

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

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4 Short title: Vascular biomarkers in CKD

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**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<sub>HA</sub>) 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<sup>2</sup>) patients were followed-up for a median of 52(36-65) months and had baseline cPWV, CPP measured by applanation tonometry, and PORH<sub>HA</sub> 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<sub>HA</sub> to CPP ( $R^2=0.45$ ), while CPP was the only parameter independently related to PORH<sub>HA</sub> ( $R^2=0.16$ , 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<sub>HA</sub> (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<sub>HA</sub>) predicted CV outcomes in CKD-ND. CPP may integrate the information provided by cPWV and PORH<sub>HA</sub>.

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2 **Summary Table**  
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4 **The significance of micro- and macrovascular biomarkers on cardiovascular**  
5 **outcome in chronic kidney disease –A prospective cohort study**

6 **What is known about topic**

- 7 – Non-dialysed chronic kidney disease (CKD-ND) is characterised by large and  
8 small vessel dysfunction, as assessed by central pulse wave velocity (cPWV),  
9 central pulse pressure, and post-occlusive reactive hyperaemia (PORH<sub>HA</sub>)  
10 – The role of these micro-and macrovascular markers in the prediction of  
11 cardiovascular (CV) outcome has not been previously evaluated in a single  
12 cohort of CKD-ND.

13 **What this study adds**

- 14 – Among cPWV, CPP and PORH<sub>HA</sub>, the impaired pulsatile component of central  
15 arteries (CPP) was the primary predictor of CV outcome.  
16 – CPP seems to integrate the information provided by cPWV and PORH<sub>HA</sub>.
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**Keywords:**

Cardiovascular mortality

Central pulse pressure

Chronic kidney disease

FGF23

Post-occlusive reactive hyperaemia

Pulse wave velocity

## 1 **Introduction**

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

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

27 The aim of our cohort study was therefore to assess the association of micro- and  
28 macrovascular biomarkers (cPWV, CPP, PORH<sub>HA</sub>) with traditional and non-traditional CV  
29 risk factors, and to evaluate their independent predictive value for CV events and mortality in  
30 “mild-to-severe” CKD-ND cases.

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## 1 **Methods**

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

### 6 7 *Patients*

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

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

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

### 28 29 *Power analysis*

30 Based on the observed difference of 7 mmHg central pulse pressure in the Strong Heart  
31 Study [15] between participants with and without incident cardiovascular events we expected  
32 a difference with similar magnitude in our population. After the enrolment of the first 25  
33 participants, we calculated the standard deviation of central pulse pressure on enrolled  
34 population (SD 12 mmHg). Furthermore, based on literature data from similar populations  
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1 with a median eGFR of 30-44 ml/min/1.73m<sup>2</sup> we expected 11 cardiovascular events per 100  
2 person years of follow-up that translates to 44 events during the planned 4-year study. Taken  
3 together all these information we needed to enrol 93 participants to have an 80% power to  
4 detect a 7 mmHg difference in central pulse pressure between cases and non-cases with an  
5 alpha of 0.05. Our observation of a difference of 8 mmHg (47.1 ± 11.7 vs. 55.2± 13.8 mmHg,  
6 p=0.004) well corresponds to the power calculations.

#### 7 8 *Macrovascular injury and blood pressure measurements*

9 Measurements of vascular markers for a given patient (cPWV and CPP or PORH<sub>HA</sub>)  
10 were performed in a random order after one another on the same morning at baseline.  
11 Subjects were asked to refrain from smoking on the day of the study and not to consume  
12 caffeine-containing drinks at least 4 hours before the start of the measurements, but to take  
13 their regular morning medication. Tests were carried out in a temperature-controlled room  
14 (24±1°C) with the subjects in supine position, after a 20 minute rest period [16].

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

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

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



### 1 *Microvascular function test*

2 For Laser Doppler Flowmetry (LDF) measurements, blood pressure and heart rate of the  
3 subjects were determined as above. Laser Doppler instrument (Periflux 5001, Stockholm,  
4 Sweden, wavelength 780 nm) was used during the study as described previously [19]. In  
5 short, during the PORH<sub>HA</sub> test after the registration of the baseline flow for 60 seconds on the  
6 volar surface of the left forearm 10 cm below the elbow with a standard LDF probe, brachial  
7 arterial occlusion was applied with 40 mmHg suprasystolic pressure by a pneumatic cuff of a  
8 sphygmomanometer for 3 minutes to reach the biological zero. After the release of the  
9 pressure, perfusion (measured as perfusion unit (PU)) rise high and rapidly above the pre-  
10 ischemic PU values. The software analysed the data automatically and calculated several  
11 indices such as the initial baseline value, slope value, peak flow, percent change in perfusion  
12 from baseline to maximum values, time to reach the maximum hyperaemia, time to reach the  
13 half value after the maximum hyperaemia, and the area of hyperaemia. This latter  
14 measurement (abbreviated as: PORH<sub>HA</sub>, unit: PU\*sec – perfusion unit \* second) seems to be  
15 the most accurate parameter to assess the hyperaemic response, as it includes three variables  
16 (speed, intensity, and duration) and this was used in the analyses as representative of the  
17 microvascular function [20, 21]. In their study, Stiefel P et al found a „cut-off” PORH<sub>HA</sub> of  
18 865 PU\*sec to have an 82% sensitivity and a 97% specificity to distinguish microvascular  
19 dysfunction of coronary artery disease patients from healthy controls [20].

20 According to previous measurements in our laboratory, the day to day variability of this  
21 system was 16-21%, which is comparable to other studies [21]. All PORH<sub>HA</sub> measurements  
22 were performed by J.E. and analysed by J.N.

### 23 *Epidemiologic and Laboratory data*

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

30 Data on haemoglobin (Hgb), serum calcium (Ca), phosphate (iP), albumin (Alb),  
31 parathormon (iPTH), creatinine, C-reactive protein (CRP), serum cholesterol (Chol),  
32 triglyceride (Tg), and LDL-cholesterol were evaluated at baseline. Routine blood chemistry  
33 measurements were performed by a Hitachi auto analyser. Intact parathyroid hormone 1-84  
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1 (iPTH) was determined by immune-chemiluminometric two-site assay (CIBA-CORNING,  
2 Frenwald, Germany). Baseline eGFR was calculated using the four-variable Chronic Kidney  
3 Disease Epidemiology Collaboration (CKD-EPI) equation [5]. Circulating concentration of  
4 fibroblast growth factor 23 (FGF23) was measured using a second-generation C-terminal  
5 ELISA (Immutopics, San Clemente, CA). Albuminuria was characterised by albumin-  
6 creatinine ratio (ACR) measured from first morning spot urine according to KDIGO  
7 recommendations [5].

## 9 **Statistical analysis**

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

16 108 people were invited to participate; however only 103 agreed it and a further 9  
17 people were excluded due to missing baseline or follow-up data. We compared baseline  
18 characteristics of participants and non-participants and found no major differences between  
19 these groups. As the number of participants with missing data was relatively low, we used  
20 complete-case analysis. No multiple imputations were performed due to the limited sample  
21 size.

22 In the baseline cross sectional analyses univariate and multivariate (stepwise, ridge)  
23 linear regressions were performed to determine the main associations of the macro- and  
24 microvascular parameters (cPWV, CPP, PORH<sub>HA</sub>). The predictor variables considered were  
25 the ones listed in table 1. The variables that showed a significant association with the given  
26 dependent variable in univariate models were considered in the final multivariate model.  
27 (Table 2).

28 To assess the predictive values of cPWV, CPP and PORH<sub>HA</sub> for CV events and CV  
29 mortality, multiple failure times Cox proportional hazard regression analyses were used with  
30 conditional risk set modelling. This method accommodates for the fact that one patient may  
31 have more than one outcome event. We first performed univariate analyses considering  
32 variables listed in table 1. Confounding was addressed in multiple linear and multivariate  
33 Cox-regression models with adjustment for potential clinical predictors. Final models were  
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1 selected using backward elimination to reach the most parsimonious models. (Table 3 and  
2 Online Data Supplement Table 1.)

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

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

## 9 10 **Ethics**

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

## 14 15 16 **Results**

### 17 *Descriptive statistical analysis at baseline*

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

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

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

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

4 Baseline cPWV, CPP, and PORH<sub>HA</sub> values were 12.5±4.5m/s, 52±13mmHg, 593  
5 PU\*sec (280-1046), respectively, with no significant differences between the less and more  
6 advanced CKD groups.

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

#### 13 14 *Cross sectional analysis at baseline*

15 Baseline associations of the vascular biomarkers (i.e. cPWV, CPP, lnPORH<sub>HA</sub>) with  
16 other baseline clinical and biochemical parameters, as assessed by uni- and multivariate linear  
17 regressions are displayed in table 2. Age, diabetes, previous CV disease, and systolic blood  
18 pressure (SBP) were related to cPWV (R<sup>2</sup>=0.3). CPP was associated with SBP and PORH<sub>HA</sub>  
19 (R<sup>2</sup>=0.45). CPP was the only parameter significantly related to PORH<sub>HA</sub> (R<sup>2</sup>=0.16) in the  
20 multivariate model.

#### 21 22 *Prospective data analysis*

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

31 In univariate Cox regression analyses all three studied vascular parameters were  
32 significantly associated with the outcome, hazard ratios for cPWV were 1.07 (1.02-1.13), for  
33 CPP 1.04 (1.01-1.07), and for PORH<sub>HA</sub> 0.70 (0.58-0.85), respectively.

1 As a result of the multivariate backward Cox regression model building, that included  
2 the other significant univariate predictors besides the three studied vascular parameters, only  
3 the presence of DM (3.06 (1.65-5.67)), lnFGF23 (1.86 (1.13-3.06)) and CPP (1.04 (1.01-  
4 1.07)) remained independent predictors of CV mortality and events (Table 3 and online data  
5 supplement Table 1) while cPWV and lnPORH<sub>HA</sub> have lost their significant predictive value  
6 for CV events.

7 To determine the sensitivity of our data we repeated all these analyses with censoring  
8 patients at the first occurrence of a CV event (a total of 31 events). In this analysis again CPP  
9 remained the only significant vascular predictor of the outcome while cPWV as well as  
10 lnPORH<sub>HA</sub> lost their initial univariate significance in predicting CV outcome. (Online data  
11 supplement Table 2.)

## 12 **Discussion**

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

23 Our population can be classified as high-risk for CV diseases, as they had an event rate  
24 of 9.8 per 100 patient years during follow up. This cannot be explained solely by the presence  
25 of Framingham risk factors [24]. Indeed, the measured cPWV (12.5±4.5m/s) and CPP  
26 (52±13mmHg) values of our patients exceeded the „cut-off” values (PWV >10 m/sec and  
27 CPP >50 mmHg, respectively) recommended by the ESH guideline and the Strong Heart  
28 Study [17,25] may in part explain this higher event rate. Our patients with an eGFR of less  
29 than 30 ml/min/1.73 m<sup>2</sup> tended to have more baseline CV diseases, such as coronary,  
30 cerebrovascular and peripheral artery disease, which likely further increased their risk of  
31 future CV events. Thus, among our patients with worsening kidney function, an increased  
32 number of comorbidities could explain the rate of events that exceeds the 3 events per 100  
33 patient year reported by Baumann et al. [11] and the 5.13 events per 100 patient year in Hoorn  
34 study [26]. While in the last decade cPWV has been proven to be an independent risk factor  
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1 of CV risk in the general and ESRD population [2,3,27] only a limited number of studies had  
2 been performed with this vascular parameter in CKD-ND. In fact, only the above two  
3 prospective cohort studies investigated cPWV in a CKD-ND population. In the first study  
4 cPWV was found to predict all-cause mortality, while in the latter study cPWV was related to  
5 cardiovascular events after 7.6 years of follow-up [11,26]. Other recent studies demonstrated  
6 that decreased arterial elasticity is related to CKD progression; long-term CV outcomes have  
7 not been reported. [28,29] There is one additional prospective cohort study by Quiroga et al  
8 [12], who found male gender, diabetes, kidney disease progression and baseline CV disease to  
9 be predictors of all-cause mortality in their Spanish cohort. It is important to note, however,  
10 that central arterial elasticity parameters, such as CPP were not examined in their study. As  
11 for the role of CPP in CKD-ND, only the cross sectional CRIC study examined and found  
12 CPP as being superior over peripheral pulse pressure to quantify the risk of CV disease and  
13 eGFR impairment [9].

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

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

23 Our cross sectional analysis suggests that CPP might represent an early functional sign  
24 of vascular injury, to which none of the metabolic markers (e.g. eGFR, anaemia, disturbed  
25 mineral metabolism) of CKD were related. We hypothesize that early stages of athero- and  
26 arteriosclerosis could lead to microstructural deterioration of central and peripheral arteries  
27 that lead to early hemodynamic dysfunction in the CKD environment. Early dysfunction of  
28 large conduit arteries (characterized by increased cPWV) may have a backward effect leading  
29 to left ventricular hypertrophy and also increase CPP. At the same time early small vessel  
30 dysfunction (characterised by decreased PORH) may increase wave reflections, contributing  
31 to the high pulsatile component of the aortic pressure (CPP) that directly damages target  
32 organs such as the heart, brain and kidneys [31]. Given the above physiological evidence, it  
33 seems reasonable to hypothesize that increased CPP could be an integrative marker of  
34 preclinical early target organ damage indicating both micro- and macrovascular injury [32].  
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1           Beyond CKD, traditional risk factors, such as diabetes also lead to a significantly worse  
2 metabolic state, hence accelerated athero- and arteriosclerosis. Thus small and large arterial  
3 injuries due to these risk factors might develop simultaneously which can be extensively  
4 described by CPP as an integrative marker of CV outcome in CKD-ND. While the presence  
5 of diabetes was expected to determine the outcome [33], in our study, it was also  
6 independently associated with CPP, rather than cPWV suggesting that CPP better described  
7 the clinically relevant vascular changes that occur in CKD. An explanation as for why  
8  $PORH_{HA}$  was not an independent predictor of outcome could be that the target organ damage  
9 characterized by increased CPP can be considered a more robust factor than a sole  
10 microvascular injury or structural central arterial stiffness marker itself. In summary, our  
11 prospective data seem to support the notion that increased CPP is indeed an early integrative  
12 marker of large and small vessel injury, with clinically relevant consequences in CKD-ND.

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

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

## 30 **Limitations**

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32           There are several limitations of our study to be acknowledged. While unique in its  
33 objectives, our study evaluated only a relatively small number of cases, that makes it difficult  
34 to homogenise the cohort, smooth the variation of group composition in each CKD stages,  
35

1 and generalize our conclusions. Furthermore, as we had small patient numbers our confidence  
2 intervals are rather wide, and we hope that other ongoing cohort studies will support our  
3 findings on the fundamental role of CPP as an integrative marker of CV risk in this  
4 hypertensive CKD ND population. It is important to note that beside diabetes and FGF23,  
5 only CPP was predictive for the outcome, suggesting its robustness despite the small patient  
6 numbers. Nonetheless, further studies are needed to confirm the reliability of CPP as a clinical  
7 marker of CV risk stratification. We acknowledge that this was a sample of patients of two  
8 tertiary care nephrology clinics, and therefore selection bias that may limit generalizability  
9 (i.e. high baseline CV disease risk burden of our patients) cannot be ruled out. We realize that  
10 the method we used to assess microvascular reactivity with LDF and  $PORH_{HA}$  is not entirely  
11 established. We, therefore, are awaiting the results of further studies with this method [35].  
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17 Hungarian Society of Hypertension, and Hungarian Society of Nephrology  
18

19 There is no **conflict of interest** to declare  
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**Table 1.** Baseline data of the participants

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

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 - triglyceride, 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,

POR<sub>HA</sub> - 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 <sub>HA</sub>		
	$\beta$	$r^2$	p	$\beta$	$r^2$	p	$\beta$	$r^2$	p
Age (years)	<b>0.38</b>	<b>0.15</b>	<b>&lt;0.001</b>	0.11	0.01	0.31	0.16	0.03	0.12
CVD	<b>0.45</b>	<b>0.21</b>	<b>&lt;0.001</b>	0.18	0.03	0.09	-0.04	0.00	0.68
DM	<b>0.32</b>	<b>0.10</b>	<b>0.002</b>	0.18	0.03	0.08	<b>-0.25</b>	<b>0.06</b>	<b>0.02</b>
BMI (kg/m <sup>2</sup> )	-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)	<b>0.32</b>	<b>0.10</b>	<b>0.002</b>	<b>0.65</b>	<b>0.42</b>	<b>&lt;0.001</b>	<b>-0.21</b>	<b>0.05</b>	<b>0.04</b>
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			<b>0.29</b>	<b>0.09</b>	<b>0.005</b>	-0.15	0.02	0.15
CPP (mmHg)	<b>0.29</b>	<b>0.09</b>	<b>0.005</b>	1			<b>-0.41</b>	<b>0.17</b>	<b>&lt;0.001</b>
ln PORH <sub>HA</sub>	-0.15	0.02	0.15	<b>-0.41</b>	<b>0.17</b>	<b>&lt;0.001</b>	1		

**Multivariate models**

	cPWV (m/s)			CPP (mmHg)			ln PORH <sub>HA</sub>		
	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p
Age (years)	<b>0.25</b>	<b>0.096</b>	<b>0.01</b>		NA			NA	
CVD	<b>0.22</b>	<b>0.099</b>	<b>0.03</b>		NA			NA	
DM	<b>0.19</b>	<b>0.089</b>	<b>0.04</b>		NA		-0.17	0.093	0.07
CPP (mmHg)	0.03	0.106	0.77		NA		<b>-0.37</b>	<b>0.115</b>	<b>0.002</b>
SBP (mmHg)	<b>0.25</b>	<b>0.105</b>	<b>0.02</b>	<b>0.51</b>	<b>0.079</b>	<b>&lt;0.001</b>	0.04	0.114	0.76
cPWV (m/s)		NA		0.08	0.079	0.29		NA	
ln PORH <sub>HA</sub>		NA		<b>-0.27</b>	<b>0.077</b>	<b>0.001</b>		NA	
	<b>Adj.R<sup>2</sup></b>	<b>SEE</b>	<b>p</b>	<b>Adj.R<sup>2</sup></b>	<b>SEE</b>	<b>p</b>	<b>Adj.R<sup>2</sup></b>	<b>SEE</b>	<b>p</b>
	<b>0.30</b>	<b>3.739</b>	<b>&lt;0.001</b>	<b>0.44</b>	<b>9.416</b>	<b>&lt;0.001</b>	<b>0.16</b>	<b>1.048</b>	<b>&lt;0.001</b>



Abbreviations: NA-not analysed,  $\beta$  -regression coefficient, p-level of significance, SE - standard error of  $\beta$ , 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 - triglyceride, 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**

	<b>Hazard Ratio</b>	<b>95% confidence interval</b>		<b>P</b>
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
<b>DM</b>	<b>3.24</b>	<b>1.7</b>	<b>6.1</b>	<b>0.0001</b>
<b>CVD</b>	<b>2.65</b>	<b>1.11</b>	<b>6.34</b>	<b>0.03</b>
BMI (kg/m <sup>2</sup> )	1.06	0.99	1.12	0.06
<b>CCI</b>	<b>1.26</b>	<b>1.11</b>	<b>1.44</b>	<b>0.0001</b>
<b>ln eGFR</b>	<b>0.41</b>	<b>0.23</b>	<b>0.73</b>	<b>0.003</b>
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
<b>ln FGF23</b>	<b>1.75</b>	<b>1.1</b>	<b>2.77</b>	<b>0.02</b>
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
<b>cPWV (m/s)</b>	<b>1.07</b>	<b>1.02</b>	<b>1.13</b>	<b>0.004</b>
<b>CPP (mmHg)</b>	<b>1.04</b>	<b>1.01</b>	<b>1.07</b>	<b>0.005</b>
<b>ln PORH<sub>HA</sub></b>	<b>0.7</b>	<b>0.58</b>	<b>0.85</b>	<b>0.0001</b>

**Final multivariate model**

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

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 - triglyceride, 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