The significance of micro- and macrovascular biomarkers on cardiovascular outcome in chronic kidney disease – A prospective cohort study

Short title: Vascular biomarkers in CKD

Orsolya Cseprekál*[a], József Egresits*[a,b], Ádám Tabák[a], János Nemcsik[c, d] , Zoltán Járai[e], Levente Babos[e], Erzsébet Fodor[f], Katalin Farkas[g], Gabriella Godina[e], Keve I. Kárpáthi[h], Lóránd Kerkovits[f], Adrienn Marton[i], Zsófia Nemcsik-Bencze[a,j], Zsófia Németh[i], László Sallai[e], István Kiss[f], András Tislér[a]

[a] *Ist. Department of Internal Medicine, Semmelweis University, Budapest, Hungary*
[b] *Department of Internal Medizine II, University Hospital Regensburg, Germany*
[c] *Department of Family Medicine, Semmelweis University, Budapest, Hungary*
[d] *Department of Emergency Medicine, Uzsoki Hospital, Budapest, Hungary*
[e] *Department of Cardiology, St Imre University Teaching Hospital, Budapest, Hungary*
[f] *Department of Nephrology and Hypertension, St Imre University Teaching Hospital, Budapest, Hungary*
[g] *Angiology Division, St Imre University Teaching Hospital, Budapest, Hungary*
[h] *IInd. Department of Internal Medicine, Semmelweis University, Budapest, Hungary*
[i] *Nephrology Division, Uzsoki Teaching Hospital, Budapest, Hungary*
[j] *Department of Radiology and Oncology, Semmelweis University, Budapest, Hungary*

*Authors contributed equally to this work

Corresponding author:
Orsolya Cseprekál MD PhD
Ist Department of Internal Medicine, Semmelweis University
2/a. Korányi Sándor Str.
H-1083 Budapest, Hungary
E-mail: cseprekal.orsolya@med.semmelweis-univ.hu
Phone/Fax: +36 210 0278 - 51526 / +3613130250
Cell: +36 20 663 2174

The authors declare no conflict of interest.

Word count: 3990

Number of tables: 3

Number of figures: 0

Number of supplementary digital content files: 2

References: 35

Abstract

Measures of small and large artery dysfunction have not been investigated in a single cohort for the prediction of cardiovascular (CV) events in patients with non-dialysed (ND) chronic kidney disease (CKD). This prospective cohort study aimed to determine whether central pulse wave velocity (cPWV), central pulse pressure (CPP) or microvascular post-occlusive reactive hyperaemia area ($PORH_{HA}$) independently predict CV events and mortality in CKD-ND.

Ninety-four stage 1-5 CKD-ND (65.3±13.1 year; eGFR 35.3(22.8-49.4) ml/min/1.73m^2) patients were followed-up for a median of 52(36-65) months and had baseline cPWV, CPP measured by applanation tonometry, and $PORH_{HA}$ by Laser Doppler Flowmetry. Multiple failure time Cox-regression models were used to determine the predictive role of vascular parameters on CV mortality and events.

Based on multiple linear regressions baseline age, diabetes, CV disease, and systolic blood pressure (SBP) were independently related to cPWV (R^2=0.3), SBP and $PORH_{HA}$ to CPP (R^2=0.45), while CPP was the only parameter independently related to $PORH_{HA}$ (R^2=0.16, all $p<0.05$). During follow up 41 CV events occurred (14 CV deaths). In univariate analyses cPWV (1.07 (1.02-1.13) per m/s), CPP (1.04 (1.01-1.07) per mmHg), and $lnPORH_{HA}$ (0.70 (0.58-0.85) per ln(mU*sec)) were all related to the outcome. Baseline diabetes (HR 3.07 (1.65-5.68)), lnFGF23 (fibroblast growth factor 23; 1.86 (1.13-3.06) per RU/mL) and CPP (1.04 (1.01-1.07) per mmHg) were independent predictors of CV events.

The impaired pulsatile component of large arteries (CPP) independently of other vascular markers (cPWV, $PORH_{HA}$) predicted CV outcomes in CKD-ND. CPP may integrate the information provided by cPWV and $PORH_{HA}$.

Summary Table

The significance of micro- and macrovascular biomarkers on cardiovascular outcome in chronic kidney disease –A prospective cohort study
What is known about topic
– Non-dialysed chronic kidney disease (CKD-ND) is characterised by large and small vessel dysfunction, as assessed by central pulse wave velocity (cPWV), central pulse pressure, and post-occlusive reactive hyperaemia ($PORH_{HA}$) – The role of these micro-and macrovascular markers in the prediction of cardiovascular (CV) outcome has not been previously evaluated in a single cohort of CKD-ND.
What this study adds
– Among cPWV, CPP and $PORH_{HA}$, the impaired pulsatile component of central arteries (CPP) was the primary predictor of CV outcome.
– CPP seems to integrate the information provided by cPWV and $PORH_{HA.}$

Keywords:
Cardiovascular mortality
Central pulse pressure
Chronic kidney disease
FGF23
Post-occlusive reactive hyperaemia
Pulse wave velocity

Introduction

The risk of cardiovascular (CV) events and all-cause mortality increases with worsening kidney function and it approaches a hundred-fold in end-stage renal disease (ESRD) compared to the general population [1]. Patients with chronic kidney disease (CKD) are more likely to die of CV disease before they get to ESRD that cannot be fully explained by decreased glomerular filtration (eGFR) and traditional risk factors. Thus CKD related vascular and metabolic alterations are suspected to explain the additional risk in this population.

Renal anaemia, disturbances in mineral metabolism and systemic inflammation are known to be potential non-traditional CV risk factors [2,3,4] and serve as part of the pathophysiological background of accelerated athero- and arteriosclerosis of the large and small arteries in ESRD. Increased central pulse wave velocity (cPWV) and central pulse pressure (CPP) independently predict target organ damage, such as coronary artery disease (CAD), acute coronary syndrome (ACS), cerebrovascular diseases (stroke or transient ischemic attack - TIA), peripheral artery disease (PAD) at a subclinical stage in ESRD [5,6]. However, the role of these vascular biomarkers of subclinical organ damage and renal comorbid conditions as risk factors of CV events and death at earlier stages of CKD is uncertain [7]. In fact, the predictive values of increased CPP and cPWV in non-dialysed (ND) CKD have been less studied [8,9,10,11,12]. Furthermore, while there is plenty of evidence that detectable microvascular injury - as the other end of the spectrum of vascular dysfunction - begins at an early stage of CKD, it is not known whether this would also be predictive for CV events in CKD. Indeed, only one follow-up study [13] is available in which post-occlusive reactive hyperaemia (PORH) measured by Laser Doppler Flowmetry (LDF) was associated with the development of CV diseases independent of Framingham and Cardiorisk cardiovascular risk scores. Furthermore, no previous study in CKD-ND considered the predictive roles of cPWV, CPP and PORH in a single cohort.

The aim of our cohort study was therefore to assess the association of micro- and macrovascular biomarkers (cPWV, CPP, $PORH_{HA}$) with traditional and non-traditional CV risk factors, and to evaluate their independent predictive value for CV events and mortality in "mild-to-severe" CKD-ND cases.

Methods

This was a prospective cohort study of stage 1-5 CKD-ND patients with baseline clinical, biochemical, micro- and macrovascular measurements, and with CV events and mortality as outcome during follow-up.

Patients

Initially 108 hypertensive CKD-ND stage 1-5 individuals were enrolled with baseline clinical biochemical and vascular measurements, however only 103 agreed to participate. Convenience sampling was used with consecutive inclusion of CKD patients presenting at the two tertiary care outpatient clinics that were included to our study (Semmelweis University Ist. Department of Internal Medicine Semmelweis University and St Imre University Teaching Hospital, Budapest, Hungary). They were then followed for a median of 52 (36-65) months from 2007 to 2013. Further 9 people were excluded due to missing baseline or follow-up data. Finally 94 patients were involved and followed-up. We compared baseline characteristics of participants and non-participants and found no major differences between these groups.

None of the patients were hospitalised or had atrial fibrillation at the time of baseline investigations. No other specific inclusion or exclusion criteria were applied. Antihypertensive treatment was tailored according to the latest recommendations of the European Society of Hypertension for reaching target values [14].

Follow-up data were collected between April and July 2013 by telephone interviews with the patients, their general practitioners or treating physicians and the information gathered were in all cases verified by original chart review. Follow up was censored at the last occurrence of a documented CV event (ACS, heart failure requiring hospitalisation, stroke or TIA, PAD verified by angiography or need for an intervention) or death due to the above CV causes. Laboratory data and vascular biomarkers were not collected during follow-up.

Power analysis

Based on the observed difference of 7 mmHg central pulse pressure in the Strong Heart Study [15] between participants with and without incident cardiovascular events we expected a difference with similar magnitude in our population. After the enrolment of the first 25 participants, we calculated the standard deviation of central pulse pressure on enrolled population (SD 12 mmHg). Furthermore, based on literature data from similar populations

with a median eGFR of 30-44 ml/min/1.73m^2 we expected 11 cardiovascular events per 100 person years of follow-up that translates to 44 events during the planned 4-year study. Taken together all these information we needed to enrol 93 participants to have an 80% power to detect a 7 mmHg difference in central pulse pressure between cases and non-cases with an alpha of 0.05. Our observation of a difference of 8 mmHg (47.1 ± 11.7 vs. 55.2± 13.8 mmHg, p=0.004) well corresponds to the power calculations.

Macrovascular injury and blood pressure measurements

Measurements of vascular markers for a given patient (cPWV and CPP or $PORH_{HA}$) were performed in a random order after one another on the same morning at baseline. Subjects were asked to refrain from smoking on the day of the study and not to consume caffeine-containing drinks at least 4 hours before the start of the measurements, but to take their regular morning medication. Tests were carried out in a temperature-controlled room (24±1°C) with the subjects in supine position, after a 20 minute rest period [16].

cPWV was measured by applanation tonometry (PulsePen device; DiaTecne s.r.l. Milan, Italy) [17] in accordance with the recommendations at that time [16] by capturing sequential recordings of the arterial pressure wave at the carotid and femoral arteries, and by measurement of the distance between the carotid and the femoral sampling sites. Since arguments for the use of 80% of the direct carotid-femoral distance as the most accurate measurement were provided in the latest expert consensus document, our data were calculated accordingly [18]. cPWV was calculated by the PulsePen software as the ratio of the distance and the transit time of the pulse pressure wave along the aorta. Pulse wave amplitude was calibrated to brachial mean and diastolic pressures, measured immediately prior to each sequence of pulse wave capture at the two sites. Mean arterial pressure was calculated by the PulsePen software as diastolic pressure + 0.4 pulse pressure. Recordings with a systolic or diastolic variability of consecutive waveforms above 10% or with the amplitude of the pulse wave signal being less than 80 mV were discarded. All measurements were done in duplicates by J.N, J. E, and Zs.N. and their averages were used in the calculations.

CPP was measured by the same device and calculated as the difference of the central systolic and diastolic blood pressure values recorded at the carotid sampling site.

All brachial blood pressure measurements throughout the study were performed by the validated oscillometric BpTRU device (VSM Meditech, Vancouver, Canada) with four sequential measurements manually averaged.

Microvascular function test

For Laser Doppler Flowmetry (LDF) measurements, blood pressure and heart rate of the subjects were determined as above. Laser Doppler instrument (Periflux 5001, Stockholm, Sweden, wavelength 780 mm) was used during the study as described previously [19]. In short, during the $PORH_{HA}$ test after the registration of the baseline flow for 60 seconds on the volar surface of the left forearm 10 cm below the elbow with a standard LDF probe, brachial arterial occlusion was applied with 40 mmHg suprasystolic pressure by a pneumatic cuff of a sphygmomanometer for 3 minutes to reach the biological zero. After the release of the pressure, perfusion (measured as perfusion unit (PU)) rise high and rapidly above the pre-ischemic PU values. The software analysed the data automatically and calculated several indices such as the initial baseline value, slope value, peak flow, percent change in perfusion from baseline to maximum values, time to reach the maximum hyperaemia, time to reach the half value after the maximum hyperaemia, and the area of hyperaemia. This latter measurement (abbreviated as: $PORH_{HA}$, unit: PU*sec – perfusion unit * second) seems to be the most accurate parameter to assess the hyperaemic response, as it includes three variables (speed, intensity, and duration) and this was used in the analyses as representative of the microvascular function [20, 21]. In their study, Stiefel P et al found a „cut-off" $PORH_{HA}$ of 865 PU*sec to have an 82% sensitivity and a 97% specificity to distinguish microvascular dysfunction of coronary artery disease patients from healthy controls [20].

According to previous measurements in our laboratory, the day to day variability of this system was 16-21%, which is comparable to other studies [21]. All $PORH_{HA}$ measurements were performed by J.E. and analysed by J.N.

Epidemiologic and Laboratory data

Baseline data on smoking habits (current), diabetes (DM, any type), hypertension, coronary artery disease (previous acute myocardial infarction or coronary intervention), chronic heart failure (previous diagnosis), Peripheral Artery Disease (PAD; documented by angiography or intervention) and cerebrovascular disease (previous stroke or TIA) were collected by health record review. The Charlson Comorbidity Index was used for overall characterization of co-morbidity of the studied population [22,23].

Data on haemoglobin (Hgb), serum calcium (Ca), phosphate (iP), albumin (Alb), parathormon (iPTH), creatinine, C-reactive protein (CRP), serum cholesterol (Chol), triglyceride (Tg), and LDL-cholesterol were evaluated at baseline. Routine blood chemistry measurements were performed by a Hitachi auto analyser. Intact parathyroid hormone 1-84

(iPTH) was determined by immune-chemiluminomtric two-site assay (CIBA-CORNING, Frenwald, Germany). Baseline eGFR was calculated using the four-variable Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [5]. Circulating concentration of fibroblast growth factor 23 (FGF23) was measured using a second-generation C-terminal ELISA (Immutopics, San Clemente, CA). Albuminuria was characterised by albumin-creatinine ratio (ACR) measured from first morning spot urine according to KDIGO recommendations [5].

Statistical analysis

All data analysis was performed by STATA (StataCorp Lp. Texas USA) and Statistica version 11.0 (StatSoft Inc. Tulsa USA). Data are given as mean and standard deviation, unless indicated otherwise. Values are presented as median and interquartile range when data did not display a normal distribution and they were transformed logarithmically for further analyses. In the group analysis of anthropometric and clinical parameters, Student's t-test for independent samples or Mann Whitney U test was used as appropriate.

108 people were invited to participate; however only 103 agreed it and a further 9 people were excluded due to missing baseline or follow-up data. We compared baseline characteristics of participants and non-participants and found no major differences between these groups. As the number of participants with missing data was relatively low, we used complete-case analysis. No multiple imputations were performed due to the limited sample size.

In the baseline cross sectional analyses univariate and multivariate (stepwise, ridge) linear regressions were performed to determine the main associations of the macro- and microvascular parameters (cPWV, CPP, $PORH_{HA}$). The predictor variables considered were the ones listed in table 1. The variables that showed a significant association with the given dependent variable in univariate models were considered in the final multivariate model. (Table 2).

To assess the predictive values of cPWV, CPP and $PORH_{HA}$ for CV events and CV mortality, multiple failure times Cox proportional hazard regression analyses were used with conditional risk set modelling. This method accommodates for the fact that one patient may have more than one outcome event. We first performed univariate analyses considering variables listed in table 1. Confounding was addressed in multiple linear and multivariate Cox-regression models with adjustment for potential clinical predictors. Final models were

selected using backward elimination to reach the most parsimonious models. (Table 3 and Online Data Supplement Table 1.)

Finally, to determine the sensitivity of our data we repeated the analyses with the more usual method of censoring patients at the first occurrence of a CV event (a total of 31 events). Our main finding, i.e. CPP is the only vascular marker that significantly and independently predicts outcome has not been altered by this analysis. (Online Data Supplement Table 2.)

A "p" value with a two-sided alpha of 0.05 was considered statistically significant. Hazard ratios are presented with their corresponding 95% confidence interval.

Ethics

CKD-ND patients in stages 1-5, who gave written, informed consent for participation, were included. The study protocol was approved by the Local Ethical Committee of the two investigation sites and it was in accordance with the principles of the Declaration of Helsinki.

Results

Descriptive statistical analysis at baseline

Table 1 displays baseline characteristics, concomitant diseases, traditional and non-traditional risk factors, metabolic and vascular parameters of our patients and divided into two subgroups by eGFR less than, or equal to and higher than 30 ml/min/1.73 m^2.

The causes of kidney disease were heterogeneous (number of cases in parentheses): glomerulonephritis (14), diabetic nephropathy (27), hypertensive nephrosclerosis (14), chronic tubulointerstitial nephritis (17), vascular cause (4), polycystic kidney disease (7), tumor (1) and unknown (10). There were two normotensive subjects with CKD with polycystic kidney disease and glomerulonephritis.

All but one patient received antihypertensive medication: (case numbers in parentheses) ACE inhibitors (84), calcium channel blockers (48), diuretics (68), ß-receptor blockers (52), α-receptor blockers (15), long-acting nitrate (14) and centrally-acting antihypertensive drugs (13), either alone or in combination. Acetylsalicylate platelet aggregation inhibitor was taken by 36 patients, while 15 individuals took clopidogrel. Thirty five patients required erythropoietin, 34 received active vitamin-D, and 8 needed calcium carbonate phosphate binder therapy.

As expected, the group with an eGFR below 30 ml/min/1.73 m^2 had a worse metabolic status as indicated by their elevated iP, iPTH, FGF23, CRP, ACR and lower haemoglobin values.

Baseline cPWV, CPP, and $PORH_{HA}$ values were 12.5±4.5m/s, 52±13mmHg, 593 PU*sec (280-1046), respectively, with no significant differences between the less and more advanced CKD groups.

At baseline there were no differences between the parameters of micro- and macrovascular damage according to the use of any antihypertensive, platelet aggregation inhibitor, erythropoietin or active vitamin-D therapy. Nearly half of our population had DM at baseline. The diabetic group had significantly higher cPWV (14.2±4.4 vs. 11.3±4.2 p=0.002), lower $PORH_{HA}$ (421 (158-999) vs. 696 (386-1139) p=0.03), but CPP was not significantly different between the groups (54±13 vs. 50±12 p=0.08).

Cross sectional analysis at baseline

Baseline associations of the vascular biomarkers (i.e. cPWV, CPP, $lnPORH_{HA}$) with other baseline clinical and biochemical parameters, as assessed by uni- and multivariate linear regressions are displayed in table 2. Age, diabetes, previous CV disease, and systolic blood pressure (SBP) were related to cPWV (R^2=0.3). CPP was associated with SBP and $PORH_{HA}$ (R^2=0.45). CPP was the only parameter significantly related to $PORH_{HA}$ (R^2=0.16) in the multivariate model.

Prospective data analysis

During a median of 52 (36-65) months of observation time no patients were lost to follow-up. In 31 participants, 41 CV events were recorded and used in the analyses. It represents an incidence rate of 9.8 events per 100 patient years. The distribution of the primary events: Fourteen patients died of CV causes (acute coronary syndrome 4, stroke 2, heart failure 7, and peripheral artery disease 1), and there were 27 additional CV events (acute coronary syndrome 4, stroke 6, heart failure 11, peripheral artery disease 6). 10 patients had a second CV event, including 7 CV deaths. All 41 primary and secondary events were used as hard end points in the multiple failure time Cox regression analysis.

In univariate Cox regression analyses all three studied vascular parameters were significantly associated with the outcome, hazard ratios for cPWV were 1.07 (1.02-1.13), for CPP 1.04 (1.01-1.07), and for $PORH_{HA}$ 0.70 (0.58-0.85), respectively.

As a result of the multivariate backward Cox regression model building, that included the other significant univariate predictors besides the three studied vascular parameters, only the presence of DM (3.06 (1.65-5.67)), lnFGF23 (1.86 (1.13-3.06)) and CPP (1.04 (1.01-1.07)) remained independent predictors of CV mortality and events (Table 3 and online data supplement Table 1) while cPWV and $lnPORH_{HA}$ have lost their significant predictive value for CV events.

To determine the sensitivity of our data we repeated all these analyses with censoring patients at the first occurrence of a CV event (a total of 31 events). In this analysis again CPP remained the only significant vascular predictor of the outcome while cPWV as well as $lnPORH_{HA}$ lost their initial univariate significance in predicting CV outcome. (Online data supplement Table 2.)

Discussion

The predictive role of macro- and microvascular biomarkers (cPWV, CPP, $PORH_{HA}$) on CV outcome in CKD-ND was investigated in our prospective cohort study, an analysis that has never been previously performed in a single cohort. Our main findings demonstrate that while there is an association between the markers of micro- and macrovascular injury and CV outcome, CPP seems to be the one that may best determine CV morbidity and mortality. Additionally, the presence of diabetes and higher FGF23, an early marker of disturbed mineral metabolism and vascular calcification were also found to be independent predictors of CV outcome in CKD-ND.

Our population can be classified as high-risk for CV diseases, as they had an event rate of 9.8 per 100 patient years during follow up. This cannot be explained solely by the presence of Framingham risk factors [24]. Indeed, the measured cPWV (12.5±4.5m/s) and CPP (52±13mmHg) values of our patients exceeded the „cut-off" values (PWV >10 m/sec and CPP >50 mmHg, respectively) recommended by the ESH guideline and the Strong Heart Study [17,25] may in part explain this higher event rate. Our patients with an eGFR of less than 30 ml/min/1.73 m^2 tended to have more baseline CV diseases, such as coronary, cerebrovascular and peripheral artery disease, which likely further increased their risk of future CV events. Thus, among our patients with worsening kidney function, an increased number of comorbidities could explain the rate of events that exceeds the 3 events per 100 patient year reported by Baumann et al. [11] and the 5.13 events per 100 patient year in Hoorn study [26]. While in the last decade cPWV has been proven to be an independent risk factor

of CV risk in the general and ESRD population [2,3,27] only a limited number of studies had been performed with this vascular parameter in CKD-ND. In fact, only the above two prospective cohort studies investigated cPWV in a CKD-ND population. In the first study cPWV was found to predict all-cause mortality, while in the latter study cPWV was related to cardiovascular events after 7.6 years of follow-up [11,26]. Other recent studies demonstrated that decreased arterial elasticity is related to CKD progression; long-term CV outcomes have not been reported. [28,29] There is one additional prospective cohort study by Quiroga et al [12], who found male gender, diabetes, kidney disease progression and baseline CV disease to be predictors of all-cause mortality in their Spanish cohort. It is important to note, however, that central arterial elasticity parameters, such as CPP were not examined in their study. As for the role of CPP in CKD-ND, only the cross sectional CRIC study examined and found CPP as being superior over peripheral pulse pressure to quantify the risk of CV disease and eGFR impairment [9].

Microvascular injury measured by LDF has not been extensively studied prospectively; Rossi et al. [30] in their cross sectional analysis found $PORH_{HA}$ values being an incremental determinant of atherosclerosis besides brachial ankle index in type 1 diabetes. The only prospective study performed among ESRD patients concluded that $PORH_{HA}$ was a determinant of coronary artery disease and all-cause mortality [13].

Our work is unique in that it is the first prospective study to investigate the combined effects of micro- and macrovascular markers, their relation to each other, and their independent effects on hard CV end points in a single cohort of CKD-ND patients, never previously performed in this population.

Our cross sectional analysis suggests that CPP might represent an early functional sign of vascular injury, to which none of the metabolic markers (e.g. eGFR, anaemia, disturbed mineral metabolism) of CKD were related. We hypothesize that early stages of athero- and arteriosclerosis could lead to microstructural deterioration of central and peripheral arteries that lead to early hemodynamic dysfunction in the CKD environment. Early dysfunction of large conduit arteries (characterized by increased cPWV) may have a backward effect leading to left ventricular hypertrophy and also increase CPP. At the same time early small vessel dysfunction (characterised by decreased PORH) may increase wave reflections, contributing to the high pulsatile component of the aortic pressure (CPP) that directly damages target organs such as the heart, brain and kidneys [31]. Given the above physiological evidence, it seems reasonable to hypothesize that increased CPP could be an integrative marker of preclinical early target organ damage indicating both micro- and macrovascular injury [32].

Beyond CKD, traditional risk factors, such as diabetes also lead to a significantly worse metabolic state, hence accelerated athero- and arteriosclerosis. Thus small and large arterial injuries due to these risk factors might develop simultaneously which can be extensively described by CPP as an integrative marker of CV outcome in CKD-ND. While the presence of diabetes was expected to determine the outcome [33], in our study, it was also independently associated with CPP, rather than cPWV suggesting that CPP better described the clinically relevant vascular changes that occur in CKD. An explanation as for why $PORH_{HA}$ was not an independent predictor of outcome could be that the target organ damage characterized by increased CPP can be considered a more robust factor than a sole microvascular injury or structural central arterial stiffness marker itself. In summary, our prospective data seem to support the notion that increased CPP is indeed an early integrative marker of large and small vessel injury, with clinically relevant consequences in CKD-ND.

The plasma level of the new early biomarker of deranged mineral metabolism and vascular calcification, FGF23, rises already at initial stages of CKD and it has been shown to relate to several target organ damages leading to cardiovascular death i.e. arterial stiffness or endothelial dysfunction. Our study confirms the role of FGF23 as an independent predictor of CV death and events as summarised recently by Xiao Y. et al. in their meta-analysis [34].

In conclusion, the impaired pulsatile component of the central arteries characterized by increased CPP, as an integrative marker of micro- and macrovascular dysfunction proved to be the sole, independent and robust vascular predictor of CV outcome in our CKD-ND population. CPP seems to integrate the information provided by cPWV and $PORH_{HA}$. Additionally, the presence of diabetes deserves special attention considering its continued predictive role for higher rate of CV events. FGF23 may also indicate increased CV risk and offer a potential future screening tool in risk stratification methods. Whether CPP can specifically be influenced by targeted vascular or metabolic therapy to alter small and large vascular function and whether all of that would have an impact on long-term CV outcome are to be seen in future diagnostic and therapeutic trials.

Limitations

There are several limitations of our study to be acknowledged. While unique in its objectives, our study evaluated only a relatively small number of cases, that makes it difficult to homogenise the cohort, smooth the variation of group composition in each CKD stages,

and generalize our conclusions. Furthermore, as we had small patient numbers our confidence intervals are rather wide, and we hope that other ongoing cohort studies will support our findings on the fundamental role of CPP as an integrative marker of CV risk in this hypertensive CKD ND population. It is important to note that beside diabetes and FGF23, only CPP was predictive for the outcome, suggesting its robustness despite the small patient numbers. Nonetheless, further studies are needed to confirm the reliability of CPP as a clinical marker of CV risk stratification. We acknowledge that this was a sample of patients of two tertiary care nephrology clinics, and therefore selection bias that may limit generalizability (i.e. high baseline CV disease risk burden of our patients) cannot be ruled out. We realize that the method we used to assess microvascular reactivity with LDF and $PORH_{HA}$ is not entirely established. We, therefore, are awaiting the results of further studies with this method [35].

Acknowledgements

This study was supported by research grants from the Hungarian Kidney Foundation, Hungarian Society of Hypertension, and Hungarian Society of Nephrology

There is no **conflict of interest** to declare

References

1 Schriffin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. Circulation 2007;116:85-97

2 Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal failure. Am J Kidney Dis 1998;32:S112–S119

3 Eknoyan G, Lameire N, Eckardt KU , Kasiske BL, Wheeler DC, Abboud OI et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int* 2013 (suppl 3): S1–S150

4 de Jager DJ, Vervloet MG, Dekker FW. Noncardiovascular mortality in CKD: an epidemiological perspective. Nature Reviews Nephrology 2014;10(4):208-214

5 Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guyonvarc'h PM et al. Central pulse pressure and mortality in end-stage renal disease. Hypertension. 2002;39(3):735-738

6 Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999;99(18):2434–2439

7 Sakuragi S, Abhayaratna WP. Arterial stiffness: methods of measurement, physiologic determinants and prediction of cardiovascular outcomes. Int J Cardiol 2010;21;138(2):112-128

8 Sengstock D, Sands RL, Gillespie BW, Zhang X, Kiser M, Eisele G. Dominance of traditional cardiovascular risk factors over renal function in predicting arterial stiffness in subjects with chronic kidney disease. Nephrol Dial Transplant 2010;25(3):853-861

9 Townsend RR, Chirinos JA, Parsa A, Weir MA, Sozio SM, Lash JP et al. Chronic Renal Insufficiency Cohort Investigators. Central pulse pressure in chronic kidney disease: a chronic renal insufficiency cohort ancillary study. Hypertension 2010;56(3):518-524

10 Ben-Shlomo Y, Spears M, Boustred C, , May M, Anderson SG, Benjamin EJ et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol 2014; 63(7):636-646

11 Baumann M, Wassertheurer S, Suttmann Y, Burkhardt K, Heemann U. Aortic Pulse wave velocity predicts mortality in chronic kidney disease stages 2-4. J of Hypertens 2014;32:899-903

12 Quiroga B, Verdalles Ú, Reque J, García de Vinuesa S, Goicoechea M, Luño J. Cardiovascular events and mortality in chronic kidney disease (stages I-IV). Nefrologia 2013;33(4):539-545

13 Kruger A, Stewart J, Sahityani R, O'Riordan E, Thompson C, Adler S et al. Laser Doppler flowmetry detection of endothelial dysfunction in endstage renal disease patients: correlation with cardiovascular risk. Kidney Int 2006;70:157-164

14 Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G et al. The task force for the management of arterial hypertension of the European Society of Hypertension, The task force for the management of arterial hypertension of the European Society of Cardiology. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2007(12):1462-1536.

15 Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T et al. Central Pressure More Strongly Relates to Vascular Disease and Outcome Than Does Brachial Pressure The Strong Heart Study. Hypertension 2007;50:197-203

16 Laurent S, Cockcroft J, Van Bortel L , Boutouyrie P, Giannattasio C, Hayoz D et al. Abridged version of the expert consensus document on arterial stiffness. Artery Res 2007;1:2-12

17 Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new non-invasive portable tonometer for determining arterial pressure wave and pulse wave velocity: the PulsePen device. J Hypertens 2004; 22(12):2285–2293

18 Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T et al. European Society of Hypertension Working Group on Vascular Structure and Function; European Network for Noninvasive Investigation of Large Arteries. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens 2012;30(3):445-448

19 Farkas K, Nemcsik J, Kolossváry E, Járai Z, Nádory E, Farsang C et al. Impairment of skin microvascular reactivity in hypertension and uraemia Nephrol Dial Transplant 2005;20(9):1821-1827

20 Stiefel P, Moreno-Luna R, Vallejo-Vaz AJ, Beltrán LM, Costa A, Gómez L et al. Which parameter is better to define endothelial dysfunction in a test of postocclusive hyperemia measured by Laser-Doppler flowmetry? Coronary Artery Disease 2012,23:57–61

21 Kubli S, Waeber B, Dalle-Ave A, Feihl F. Reproducibility of laser Doppler imaging of skin blood flow as a tool to assess endothelial function. J Cardiovasc Pharmacol 2000;36(5):640-648

22 Prasad N, Sinha A. Clinical Queries: Malnutrition and co-morbidity in diabetic kidney disease patients. Nephrology 2012;1: 138–143

23 Huang Y, Gou R, Diao Y , Yin Q, Fan W, Liang Y et al. Charlson comorbidity index helps predict the risk of mortality for patients with type 2 diabetic nephropathy. J Zhejiang Univ-Sci B (Biomed & Biotechnol) 2014; 15(1):58-66

24 Lieb W, Larson MG, Benjamin EJ, Yin X, Tofler GH, Selhub J et al. Multimarker approach to evaluate correlates of vascular stiffness: the Framingham Heart Study. Circulation 2009;119(1):37-43

25 Roman MJ, Devereux RB, Krzer JR, Okin PM, Lee ET, Wang WN et al. High central pulse pressure is independently associated with adverse cardiovascular outcome: the strong heart study. J Am Coll Cardiol 2009;54:1730-1734

26 van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RM et al. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality: the Hoorn study. J Am Coll Cardiol 2014;63(17):1739-47

27 Vlachopoulos C, Aznaouridis K, Stefanidis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness A systematic review and meta-analysis. J Am Coll Cardiol 2010; 30;55(13):1318-1327

28 Taal MW, Sigrist MK, Fakis A, Fluck RJ, McIntyre CW. Markers of arterial stiffness are risk factors for progression to end stage renal disease among patinets with chronic kidney disease stage 4 and 5. Nephron Clin Prac 2007;107:c177-c181

29 Briet M, Collin C, Karras A, Laurent S, Bozec E, Jacquot C et al. Nephrotest Study Group Arterial remodeling associates with CKD progression. J Am Soc Nephrol 2011;22(5):967-974

30 Rossi M, Matteucci E, Pesce M, Consani C, Franzoni F, Santoro Get al. Peripheral microvascular dysfunction as an independent predictor of atherosclerotic damage in type 1 diabetes patients: a preliminary study. Clin Hemorheol Microcirc 2013;54(4):381-391

31 Feihl F. Liaudet L, Waeber B. The macrocirculation and microcirculation of hypertension. Curr Hypertens Rep 2009;11(3):182-189

32 Safar ME, Jankowski P. Central blood pressure and hypertension: role in cardiovascular risk assessment. Clinical Science 2009;116:273-282.

33 Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106(16):2085-2090

34 Xiao Y, Luo X, Huang W, Zhang J, Peng C. FGF 23 and risk of all-cause mortality and cardiovascular events: A meta-analysis of prospective cohort studies. Int J Cardiol 2014; 174(3):824-828

35 Turner J, Belch JJF, Khan F. Current concepts in assessment of microvascular endothelial function using laser doppler imaging and iontophoresis. Trends Cardiovasc Med. 2008;18:109-116.

Table 1. Baseline data of the participants

	All CKD patients		eGfr ≥ 30ml/min/1.73 m^2		eGfr < 30ml/min/1.73 m^2	
n (%)	94		56		38	
Males (%)	45 (48)		32 (57)		13 (34)	
Smoke (current) (%)	9 (10)		4 (7)		5 (13)	
Diabetes mellitus (%)	41 (44)		23 (41)		18 (47)	
Cardiovascular disease (%)	61 (65)		33 (59)		28 (74)	
Coronary artery disease (%)	13 (14)		5 (9)		8 (21)	
Chronic heart failure (%)	18 (19)		8 (14)		10 (26)	
Cerebrovascular disease (%)	23 (24)		9 (16)		14 (37)	
Peripheral artery disease (%)	50 (53)		28 (50)		22 (58)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (years)	65.3	13.1	64.1	14.6	67.1	10.6
BMI (kg/m^2)	28.16	4.83	28	4.29	28.39	5.58
CCI* #	4	(1-6)	3	(1-5)	5	(2-7)
eGFR ($ml/min/1.73m^2$) * #	35.3	(22.8-49.4)	44.9	(37.7-55.3)	21.9	(15.5-26.3)
Hgb (g/L) #	126	14	130	14	122	12
Chol (mmol/L)	4.89	1.1	4.88	1.03	4.91	1.21
Tg (mmol/L)	2.15	1.3	2.19	1.44	2.09	1.07
LDL (mmol/L)	2.55	0.82	2.57	0.85	2.51	0.8
Ca (mmol/L)	2.36	0.12	2.37	0.1	2.35	0.15
iP (mmol/L) #	1.22	0.24	1.15	0.2	1.33	0.25
FGF23 (RU/mL) * #	29.2	(20.9-55.3)	23.63	(19.3-34.8)	47.5	(28.3-80.5)
iPTH (pg/mL) * #	56	(38-104)	48	(28-65)	100	(50-180)
Alb (g/L)	45.3	4.2	45.4	4.4	45.1	4.0
CRP (mg/L) * #	2.2	(0.9-4)	1.6	(0.6-3.1)	3.1	(1.1-6)
ACR (mg/mmol) * #	8.43	(1.84-58.71)	5.02	(0.98-31.29)	20.49	(4.89-97.71)
SBP (mmHg)	135	16	135	15	134	17
DBP (mmHg)	73	10	74	9	72	11
HR (1/min)	67	12	65	10	69	14
cPWV (m/s)	12.52	4.47	12.58	4.69	12.43	4.22
CPP (mmHg)	52	13	52	13	52	12
$PORH_{HA}$ (PU*sec)*	593	(280-1046)	523	(248-1007)	846	(370-1116)

***** Data with non-normal distribution are given as median and interquartile range.
# $p < 0.05$ difference between groups of eGFR <and ≥ 30 ml/min/1.73 m2
No missing data.

Abbreviations: n -case number, SD - standard deviation, CCI - Charlson Comorbidity Index,
BMI - body mass index, eGFR - estimated glomerular filtration rate (EPI),
Hgb - hemoglobin, Chol-cholesterol, Tg - triglyreride, LDL – low density lipoprotein,
Ca - serum calcium,iP – serum inorganic phosphate,
ACR - albumin creatinin ratio, SBP - peripheral systolic
blood pressure, DBP - peripheral diastolic blood pressure, HR - heart rate,
cPWV - central pulse wave velocity, CPP - central pulse pressure,
$PORH_{HA}$ - post occlusive reactive hyperaemia area, PU-perfusion unit

Cardiovascular disease is defined as a documented baseline history of coronary artery disease, chronic heart failure, cerebrovascular or peripheral vascular disease.

Table 2. Baseline associations of the vascular biomarkers
– Uni- and multivariate linear regression models

Univariate models

	c PWV (m/s)			CPP (mmHg)			$lnPORH_{HA}$		
	ß	**r^2**	**p**	**ß**	**r^2**	**p**	**ß**	**r^2**	**p**
Age (years)	***0.38***	***0.15***	***<0.001***	0.11	0.01	0.31	0.16	0.03	0.12
CVD	***0.45***	***0.21***	***<0.001***	0.18	0.03	0.09	-0.04	0.00	0.68
DM	***0.32***	***0.10***	***0.002***	0.18	0.03	0.08	***-0.25***	***0.06***	***0.02***
BMI (kg/m^2)	-0.11	0.01	0.3	0.14	0.02	0.18	-0.08	0.01	0.47
ln eGFR	-0.18	0.01	0.3	-0.12	0.01	0.38	0.03	0.00	0.88
Hgb (g/L)	-0.14	0.02	0.19	-0.1	0.01	0.33	0.08	0.01	0.45
Chol (mmol/L)	-0.01	0.00	0.89	0.1	0.01	0.36	-0.07	0.00	0.52
Tg (mmol/L)	-0.15	0.02	0.15	0.02	0.00	0.84	-0.003	0.00	0.97
LDL (mmol/L)	0.11	0.01	0.32	0.14	0.02	0.19	-0.04	0.00	0.73
Ca (mmol/L)	-0.14	0.02	0.19	-0.01	0.00	0.96	-0.17	0.03	0.11
iP (mmol/L)	-0.12	0.02	0.24	0.07	0.00	0.52	-0.08	0.01	0.46
ln FGF23	-0.001	0.00	0.99	0.03	0.00	0.78	0.006	0.00	0.95
ln iPTH	0.14	0.02	0.19	0.04	0.00	0.69	0.15	0.02	0.16
Alb (g/L)	-0.09	0.01	0.39	0.02	0.00	0.86	-0.06	0.00	0.6
ln CRP	-0.03	0.00	0.59	-0.16	0.01	0.39	0.08	0.01	0.3
ln ACR	0.02	0.00	0.83	0.07	0.00	0.51	-0.15	0.02	0.15
SBP (mmHg)	***0.32***	***0.10***	***0.002***	***0.65***	***0.42***	***<0.001***	***-0.21***	***0.05***	***0.04***
DBP (mmHg)	0.01	0.00	0.93	0.19	0.04	0.07	0.14	0.02	0.19
HR (1/min)	0.07	0.00	0.52	-0.13	0.02	0.24	-0.09	0.01	0.42
cPWV (m/s)	1			***0.29***	***0.09***	***0.005***	-0.15	0.02	0.15
CPP (mmHg)	***0.29***	***0.09***	***0.005***	1			***-0.41***	***0.17***	***<0.001***
ln $PORH_{HA}$	-0.15	0.02	0.15	***-0.41***	***0.17***	***<0.001***	1		

Multivariate models

	cPWV (m/s)			CPP (mmHg)			ln $PORH_{HA}$		
	ß	**SE**	**p**	**ß**	**SE**	**p**	**ß**	**SE**	**p**
Age (years)	***0.25***	***0.096***	***0.01***		NA			NA	
CVD	***0.22***	***0.099***	***0.03***		NA			NA	
DM	***0.19***	***0.089***	***0.04***		NA		-0.17	0.093	0.07
CPP (mmHg)	0.03	0.106	0.77		NA		***-0.37***	***0.115***	***0.002***
SBP (mmHg)	***0.25***	***0.105***	***0.02***	***0.51***	***0.079***	***<0.001***	0.04	0.114	0.76
cPWV (m/s)		NA		0.08	0.079	0.29		NA	
ln $PORH_{HA}$		NA		***-0.27***	***0.077***	***0.001***		NA	

$Adj.R^2$	**SEE**	**p**	**$Adj.R^2$**	**SEE**	**p**	**$Adj.R^2$**	**SEE**	**p**
0.30	***3.739***	***<0.001***	***0.44***	***9.416***	***<0.001***	***0.16***	***1.048***	***<0.001***

Abbreviations: NA-not analysed, ß –regression coefficient, p-level of significance,
SE - standard error of ß, SEE - standard error of estimate, BMI - body mass index,
ln - natural based logarithm, DM - diabetes mellitus, CVD - cardiovascular disease
(coronary artery disease+chronic heart failure+peripheral artery disease+cerebrovascular
disease), eGFR - estimated glomerular filtration rate, Hgb - hemoglobin, Chol-cholesterol,
Tg - triglyreride, LDL – low density lipoprotein, Ca - serum calcium,
iP – serum inorganic phosphate, ACR - albumin creatinin ratio,
SBP - peripheral systolic blood pressure, DBP - peripheral diastolic blood pressure,
HR - heart rate, cPWV - central pulse wave velocity, CPP - central pulse pressure,
PORHHA - post occlusive reactive hyperaemia area

Table 3. Uni- and multivariate multiple failure time Cox-proportional hazards regression analysis of cardiovascular morbidity and mortality risk predictors

Univariate model

	Hazard Ratio	95% confidence interval		P
Age (years)	1.01	0.99	1.04	0.2
Gender (male)	0.88	0.48	1.63	0.69
Smoking	0.84	0.31	2.31	0.74
DM	***3.24***	***1.7***	***6.1***	***0.0001***
CVD	***2.65***	***1.11***	***6.34***	***0.03***
BMI (kg/m2)	1.06	0.99	1.12	0.06
CCI	***1.26***	***1.11***	***1.44***	***0.0001***
ln eGFR	***0.41***	***0.23***	***0.73***	***0.003***
Hgb (g/L)	0.98	0.97	1.01	0.19
Chol (mmol/L)	0.88	0.72	1.08	0.23
Tg (mmol/L)	0.98	0.81	1.18	0.87
LDL (mmol/L)	0.94	0.69	1.27	0.69
Ca (mmol/L)	1.91	0.1	35.21	0.66
iP (mmol/L)	1.37	0.42	4.42	0.6
Alb (g/L)	1.03	0.94	1.11	0.5
ln CRP	1.31	0.80	2.16	0.28
ln FGF23	***1.75***	***1.1***	***2.77***	***0.02***
ln iPTH	1.31	0.90	1.89	0.16
ln ACR	1.04	0.89	1.21	0.64
SBP (mmHg)	1.02	1	1.04	0.12
DBP (mmHg)	0.99	0.96	1.03	0.97
HR (1/min)	1.02	1	1.04	0.13
cPWV (m/s)	***1.07***	***1.02***	***1.13***	***0.004***
CPP (mmHg)	***1.04***	***1.01***	***1.07***	***0.005***
ln PORH $_{HA}$	***0.7***	***0.58***	***0.85***	***0.0001***

Final multivariate model

	Hazard Ratio	CI 95%		P=
DM	***3.06***	***1.65***	***5.67***	***0.0001***
ln FGF23	***1.86***	***1.13***	***3.06***	***0.01***
CPP (mmHg)	***1.04***	***1.01***	***1.07***	***0.005***

Abbreviations: CI - confidence interval, BMI - body mass index, ln - natural based logarithm, CCI - Charlson comorbidity index, DM - diabetes mellitus, CVD - cardiovascular disease (coronary artery disease+chronic heart failure+peripheral artery disease+cerebrovascular disease), eGFR - estimated glomerular filtration rate (EPI), Hgb - hemoglobin, Chol-cholesterol, Tg - triglyreride, LDL – low density lipoprotein, Ca - serum calcium, iP - serum inorganic phosphate, ACR - albumin creatinin ratio, SBP - peripheral systolic blood pressure, DBP - peripheral diastolic blood pressure, HR - heart rate, cPWV - central pulse wave velocity, CPP - central pulse pressure, PORHHA - post occlusive reactive hyperaemia area