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## New roles for renin in heart failure and cardio-renal interaction

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Schroten, N. (2015). *New roles for renin in heart failure and cardio-renal interaction*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

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PLASMA RENIN AND ALDOSTERONE CONCENTRATIONS  
AND RENAL FUNCTION DECLINE IN A COMMUNITY  
BASED COHORT

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Manuscript in preparation

## ABSTRACT

**Aims** – The renin-angiotensin-aldosterone-system (RAAS) plays a pivotal role in hypertension, cardiovascular (CV), and kidney disease. However, it is unclear if elevated RAAS activity contributes to an accelerated renal function decline in healthy subjects, independent from other risk factors.

**Methods** – We studied 5,109 subjects not on antihypertensive medication from the community based cohort Prevention of REnal and Vascular ENd-stage Disease (PREVEND). Generalized estimating equations (GEE) were used to examine the association of plasma renin concentration (PRC) and aldosterone (PAC) with the decline of estimated glomerular filtration (eGFR, calculated using the CKD-EPI cystatin C formula). Cox-regression analysis were performed for the outcome eGFR<60 and < 45 ml/min/1.73m<sup>2</sup>.

**Results** – At baseline, mean age was 48 (±12) years, 46% was male, mean eGFR was 95±18 ml/min/1.73m<sup>2</sup>, PRC 17.3 (10.6-26.7) µIU/mL and PAC 117 (92-151) pg/mL. After 12 years of follow-up 344 subjects reached an eGFR < 60 and 103 subjects < 45ml/min/1.73m<sup>2</sup>. Univariable cox regression analysis showed an association of PRC (HR 0.79 p < 0.001) and ARR (HR 1.2 p=0.001) with incidence for CKD (< 60 ml/min/1.73m<sup>2</sup>). However, after correction for age, sex and baseline eGFR this association was not significant anymore. GEE analysis confirmed that PRC, PAC and ARR are not associated with accelerated eGFR decline. Only in subjects with low NT-proBNP, the association between PRC and eGFR remained significant long-term.

**Conclusion** – In this community based cohort, high PRC and PAC are associated with a lower eGFR at baseline, but not with long-term eGFR decline. These data do not support screening for PRC or PAC to identify patients at risk for the development of CKD.

## INTRODUCTION

During the last decades the prevalence of chronic kidney disease (CKD) has risen. (1) CKD is associated with an increased risk for cardiovascular (CV) events and mortality – even minor changes in glomerular filtration rate and urinary albumin excretion (UAE) strongly suggest imposing an increased risk (2). Identification of patients at risk to develop CKD is therefore feasible.

Medical interventions in the renin-angiotensin-aldosterone system (RAAS), with angiotensin-converting-enzyme inhibitors (ACEi), angiotensin-II receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs), (3) have shown to be effective in the treatment of elevated albuminuria and renal function decline. (4,5) Furthermore it appears that the beneficial effects are independent from blood pressure. (6,7) However, other trial demonstrated that double RAAS blockade may be harmful. (8,9) Nonetheless it is clear that the RAAS plays a pivotal role in the development of CKD. However, only a few studies have investigated the role of circulating hormones of the RAAS in the development of CKD in the general population, and they have shown conflicting results. In the Framingham Offspring study high aldosterone levels, but not renin, were associated with increased incidence for CKD. (3) In contrast, high renin levels were associated with increased incidence of CKD in treated hypertensive patients from the ASCOT trial and the Kaiser Permanente Southern California cohort. (10,11) A major confounder for plasma levels of renin and aldosterone is the use of anti-hypertensive medication – and nearly all published studies included patients on medication. Secondly, these studies analyzed CKD as a dichotomous outcome (eGFR < 60 or 50% increase in creatinine), which is also largely dependent on baseline creatinine and are therefore not suitable to study both patients high and low creatinine at baseline.

Given the profound effects of ACEi on renal function protection, which appears to be blood pressure independent, (7) we hypothesized that (low key) activation of the renin angiotensin system would be associated with accelerated renal function decline over time, especially in patients not on anti-hypertensive medication. Surprisingly, there are to date no studies that link circulating levels of hormones of the RAAS in normotensive subjects with renal function changes. Therefore, in the current study we investigate the association between plasma renin concentration (PRC), plasma aldosterone concentration (PAC) and the aldosterone-renin-ratio (ARR) with renal function decline in a large community based cohort, not using anti-hypertensive medication.

## METHODS

The current study is an analysis in the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort. Details have been described previously. (12,13) In brief, PREVEND is a community based cohort in the city of Groningen, the Netherlands. In the years 1997 and 1998 all inhabitants between 28 and 75 years of age were asked to collect a vial of early morning urine and fill out a questionnaire. The response rate was 47.8% (n=40,856 persons). Individuals with type 1 diabetes mellitus and pregnant women were excluded. Urinary albumin was measured in a central laboratory. Six thousand subjects with a UAE equal or greater than 10 mg/L were selected. A control group of 2,592 individuals was randomly selected from individuals with an UAE below 10 mg/L. All selected individuals were asked to collect two consecutive 24-hour urines and were followed up after 4, 6, 9 and 12 years in the outpatient clinic. For the current analyses we selected individuals with available baseline PRC and PAC measurements, and that did not take anti-hypertensive medication (n = 5,109), since anti-hypertensive medication strongly influences RAAS activation. (13)

### LABORATORY MEASUREMENTS

EDTA-anticoagulated plasma was frozen in vials and stored at -80C directly after collection and never thawed before assaying. We measured PRC using an automated sandwich immunochemiluminescent assay (LIAISON<sup>®</sup>, Diasorin, DiaSorin Ltd, Schiphol Rijk, and The Netherlands), with an intra-assay precision of 7.2% and inter-assay precision of < 10.4%. Plasma aldosterone concentration was measured using an ELISA kit (Alpco, Salem, NH, USA). Intra-assay precision 6.6% and inter-assay precision 9.6%, as described previously. (13)

Cystatin-C (mg/L) was measured using a BNII nephelometer (Dade Behring Inc., Deerfield, IL). Intra-assay coefficients of variation is between 2.0 and 2.8% and inter-assay between 2.3 and 3.1%. Creatinine, plasma glucose and serum cholesterol were measured by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York). Urinary albumin concentrations were measured by nephelometry, with a threshold of 2.3 mg/L and intra- and inter-assay coefficients of variation of 4.3% and 4.4%, respectively (Dade Behring Diagnostic, Marburg, Germany).

### DEFINITIONS AND CALCULATIONS

Blood pressure was calculated as the mean of two measurements. Body mass index (BMI) was calculated as the ratio of weight and height squared (kg/m<sup>2</sup>). Type

2 diabetes mellitus (T2DM) was defined as a fasting glucose level of  $\geq 7.0$  mmol/L (126 mg/dL) or a non-fasting glucose level of  $\geq 11.1$  mmol/L (200 mg/dL), or the use of anti-diabetic drugs. Smoking was defined as current smoking or stopped smoking within the previous year. Urinary albumin excretion (UAE) was calculated as the average UAE in the two consecutive baseline 24 h urine collections (mg/L). An estimate of the glomerular filtration rate (GFR) was calculated using the CKD-epi equation, which provides a more accurate estimation of GFR than the MDRD formula. (14)

## STATISTICAL ANALYSIS

Normally distributed variables are presented as means $\pm$ SD; non-normally distributed variables are presented as medians (with 25<sup>th</sup> percentile – 75<sup>th</sup> percentile), and transformed using the natural logarithm when appropriate for statistical tests. The association of PRC, aldosterone and traditional risk factors for CKD with baseline kidney function (CKD-EPI cystatin C) was tested using linear regression. Variables for the multivariable analysis were selected by stepwise backward selection with a threshold of  $p < 0.1$ . Interaction of the co-variables with renin and aldosterone were tested by adding the product of PRC, PAC or ARR with the variable of interest to the model.  $P < 0.05$  was considered statistically significant. Collinearity was tested by computation of the uncentered variance inflation factors.

To study the associations of PRC and PAC with renal function over time, first we used a generalized estimating equations (GEE) analysis with an exchangeable correlation structure. This longitudinal analysis technique makes it possible to include factor variables and study the relation of the baseline variables with time, also in a non-linear fashion. The relation of age with renal function was tested using age as a continuous variable and an ordinal variable per 10 years to study a possible non-linear relation. The association of PRC, PAC and ARR with renal function decline was investigated by adding the interaction of PRC, PAC or ARR with follow-up time to the model. Correction for traditional risk factors was done by adding the variable of interest to the model and by adding the interaction with follow-up time and PRC, PAC or ARR to the model. A negative interaction represents accelerated renal function decline in ml/min/1.73 m<sup>2</sup> per SD increase of the risk variable at baseline. This analysis was repeated with follow up visits as categorical variables instead of follow up time to investigate non-linear relationships in time.

In order to further clarify the associations of baseline PRC, PAC and ARR with eGFR in time we performed a COX-regression analysis for the outcome CKD defined as CKD-EPCys  $< 60$  and  $< 45$  ml/min/1.73 m<sup>2</sup> was performed. Three models were tested; Model 1 using PRC, aldosterone or ARR as univariate predictors; Model 2 was additionally corrected for age, gender and baseline CKD-EPI. Model

3 was corrected for age, gender, baseline CKD-EPI and traditional risk factors for CKD (systolic blood pressure, diabetes, BMI, history of myocardial infarction and hypercholesterolemia). Proportional hazard assumption was tested on the basis of Schoenfeld residuals.

## SENSITIVITY ANALYSES

A previous study demonstrated gender differences in the association of risk factors with kidney disease (15), therefore we also performed gender stratified analyses. The analyses were also repeated stratified for baseline 24-hour UAE below and above 10 mg/L, to account for enrichment of the cohort with subjects with higher albuminuria.

## RESULTS

Baseline characteristics of the individuals included in the analyses are shown in table 1. In brief, mean age was 48 ( $\pm 12$ ) years, 46% was male, mean eGFR-cys was  $95 \pm 18$  ml/min/ $1.73\text{m}^2$  and median 24h-UAE was 9.1 (6.3-16.2) mg/24h, PRC 17.3 (10.6-26.7)  $\mu\text{IU/mL}$  and PAC 117 (92-151) pg/mL.

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### ASSOCIATION OF PRC AND PAC WITH BASELINE CKD-EPI

Linear regression of PRC (corrected for age and gender) showed that high baseline PRC was associated with an increased cystatin C and decreased CKD-EPI at baseline ( $p < 0.001$ ). High PAC was associated with high creatinine and cystatin C and decreased CKD-EPI at baseline (all  $p < 0.001$ ).

The relation of PRC and PAC with CKD-EPI remained significant ( $p < 0.001$ ) in a multivariate model including traditional risk factors such as age, sex, diabetes, heart rate, smoking, hypercholesterolemia, BMI, and markers for volume homeostasis including 24h urinary sodium, pro-AVP and NT-proBNP (table 2). Using backward selection, systolic blood pressure, UAE and history of myocardial infarction were excluded from the model at  $p > 0.1$ . There was a significant interaction of NT-proBNP (beta 0.49  $p = 0.011$ ) and smoking (beta = -1.22,  $p = 0.003$ ) with PRC for the outcome CKD-EPI. Aldosterone had a significant interaction with BMI (beta = 0.43  $p = 0.03$ ), hypercholesterolemia (beta = -1.15  $p = 0.015$ ) and smoking (beta = -1.16  $p = 0.005$ ) for the outcome CKD-EPI.

**TABLE 1: BASELINE CHARACTERISTICS**

<b>Characteristics</b>	<b>Total (n = 5,109)</b>
Age (years)	48.3 (±12.1)
Female, n (%)	2766 (54%)
Currently smoking, n (%)	2035 (40%)
History of CVD, n (%)	60 (1%)
Diabetes, n (%)	155 (3%)
Systolic BP (mmHg)	126.8 (±19.2)
Diastolic BP (mmHg)	73.3 (±9.5)
Heart rate (bpm)	69.7 (±10.0)
Waist circumference (cm)	87.4 (±12.8)
BMI (kg/m <sup>2</sup> )*	25.8 (±4.1)
Renin (PRC, µIU/ml)*	17.3 (10.6-26.7)
Aldosterone (PAC, pg/ml)*	117.1 (91.9-150.5)
ARR*	7.0 (4.5-11.4)
Plasma glucose (mmol/l)*	4.7 (4.4-5.1)
Serum Cholesterol (mmol/l)*	5.5 (4.9-6.3)
Triglycerides (mmol/l)*	1.1 (0.8-1.6)
hs-CRP (mg/L)	2.6 (±5.1)
NT-proBNP (pg/ml)*	36.8 (16.7-68.6)
Urinary sodium (mmol/24h)*	135.1 (104.9-169.4)
24h UAE (mmol/24h)*	9.1 (6.3-16.2)
Serum Creatinine (umol/l)	76.3 (±14.6)
eGFR-MDRD (ml/min/1.73m <sup>2</sup> )	89.2 (±16.5)
eGFR-Cys (ml/min/1.73m <sup>2</sup> )	94.9 (±17.9)
eGFR-CysCr (ml/min/1.73m <sup>2</sup> )	92.5 (±14.7)

*Normally distributed variables are presented as means±SD.*

*\* Non-normally distributed continuous variables are presented as median value (25-75th percentiles); ARR, aldosterone/renin ratio; BP, blood pressure; bpm, beats per minute. eGFR, estimated glomerular filtration rate by simplified Modification of Diet in Renal Disease (eGFR) formula; UAE, urinary albumin excretion; Conversion of N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels: 100 pg/mL equates to 11.82 pmol/L.*



TABLE 2: MULTIVARIATE ASSOCIATION OF RISK FACTORS WITH RENAL FUNCTION (EGFR-CYS) AT BASELINE

	Beta	95% confidence interval	T	p
Plasma renin ( $\mu$ U/ml)*	-1.04	(-1.47 to -0.61)	-4.72	0.000
Plasma aldosterone (pg/ml)*	-0.86	(-1.27 to -0.46)	-4.17	0.000
Age (years)	-10.24	(-10.72 to -9.77)	-42.13	0.000
Female sex	0.97	(-0.01 to 1.95)	1.95	0.051
Smoking	-5.50	(-6.33 to -4.67)	-13.03	0.000
Diabetes	3.48	(1.07 to 5.89)	2.84	0.005
BMI (kg/m <sup>2</sup> )	-0.43	(-0.53 to -0.32)	-7.92	0.000
NT-proBNP (pg/ml)*	-1.49	(-1.80 to -1.19)	-9.66	0.000
Heartrate (bpm)	-0.09	(-0.14 to -0.05)	-4.43	0.000
Hypercholesterolemia	-1.16	(-2.16 to -0.17)	-2.29	0.022
24h urinary sodium (mg/24)*	2.26	(1.45 to 3.07)	5.48	0.000
Copeptin (pmol/l)*	-1.62	(-2.06 to -1.19)	-7.3	0.000

\* skewed data were log transformed before standardization

TABLE 3: RENAL FUNCTION AT FOLLOW UP VISITS

Characteristics	NC1 (n = 5109)	NC2 (n = 4172)	NC3 (n = 3575)	NC4 (n = 3169)	NC5 (n = 3169)
<b>Follow-up (days)</b>	NA	1533 (1464-1643)	2332 (2232-2526)	3393 (3276-3550)	4306 (4189-4463)
<b>Age (years)</b>	48.3 ( $\pm$ 12.1)	52.5 ( $\pm$ 11.6)	54.4 ( $\pm$ 11.3)	57.1 ( $\pm$ 10.9)	59.6 ( $\pm$ 10.9)
<b>Cystatin-C (mg/L)</b>	0.86 (0.77-1.0)	0.86 (0.78-0.97)	0.88 (0.79-0.98)	0.88 (0.79-0.99)	0.89 (0.80-1.00)
<b>CKD-EPI (ml/min/1.73m<sup>2</sup>)</b>	94.9 ( $\pm$ 17.89)	92.3 ( $\pm$ 18.3)	90.1 ( $\pm$ 18.6)	88.9 ( $\pm$ 18.9)	86.0 ( $\pm$ 18.6)

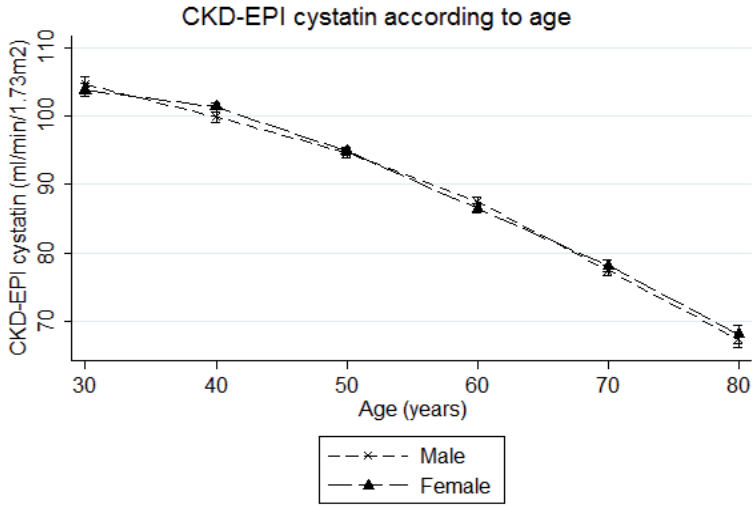
## GEE ANALYSIS FOR CHANGE OF CKD-EPI IN TIME

GEE analysis showed a clear relation of age with CKD-EPI and a relation of age and sex with cystatin C (figure 1). Although the relation of age with CKD-EPI was cubic addition of the cubic term on top of age as a linear variable had only a marginal effect on estimates of the following analyses. Univariable GEE analysis showed a positive interaction of PRC with follow-up time in years for the outcome CKD-EPI (+0.14 ml/min/1.73m<sup>2</sup> per SD of PRC per year  $p < 0.001$ ), suggesting a slower decline of CKD-EPI in patients with high PRC. This interaction remains significant after correction for age and gender (model 2; +0.24 ml/min/1.73m<sup>2</sup> per SD of PRC per year  $p=0.009$ ). However, in line with the linear regression analysis, there is also a significant negative association of PRC with CKD-EPI at baseline, (model 2; -2.6 ml/min/1.73m<sup>2</sup> per SD of PRC;  $p = 0.043$ ). This suggests that subjects with high PRC at baseline start with a lower CKD-EPI, but show slower decline over time.

Likewise, PAC showed a positive interaction with follow-up time for CKD-EPI (model 1; +0.11 ml/min/1.73m<sup>2</sup> per SD of PAC per year;  $p < 0.001$ ). After correction for age and gender this remained significant (model 2; +0.08 ml/min/1.73m<sup>2</sup> per SD of PAC per year;  $p < 0.001$ ). Baseline PAC was also associated with CKD-EPI at baseline (-0.77 ml/min/1.73m<sup>2</sup> per SD of PAC;  $p < 0.001$ ), again suggesting a lower CKD-EPI at baseline for subjects with high PAC, but a slower decline over time.

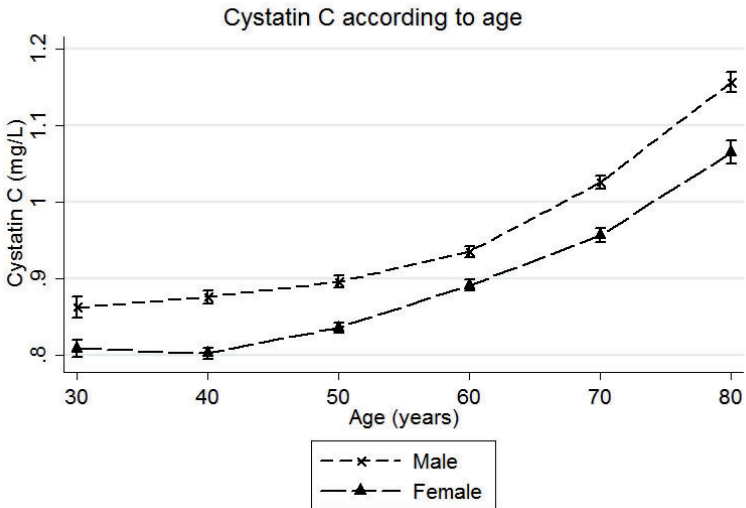
ARR on the other hand showed a negative interaction with follow-up time (model 1; -0.07 ml/min/1.73m<sup>2</sup> per SD of PAC per year;  $p < 0.001$ ). After correction for age and gender the interaction remained significant (model 2; -0.06 ml/min/1.73m<sup>2</sup> per SD of PAC per year;  $p < 0.001$ ) and association of baseline ARR with CKD-EPI was positive (+0.94 ml/min/1.73m<sup>2</sup> per SD of PAC;  $p = 0.002$ ).

FIGURE 1A – GEE ANALYSIS OF CKD-EPI CYSTATIN WITH AGE



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FIGURE 1B – GEE ANALYSIS OF CYSTATIN WITH AGE



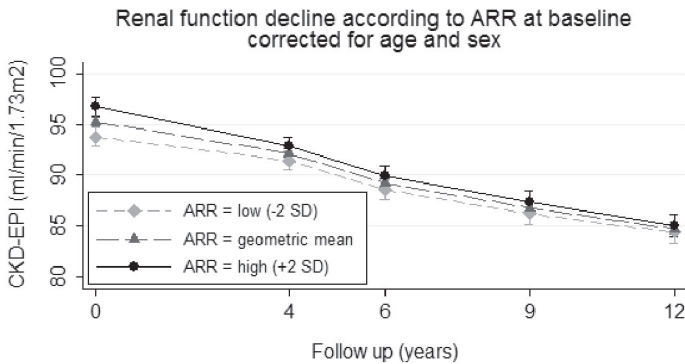
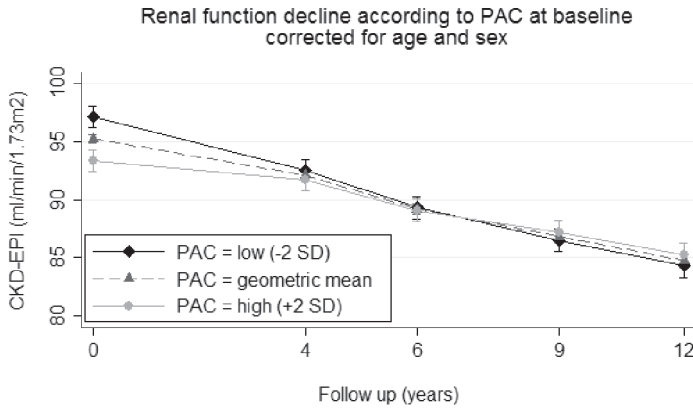
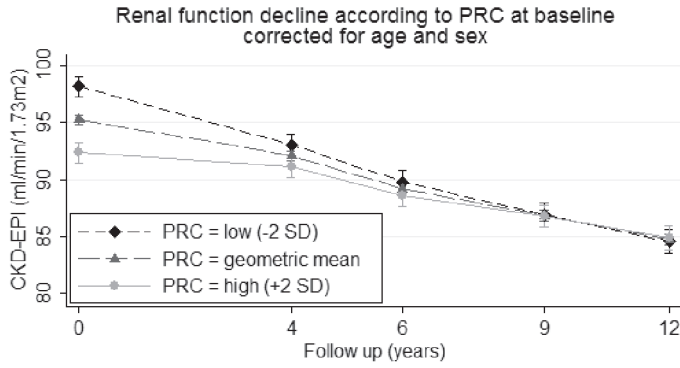
In order to further investigate if the relation of PRC, PAC and ARR with CKD-EPI decline changed over time we performed a GEE analysis including visit as an ordinal variable. These analyses showed that the relation between PRC, PAC and ARR and CKD-EPI at baseline was no longer apparent at visit 2 (mean follow up time 4 years) and that PRC, PAC and ARR had no effect on further CKD-EPI decline during the following visits (Figure 2).

## MULTIVARIATE ANALYSIS OF PRC AND PAC WITH RENAL FUNCTION DURING FOLLOW-UP

A multivariate GEE analysis of PRC with renal function was performed, including age, sex, diabetes, smoking, hypercholesterolemia, BMI, and systolic blood pressure. This did not affect the observed relations between PRC, PAC and CKD-EPI. Subsequently these parameters and markers for volume homeostasis including 24-hour urinary sodium, pro-AVP and NT-proBNP were added separately as interaction terms with PRC and visit. These did not substantially affect the relation of PRC, PAC and ARR. Adding interaction terms did not alter the relation between PRC and renal function decline

Multivariate GEE analysis of PAC including diabetes, smoking, hypercholesterolemia, BMI and systolic blood pressure did not change the observed relations either. Interaction analysis showed a significant interaction with BMI and 24 h urinary sodium (figure 3b). There was a borderline significant interaction with hypercholesterolemie ( $p = 0.07$ ) and smoking ( $p = 0.051$ ), while UAE only showed a significant interaction on visit 5.

**FIGURE 2 – GEE ANALYSIS OF RENAL FUNCTION (EGFR-CYS) DECLINE ACCORDING TO PRC, PAC AND ARR AT BASELINE CORRECTED FOR AGE AND SEX**



## SENSITIVITY ANALYSES WITH CORRECTION FOR SEX, BASELINE HYPERTENSION AND UAE

Sex stratified analysis confirmed that the aforementioned relations were present in both males and females. Analyses stratified for UAE at baseline showed no differences between the high UAE group (> 10mg/L) and the randomly selected control group with low UAE (< 10mg/L) for the aforementioned correlations.

## COX-REGRESSION ANALYSIS FOR THE OUTCOME CKD

Of the 3840 subjects with CKD-EPI cystatin  $> 60$  ml/min/1.73m<sup>2</sup> at baseline and a valid follow up cystatin-C measurements 344 subjects developed CKD defined as CKD-EPIcys  $< 60$  ml/min/1.73m<sup>2</sup> and 103 subjects  $< 45$  ml/min/1.73m<sup>2</sup>. In the univariable cox regression analysis high PRC was associated with a decreased incidence for CKD ( $< 60$  ml/min/1.73m<sup>2</sup>) and ARR with an increased incidence for CKD ( $< 60$  ml/min/1.73m<sup>2</sup>). However, after correction for age, sex and baseline CKD-EPIcys this association was not significant anymore (table 4). Neither was it after correction for other traditional risk factors.

TABLE 4: HAZARD RATIO'S FOR THE FOR THE OUTCOME  
CKD-EPI  $< 60$  OR  $< 30$  ML/MIN/1.73M<sup>2</sup> BY PRC, PAC AND ARR

<b>Outcome CKD-EPI cystatin <math>&lt; 60</math> ml/min/1.73m<sup>2</sup></b>						
<b>Plasma renin concentration</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	0.79	0.04	-4.48	0.000	0.72	0.88
Model 2	0.91	0.05	-1.69	0.09	0.82	1.01
Model 3	0.95	0.05	-0.93	0.35	0.85	1.06
<b>Plasma aldosterone concentration</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	0.90	0.05	-1.90	0.06	0.81	1.00
Model 2	0.95	0.05	-0.89	0.38	0.85	1.06
Model 3	0.96	0.06	-0.69	0.49	0.86	1.08
<b>Aldosterone-Renin ratio</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	1.19	0.06	3.27	0.001	1.07	1.32
Model 2	1.06	0.05	1.05	0.29	0.95	1.17
Model 3	1.02	0.05	0.46	0.65	0.92	1.14

TABLE 4 (CONT)

<b>Outcome CKD-EPI cystatin &lt; 45 ml/min/1.73m<sup>2</sup></b>						
<b>Plasma renin concentration</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	0.84	0.10	-1.47	0.14	0.66	1.06
Model 2	1.05	0.13	0.38	0.70	0.82	1.35
Model 3	1.13	0.16	0.89	0.38	0.86	1.49
<b>Plasma aldosterone concentration</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	0.72	0.08	-3.04	0.002	0.58	0.89
Model 2	0.75	0.11	-2.01	0.04	0.57	0.99
Model 3	0.81	0.11	-1.51	0.13	0.62	1.07
<b>Aldosterone-Renin ratio</b>	<b>HR</b>	<b>SE</b>	<b>z</b>	<b>p-value</b>	<b>95% CI</b>	
Model 1	0.99	0.13	-0.07	0.95	0.77	1.27
Model 2	0.83	0.11	-1.44	0.15	0.65	1.07
Model 3	0.81	0.11	-1.58	0.12	0.63	1.05

*Model 1: unadjusted*

*Model 2: adjusted for age, gender and baseline CKD-EPI*

*Model 3: adjusted for age, gender, baseline CKD-EPI, systolic blood pressure, diabetes, BMI, history of myocardial infarction and hypercholesterolemia*

## DISCUSSION

In this analysis of the PREVEND study, we show a clear gradual decline of CKD-EPI<sub>cys</sub> over time. Both high PRC and PAC showed a negative association with CKD-EPI<sub>cys</sub> at baseline and both high PRC and PAC were associated with decreased CKD-EPI<sub>cys</sub>, independently of each other and of other risk factors for CKD. However, neither PRC nor PAC was associated with long term renal function decline.

Previous studies have shown conflicting results on the relation of renin and aldosterone levels with development of kidney disease. In the Framingham Offspring study high aldosterone levels, but not renin, were associated with increased incidence for CKD (defined as an eGFR < 60 ml/min/1.73m<sup>2</sup> or an increased urine albumin-to-creatinine) during 9.5 year follow-up. (3) In this study 35% of all patients were using anti-hypertensive medication. Interestingly, in a subgroup analysis of 1575 patients without hypertension, aldosterone was

no longer significantly associated with CKD, suggesting that blood pressure was a main driver, not aldosterone. Other studies reported an association between high renin levels and increased incidence of CKD. The ASCOT trial studied 2240 treated hypertensive patients during 5.5 years. (10) Although plasma renin activity (PRA) was not associated with CKD incidence in an unadjusted model, after full adjustment (for 12 factors) they observed a significant odds ratio of 1.39 per SD increase of PRA. From this study it remains, however, unclear which covariate accounts for this difference, furthermore nearly all patients were on antihypertensive medication, which showed a strong association with PRA levels. Only one Japanese study examined the association of renin and aldosterone in subjects not using anti-hypertensive medication. (16) In contrast to the previous studies, they found that low renin and a high aldosterone-renin-ratio (ARR) were associated with increased incidence of CKD in 689 subjects followed for 9 years. Aldosterone levels alone were not predictive.

A major limitation of all published work is that kidney disease always was presented as a dichotomized outcome (yes or no), usually the number of individuals that reach an eGFR < 60 or < 30. Clearly, renal function decline is better assessed by a continuous scale that is measured over time. So, our study is the largest longitudinal study investigating the association between PRC and PAC and renal function decline, measured in a continuous fashion, in normotensive subjects. We demonstrate that initially renal function is significantly, but marginally, worse in individuals with high PRC and PAC at baseline. But these differences in eGFR are no longer present after > 5 years of follow-up.

It is well known that renin release is stimulated by beta-adrenergic stimulation, decreased renal perfusion pressure, low sodium delivery to the macula densa, and a decreased negative angiotensin II feedback. (17) In our previous analysis, (13) we demonstrated that PRC shows a strong association with blood pressure, NT-proBNP, and sodium intake. Several of these factors are important determinants of renal physiology and (hypo)perfusion. This would explain both high PRC, due to decreased renal perfusion pressure, and the decreased eGFR-cys at baseline. It may be speculated that the association of high PRC and PAC with decreased CKD-EPI at baseline may reflect temporary changes in renal hemodynamics rather than long term kidney damage. However, correction for volume status parameters including urinary sodium, pro-AVP and NT-proBNP could not fully explain this relationship. although the effect is still only moderate and the underlying pathophysiology unclear. Subgroup analysis did not reveal a subgroup group in which measuring PAC could predict long term renal function decline, although it did appear that urinary sodium excretion, which is a marker for sodium intake and volume, may explain part of the baseline association. Likewise, low BMI may also reflect dehydration. By default, BMI is a poor marker for volume status, but since we observed that subjects with low BMI and high aldosterone had a lower eGFR at baseline, and this recovered in subsequent visits, we hy-



pothesize that this might reflect rehydration. Naturally, we cannot exclude other factors between visits, like the use of medication or differences in diet or lifestyle and regression to the mean.

## CLINICAL IMPLICATIONS

Nowadays, there is much more awareness that renal function decline can be a silent process, which only presents after years of asymptomatic disease. Early detection and intervention may delay or offset the development of renal disease. From our data, we cannot recommend to use PRC, PAC or ARR for screening purposes. Clearly, assessment of eGFR is a good tool to measure cross-sectional renal function, but addition of PRC and/or PAC did not provide useful information for the long-term renal outcome. The association between PRC and PAC with lower baseline eGFR suggest that activation of the RAAS may play a role in this stage of renal impairment. Current guidelines recommend to preferentially use RAAS inhibitors in subjects with signs of renal disease, so the availability of PRC and/or PAC will not guide this decision making.

## STRENGTHS AND LIMITATIONS

We describe the largest study to date reporting on the relation between RAAS and kidney function decline in healthy community-dwelling subjects, not using anti-hypertensive medication. It is unique for its measurements on renal function decline in time as a continuous outcome, rather than dichotomized. This gives us the opportunity to study short and long term correlations, as well as non-linear correlations over time in more detail. Furthermore it provides insight in the development of renal function well above 30 or 60/ml/min, which were used as a cut-off in most studies. We used the CKD-EPI formula for calculation of eGFR, which has shown an excellent correlation with actual GFR. (14) We measured all samples of each subject in a single run, minimizing inter-assay variability.

Limitations are that although we attempted to correct for the traditional CV risk factors, we cannot exclude residual confounding because of the observational nature of the study. Second, the PRC assay is a less costly and laborious measurement than plasma renin activity, and shows an excellent correlation of plasma renin activity, (18) however, PRC may be affected by cryo-activation, and may be elevated in specific disease. The latter, however, are unlikely to have influenced our results in this healthy cohort. Finally, we only have PRC and PAC values from the baseline visit; therefore we cannot investigate the effect of long term elevated PRC and/or PAC on kidney function. Possibly, the predictive value of PRC and PAC would be stronger if repeated measures were available, closer to the eGFR assessment during follow up.

## CONCLUSION

We found high baseline PRC and PAC levels to be associated with a decreased renal function, but PRC nor PAC had no effect on long term kidney function over the course of 12 years. From our data, we cannot recommend addition of screening of PRC and PAC to standard screening strategies in order to identify patients at increased risk for CKD.

## ACKNOWLEDGEMENTS

This work was supported by the Netherlands Heart Foundation (grant 2007T046 to Dr. de Boer) and the Innovational Research Incentives Scheme program of the Netherlands Organization for Scientific Research (NWO VENI, grant 916.10.117, also to Dr. de Boer). We would like to thank Rob Faber and Tyrone Eckmeyer (Department of Microbiology, Section of Virology, UMCG, Groningen, the Netherlands) and Martin Dokter, Mariusz Szymanski, and Hasan Mahmud (Department of Cardiology, UMCG, Groningen, the Netherlands) for excellent technical support.

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