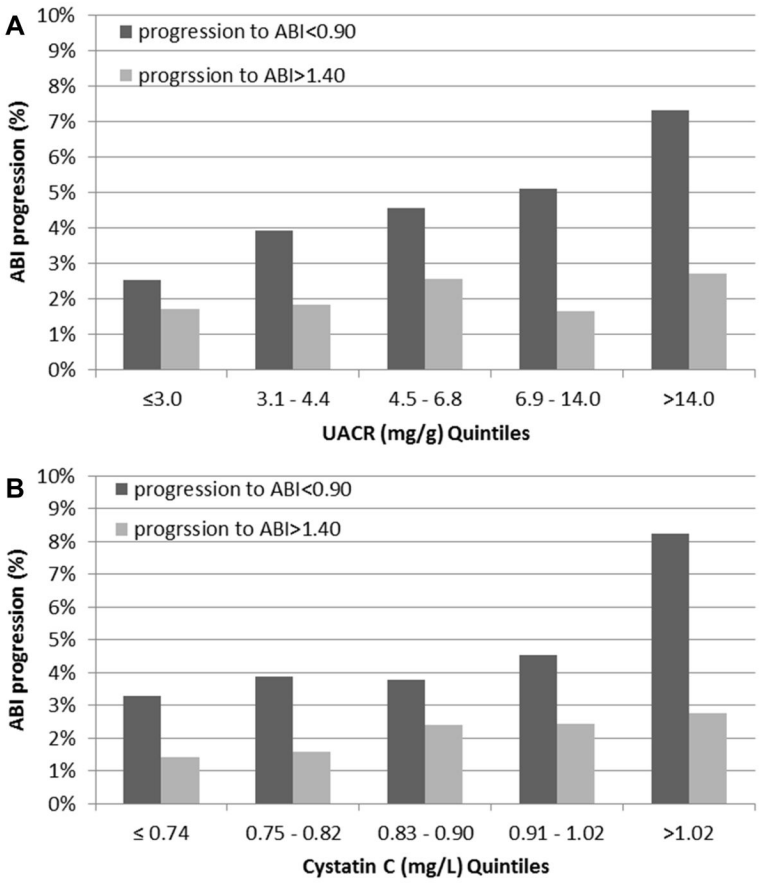


**Figure 1.**

A. Incidence of progression to low and high ABI, by quintiles of ACR B. Incidence of progression to low and high ABI by quintiles of Cystatin C Abbreviations: ABI- ankle-brachial index, ACR- albumin-creatinine ratio

## Table 1

Baseline characteristics stratified by ABI outcome

| Characteristic | Progression to ABI <0.90 | ABI remained 0.90–1.40 | Progression to ABI >1.40 | P * |
|---|---|---|---|---|
| No. of participants | 221 | 5281 | 89 | |
| Age (y) | 68 ±9 | 61 ±10 | 63 ±10 | <0.001 |
| Male sex | 87 (39) | 2498 (47) | 67 (75) | <0.001 |
| Race/Ethnicity | | | | <0.001 |
| White | 74 (34) | 2092 (40) | 51 (57) | |
| Chinese | 16 (7) | 666 (13) | 3 (3) | |
| Black | 91 (41) | 1368 (26) | 13 (15) | |
| Hispanic | 40 (18) | 1155 (22) | 22 (25) | |
| BMI (kg/m$^2$) | 28.9 ±5.8 | 28.2 ±5.3 | 29.7 ±4.9 | 0.006 |
| Diabetes | 47 (21) | 538 (10) | 17 (19) | <0.001 |
| Hypertension | 141 (64) | 2196 (42) | 37 (42) | <0.001 |
| SBP (mm Hg) | 136 ±22 | 125 ±21 | 124 ±18 | <0.001 |
| DBP (mm Hg) | 71 ±11 | 72 ±10 | 72 ±10 | 0.7 |
| Smoking | | | | 0.002 |
| Never | 90 (41) | 2732 (52) | 45 (51) | |
| Former | 90 (41) | 1897 (36) | 39 (44) | |
| Current | 40 (18) | 639 (12) | 5 (6) | |
| LDL cholesterol (mg/dL) | 119 ±34 | 117 ±31 | 110 ±31 | 0.07 |
| HDL cholesterol (mg/dL) | 49 ±14 | 51 ±15 | 47 ±14 | 0.002 |
| hsCRP (mg/dL) | 2.95 [1.01–5.05] | 1.80 [0.80–4.08] | 1.89 [0.94–3.25] | <0.001 |
| Cystatin C (mg/L) | 0.99 ±0.34 | 0.88 ±0.19 | 0.92 ±0.19 | <0.001 |
| Urine ACR (mg/g) | 7.0 [4.3–19.9] | 5.0 [3.2–9.7] | 6.1 [4.0–15.0] | <0.001 |
| Baseline ABI | 1.05 ±0.08 | 1.13 ±0.09 | 1.20 0.10 | <0.001 |

* p for difference between groups

Note: Values for categorical variables are given as number (percentage); values for continuous variables are given as mean ± standard deviation or median [interquartile range].

ABI- ankle-brachial index, BMI- body mass index, SBP- systolic blood pressure, DBP- diastolic blood pressure, LDL-low density lipoprotein, HDL- high density lipoprotein, hsCRP- high-sensitivity C-reactive protein, ACR- albumin-creatinine ratio

**Table 2**

Association of ACR and cystatin C with progression to low ABI

| | N | No. of progressors | Demographic adjusted * | Risk factor adjusted** | Kidney function adjusted† | Further adjusted for baseline ABI |
|---|---|---|---|---|---|---|
| ACR | | | | | | |
| Continuous, per doubling | 5591 | 221 | 1.18 (1.10, 1.28) | 1.10 (1.02, 1.20) | 1.09 (1.00, 1.18) | 1.08 (0.99, 1.18) |
| Quintiles | | | | | | |
| ≤ 3.0 mg/g | 1211 | 21 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| 3.1 – 4.4 mg/g | 1184 | 41 | 2.10 (1.23, 3.61) | 2.10 (1.21, 3.66) | 2.14 (1.23, 3.72) | 2.14 (1.22, 3.74) |
| 4.5 – 6.8 mg/g | 1126 | 46 | 2.08 (1.22, 3.55) | 1.87 (1.08, 3.24) | 1.90 (1.10, 3.29) | 1.86 (1.07, 3.25) |
| 6.9 – 14.0 mg/g | 1083 | 49 | 2.00 (1.17, 3.39) | 1.72 (0.99, 2.97) | 1.73 (1.00, 3.00) | 1.71 (0.98, 2.99) |
| > 14.0 mg/g | 987 | 64 | 2.63 (1.58, 4.40) | 1.89 (1.09, 3.26) | 1.85 (1.07, 3.19) | 1.79 (1.03, 3.12) |
| ACR ≥ 30 vs <30 mg/g | 447 | 39 | 1.97 (1.35, 2.87) | 1.43 (0.96, 2.14) | 1.35 (0.90, 2.03) | 1.24 (0.81, 1.89) |
| Cystatin C | | | | | | |
| Continuous, per 0.2-mg/L greater^ | 5591 | 221 | 1.22 (1.10, 1.36) | 1.15 (1.03, 1.29) | 1.13 (1.01, 1.26) | 1.12 (1.00, 1.26) |
| Quintiles | | | | | | |
| ≤ 0.74 mg/L | 1269 | 32 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| 0.75 – 0.82 mg/L | 1172 | 33 | 0.97 (0.59, 1.61) | 0.91 (0.55, 1.51) | 0.92 (0.55, 1.53) | 0.92 (0.55, 1.54) |
| 0.83 – 0.90 mg/L | 1079 | 38 | 1.09 (0.67, 1.78) | 1.00 (0.61, 1.66) | 1.02 (0.62, 1.69) | 1.07 (0.64, 1.78) |
| 0.91 – 1.02 mg/L | 1133 | 44 | 0.99 (0.61, 1.60) | 0.83 (0.50, 1.37) | 0.84 (0.51, 1.39) | 0.85 (0.51, 1.42) |
| > 1.02 mg/L | 938 | 74 | 1.66 (1.04, 2.64) | 1.25 (0.77, 2.03) | 1.23 (0.76, 2.01) | 1.20 (0.73, 1.98) |
| eGFR <60 vs ≥60 mL/min/1.73 $m^2$ | 415 | 39 | 1.43 (0.97, 2.11) | 1.31 (0.80, 2.13) | 1.21 (0.74, 1.99) | 1.13 (0.67, 1.89) |

Note: Values are given as odds ratio (95% confidence interval). Low ABI defined as ABI < 0.90.

* Adjusted for age, gender, race

** further adjusted for systolic blood pressure, antihypertensive medications, diabetes, smoking, body mass index, low-density lipoprotein cholesterol, high -density lipoprotein cholesterol and C-reactive protein, antiplatelet agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and statin use

† further adjusted for ACR in cystatin C models and cystatin C in ACR models

^ increment equivalent to 1 standard deviation.

ABI- ankle-brachial index, ACR, albumin-creatinine ratio; eGFR- estimated glomerular filtration rate

**Table 3**

Association of ACR and cystatin C with progression to high ABI

| | N | No. of progressors | Demographic adjusted* | Risk factor adjusted** | Kidney function adjusted† | Further adjusted for baseline ABI |
|---|---|---|---|---|---|---|
| ACR | | | | | | |
| Continuous, per doubling | 5591 | 89 | 1.17 (1.04, 1.31) | 1.15 (1.01, 1.31) | 1.15 (1.01, 1.32) | 1.16 (1.01, 1.32) |
| Quintiles | | | | | | |
| ≤ 3.0 mg/g | 1211 | 15 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| 3.1 – 4.4 mg/g | 1184 | 14 | 1.05 (0.50, 2.20) | 1.19 (0.56, 2.53) | 1.19 (0.56, 2.53) | 1.13 (0.53, 2.41) |
| 4.5 – 6.8 mg/g | 1126 | 23 | 2.18 (1.12, 4.26) | 2.42 (1.22, 4.82) | 2.43 (1.22, 4.84) | 2.42 (1.21, 4.83) |
| 6.9 – 14.0 mg/g | 1083 | 13 | 1.32 (0.62, 2.85) | 1.44 (0.65, 3.18) | 1.44 (0.60, 3.18) | 1.37 (0.62, 3.04) |
| > 14.0 mg/g | 987 | 24 | 2.57 (1.31, 5.06) | 2.74 (1.31, 5.70) | 2.74 (1.31, 5.70) | 2.76 (1.32, 5.77) |
| ACR ≥ 30 vs < 30 mg/g | 447 | 13 | 2.21 (1.19, 4.10) | 2.00 (1.04, 3.83) | 2.00 (1.04, 3.86) | 2.08 (1.07, 4.06) |
| Cystatin C | | | | | | |
| Continuous, per 0.2-mg/L greater^ | 5591 | 89 | 1.08 (0.88, 1.33) | 1.03 (0.82, 1.28) | 1.00 (0.80, 1.24) | 1.01 (0.81, 1.26) |
| Quintiles | | | | | | |
| ≤ 0.74 mg/L | 1269 | 13 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| 0.75 – 0.82 mg/L | 1172 | 13 | 0.87 (0.40, 1.90) | 0.86 (0.39, 1.89) | 0.88 (0.40, 1.93) | 0.87 (0.40, 1.92) |
| 0.83 – 0.90 mg/L | 1079 | 22 | 1.42 (0.70, 2.87) | 1.33 (0.65, 2.73) | 1.39 (0.67, 2.85) | 1.33 (0.64, 2.76) |
| 0.91 – 1.02 mg/L | 1133 | 20 | 1.17 (0.56, 2.43) | 1.02 (0.47, 2.17) | 1.06 (0.50, 2.28) | 1.04 (0.48, 2.24) |
| > 1.02 mg/L | 938 | 21 | 1.46 (0.69, 3.10) | 1.20 (0.55, 2.65) | 1.17 (0.53, 2.58) | 1.25 (0.56, 2.78) |
| eGFR <60 vs ≥60 mL/min/1.73 $m^2$ | 415 | 7 | 0.97 (0.43, 2.18) | 0.91 (0.35, 2.35) | 0.80 (0.31, 2.10) | 0.89 (0.34, 2.32) |

Note: Values are given as odds ratio (95% confidence interval). High ABI defined as ABI > 1.40.

*Adjusted for age, gender, race

**further adjusted for systolic blood pressure, antihypertensive medications, diabetes, smoking, body mass index, low-density lipoprotein cholesterol, high -density lipoprotein cholesterol and C-reactive protein, antiplatelet agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and statin use

†further adjusted for ACR in cystatin C models and cystatin C in ACR models

^increment equivalent to 1 standard deviation.

ABI- ankle-brachial index, ACR, albumin-creatinine ratio; eGFR- estimated glomerular filtration rate