ARTERIAL STIFFNESS AND CARDIOVASCULAR COMPLICATIONS IN CHRONIC KIDNEY DISEASE

Ph.D. theses

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Abbreviations

ABPM ambulatory blood pressure monitor

ACEI angiotensin converting enzim inhibitor

ADPKD autosomal dominant polycystic kidney disease

AIx augmentation index

ARB angiotensin receptor blocker

BMI body mass index

CH carbohydrate

CKD chronic kidney disease

CKD-MBD krónikus vesebetegséghez társuló csont-és ásványianyagcsere-zavar

CRP C-reaktive protein

CVD cardiovascular disease

DBP diasztolic blood pressure

DVP digital volume pulse

eGFR estimated glomerular filtration rate

ESRD end-stage renal disease

FMC Fresenius Medical Care

HD hemodialysis

IgAN IgA nephropathy

IL-6 interleukin-6

iPTH intact parathormon

IR insulin resistance

MAP mean atrerial pressure

MDRD Modification of Diet in Renal Disease

MetS metabolic syndrome

NO nitrogen oxide

NYHA New York Heart Association

OC osteocalcin

OP osteopontin

OPG osteoprotegerin

PAD periferial artery disease

PC polycystin

PWV pulse wave velocity

RAAS renin-angiotensin-aldosteron system

RANK receptor activator of nuclear factor kappa-B

RR blood pressure

SBP systolic blood pressure

SD standard deviation

SI_{DVP} stiffness index

SPSS Statistical Package for the Social Sciences

TNF tumor necrosis factor

VC vascular calcification

VS vascular stiffness

VSMC vascular smooth muscle cell

1. Introduction

It is well established that cardiovascular complications are more frequent in patients with chronic kidney disease (CKD) than in the general population, as a result of accelerated atherosclerosis. CKD is an independent risk factor of cardiovascular disease (CVD). Premature death in CKD occurs mainly as a consequence of cardiovascular disease that develops during the disease course, and not owing to renal failure causes. It is estimated that risk of cardiovascular events may increase from 40% to 100% even in early CKD stages.

The development of vascular injury and increased vascular stiffness due to, in part, vascular calcification is observed in CKD with decreasing renal function, and myocardial function also worsen leading to increased arrhythmia risk, as well as left ventricular systolic and diastolic dysfunction. In addition, mineral bone metabolic disturbances (CKD-MBD) may also develop in CKD parallel with the deterioration of renal function contributing to the increased vascular stiffness via extraosseal calcification in the vessels. In CKD patients the risk of cardiac death is 10-20 times higher compared to non-renal patients. Although underlying mechanisms in the development and progression of CVD are not precisely characterized in CKD, both traditional risk factors (hypertension, diabetes, dyslipidemia, obesity, age) and non-traditional risk factors play important roles in the CKD-related cardiovascular complications, such as endothelial dysfunction, increased sympathetic activity, oxidative stress, hyperhomocysteinemia, anemia, advanced glycated end product upsurge, CKD-MBD, secondary hyperparathyroidism, and subclinical inflammation.

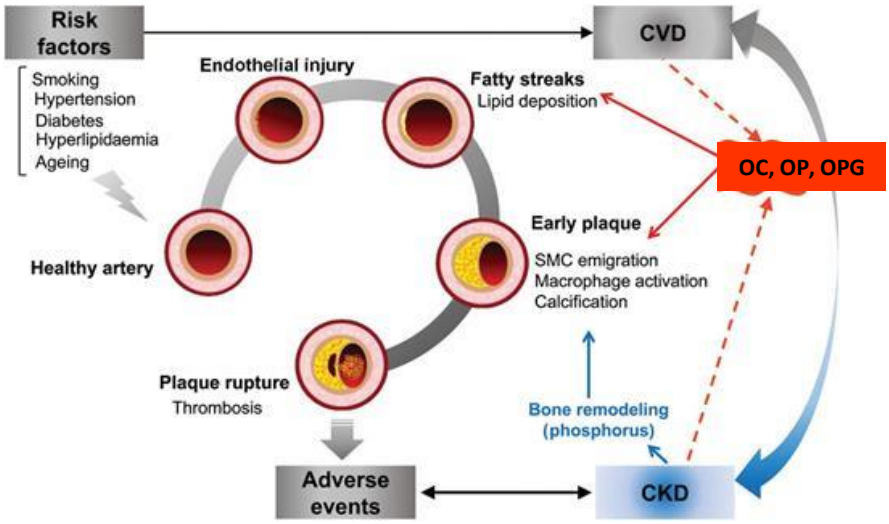
Arterial stiffness

To determine the arterial vascular stiffness, the most accepted and evidenced gold standard parameter is the pulse wave velocity (PWV), which is calculated from the pulse waves of carotid and femoral arteries by means of the distance between the two points. The increased pulse wave velocity (cfPWV) is a well accepted CV risk factor. In comparison with several, other conventional variable parameters used for CV risk estimation, including blood pressure, blood glucose, or cholesterol levels, the PWV appears more stable, constant, and reproducible parameter. Based on our recent knowledge, the value of PWV reflects the common effects of both known and still unknown causes and genetic factors that could affect arterial wall injury. The predictive value of PWV for CV mortality risk was shown in numerous follow-up studies with different patient populations (general population, elderly, hypertension, diabetes, and end stage kidney disease /ESRD/).

Vascular calcification

As previous studies showed, vascular calcification (VC) plays a key role in the development of cardiovascular disease in CKD. Vascular calcification is most evidently present in patient with ESRD. The vascular smooth muscle cells (VSMC) have been implied principally. Recent studies suggested that interaction of endothelial cells and VSMC could also regulate VC progression. The bone-vascular axis dysfunction induced harmful relationship between bone loss and VC is amplified by CKD. Various inhibitors and promoters were identified that have roles in mediating the signaling between the skeletal muscle and the vascular system. In recent years, special attention has been focused to bone-related proteins, including osteoprotegerin (OPG), osteocalcin (OC), and osteopontin (OP) which could serve as a common point linking the development of vascular complications in chronic CVD and CKD (Figure 1).

Figure 1: The role of bone- associated proteins in vascular calcification



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2. Aim of the studies

Before of our studies, there have been scarce data available on the prognostic value of arterial stiffness in chronic but non-uremic renal patients, or homogeneous etiologic groups of CKD patients. In chronic dialysis patients the relationship between selected bone related hormones and arterial stiffness has not been determined yet. Our objective was to establish the prevalence of certain non-traditional risk factors and clarify their role in the development of cardiovascular disorders in CKD.

In the first study, we determined in IgA patients representing homogeneous group of CKD

a / the prognostic role of stiffnes index

b / the outcome influencing factors

c / the relationship between vascular stiffness and renal function (estimated GFR)

In the second study, we investigated in autosomal dominant polycystic kidney disease (ADPKD) patients

a / the prognostic value of arterial stiffness for renal and CV progression

b / the outcome modifyling factors

c / whether metabolic syndrome had any impact on the endpoints

In the third study, we examined in renal patients on regular hemodialysis (HD) treatments

a / the extent and severity of arteriosclerotic lesions by measuring the atrial stiffness

b / the correlations between atrial rigidity parameters and OPG, OC, and OP

c / the relationships between selected bone-related proteins and clinical/biochemical CVD risk factors

3. Subjects of the studies

In our first study, conducted at the Univ. of Pécs, 2nd Dept. of Internal Medicine and Nephrological Centre, we examined 108 IgAN patients and followed-up for the average of 65 (6-107) months. During the study period, 5 patients did not attend any control visits, therefore, 103 patients were studied. The diagnosis of IgAN was proved by kidney biopsy in all patient. At the enrollment visit, all classical CV risk factors (hypertension, carbohydrate metabolic disorders, obesity, lipid abnormalities, smoking), and current therapy of patients were recorded.

In our second study, 60 ADPKD patients were involved, 5 patients were dropped out from analyses as they did not attend the control visits. Patients were followed-up for the of 63 (1-99) months. The diagnosis of ADPKD was defined of the clinical picture, physical examination, and the typical ultrasonic morphology. Serious clinical conditions (NYHA III-IV heart failure, stroke and myocardial infarction within 3 months, uncontrolled cardiac arrhythmia, malignant disease requiring active treatment, infection, fever) were the exclusion criteria. Patients with ESRD (stage CKD 5), renal replacement therapy, or kidney transplantation were also excluded from the study.

In the third study, 68 patients on chronic hemodialysis (HD) treatments at the FMC dialysis center in Pécs with stable clinical status were investigated. Exclusion criteria were as follows: lower limb amputation, acute infection, malignancy, acute myocardial infarction, pulmonary edema, or hemodynamic instability. The main etiologies of ESRD were the following kidney diseases: diabetic nephropathy (26%), benign nephrosclerosis (23%), chronic glomerulonephritis (15%), polycystic kidney disease (13%), chronic interstitial nephritis (10%), renovascular disease 1%) and other / unknown reasons (12%). Majority of patients (66/68) received antihypertensive treatment. Data are shown as predialysis values. Control 35 subjects were non-cardiovascular, non-metabolic, and non-renal healthy individuals (healthcare workers) for the analyses of cardiovascular and biochemical parameters.

4. The prognostic role of stiffness index determined by finger photoplethysmography in IgA nephropathy

Methods

Vascular stiffness determinations

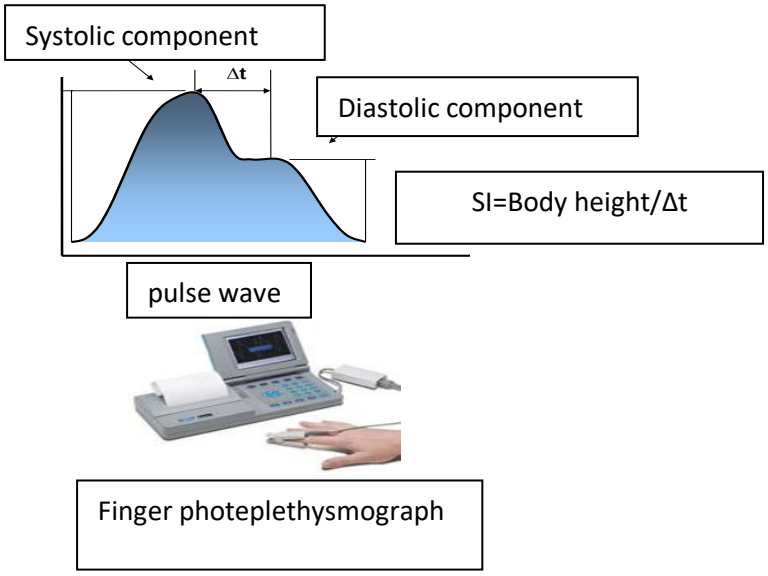
The finger photoplethysmography method with the Pulse Trace System (Micro Medical Ltd., Rochester, UK) was used to assess pulse-wave velocity (PWV), as described earlier. This method enables to determine the stiffness index (SI) which can be derived from the digital volume pulse (DVP), and is reflected as SI_{DVP}. Patients and controls were allowed to take their regular medications as necessary.

The participants were asked not to smoke and not to drink coffee on the day of the examination. All subjects were examined in supine position after at least 10 min of resting. A single waveform was obtained by averaging DVP contours during 30 sec of period. To enhance the accuracy of the investigation of SI_{DVP}, five period samples were taken and the upper and lower merit of DVP was deleted. The remaining three merits were averaged and used for further analyses. Variability test was then performed with the remaining three merits. All measurements were conducted in the morning hours between 9 and 11 am, thereby eliminating confounding effects of the circadian variability (Figure 2). The person analyzing the data was unaware of any clinical information of the subjects.

Primary and secondary endpoints

As a primary composite endpoint of the study were mortality from any origin, acute coronary event, acute myocardial infarction, stroke as cardiovascular endpoints and end-stage renal disease together. As a secondary endpoints the cardiovascular and renal endpoints were analyzed separately.

Figure 2. Pulse Trace system. Above is a sketch of the pulse wave curve and below the scan of the finger photoplethysmograph. SI = stiffness index.



Statistical analysis

The value of arterial stiffness may be different, at certain levels, depending on the test method used. For statistical analyses, we assigned the patients into two groups by the accepted 10 m / s limit, as known in the international literature. All values were expressed as mean ± SD format unless otherwise indicated. Survival was examined by Mantel-Cox log-rank test. Cox regression analysis was used to evaluate the effects of survival factors. ROC analysis was also performed in order to determine which SI was the limit value by our method that separated better the two groups for the outcome. Multivariate analysis was used to investigate the factors that influence CV events and impaired renal function. Statistical analyses were performed using the SPSS software version 22.0. P <0.05 was considered as statistically significant.

Results

Of the 103 IgAN patients involved in our study, 67 were male, the mean age was 45 ± 11 years. The follow-up time was in average 65 ± 32 months. The majority of patients (74%) was hypertensive, and approximately one-third was diabetic (29%). The mean value of SI_{DVP} was 9.98 m/s. The main clinical characteristics and occurrence of risk factors are summarized in Table 1.

Table 1. Baseline characteristics of IgAN patient

Clinical data	All patients	SIDVP ≤10m/s	SIDVP >10 m/s	P value
Male/Female (n=/%)	67/36	39/21 (65/35)	28/15 (70/30)	NS
Average age (years)	45±11	40.4±10.5	51.4±9.9	0.001
SIDVP (m/s)	9.98±2.48	8.35	12.25	0.001
Metabolic syndrome (n, %)	27 (27)	11 (18)	16 (37)	0.01
Average blood pressure (Hgmm)	128/81	128/80	130/82	NS
Hypertension (n, %)	75 (74)	38 (63)	39 (91)	0.001
Dyslipidemia (n, %)	49 (48)	23 (38)	26 (60)	0.013
Obesity (n, %)	28 (27)	14 (23)	14 (32)	NS
Carbohydrate metabolic disturbance (n, %)	30 (29)	13 (22)	17 (39)	0.02
eGFR (ml/min)	87 ± 35	95.9±35.7	75.0±32.3	0.001
Smoking (n, %)	16 (16)	9 (15)	7 (16)	NS
ACEI/ARB therapy (n, %)	84 (83)	43 (72)	41 (95)	<0.001

p<0.05

IgAN patients were divided into two groups based on the stiffness index (SI_{DVP}): (i) SI_{DVP} lower or equal of 10m/s; and (ii) SI higher of 10 m/s groups, as shown in Table 1. The two groups were significantly different in age, renal function, metabolic parameters (hypertension, dyslipidemia, and carbohydrate metabolism), the incidence of metabolic syndrome, and ACEI/ARB use. There were no differences between the groups in gender, actual average blood pressure, smoking, and obesity.

The probability of combined (cardiovascular + renal) primary endpoints was significantly higher (Chi square: 5.860; P = 0.015) in the increased vascular stiffness (SI_{DVP} > 10 m/s) group, as illustrated in Figure 3. After the analysis of secondary endpoints (cardiovascular or renal separately), we found significant difference with the renal endpoint (Chi-square: 4.788; P = 0.029), while there was no difference with the cardiovascular endpoint (Chi square: 1.363; P = 0.243), as shown in Figure 4 and Figure 5, respectively.

Figure 3. Primary combined end points in IgAN patients with SI_{DVP} > 10 m/s versus SI_{DVP} ≤ 10 m/s.

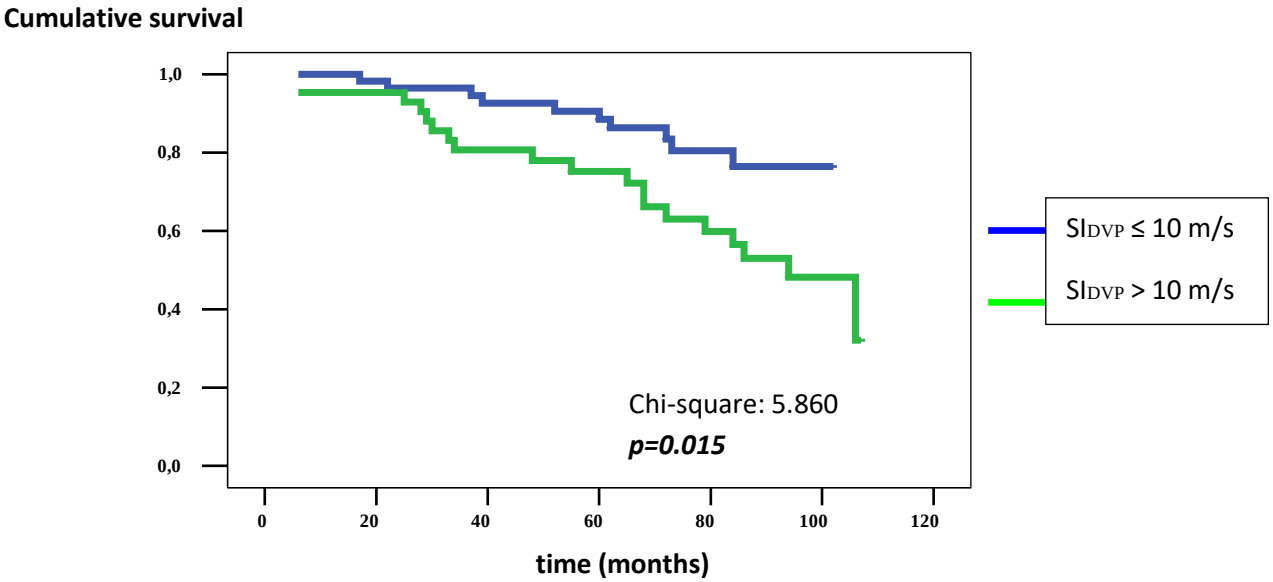


Figure 4. Secondary, renal end points in IgAN patients with SI_{DVP} > 10 m/s versus SI_{DVP} ≤ 10 m/s.

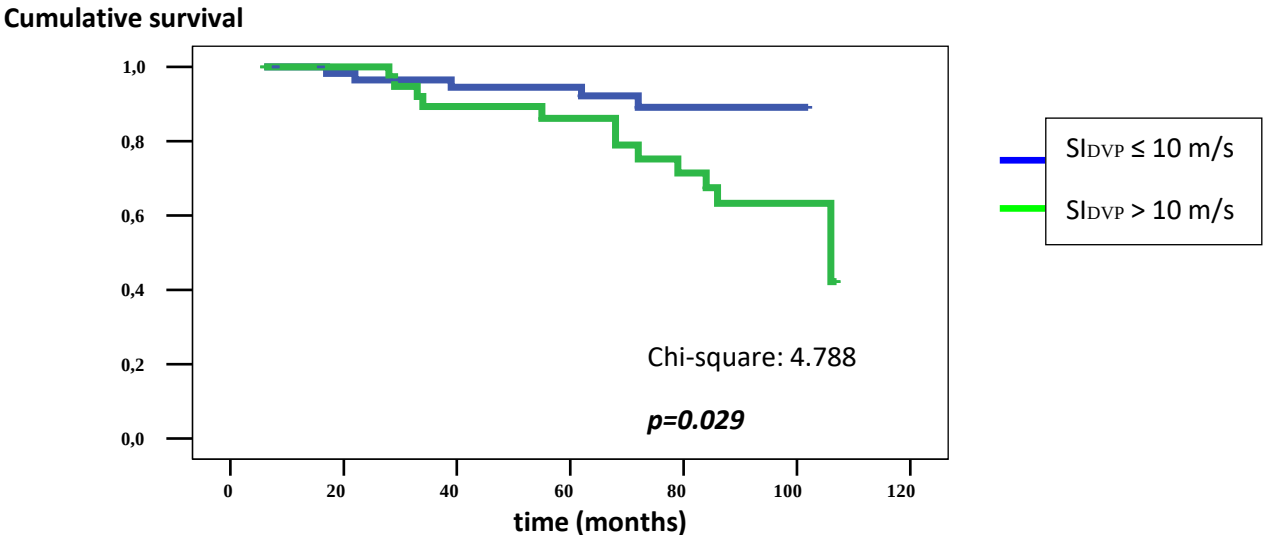
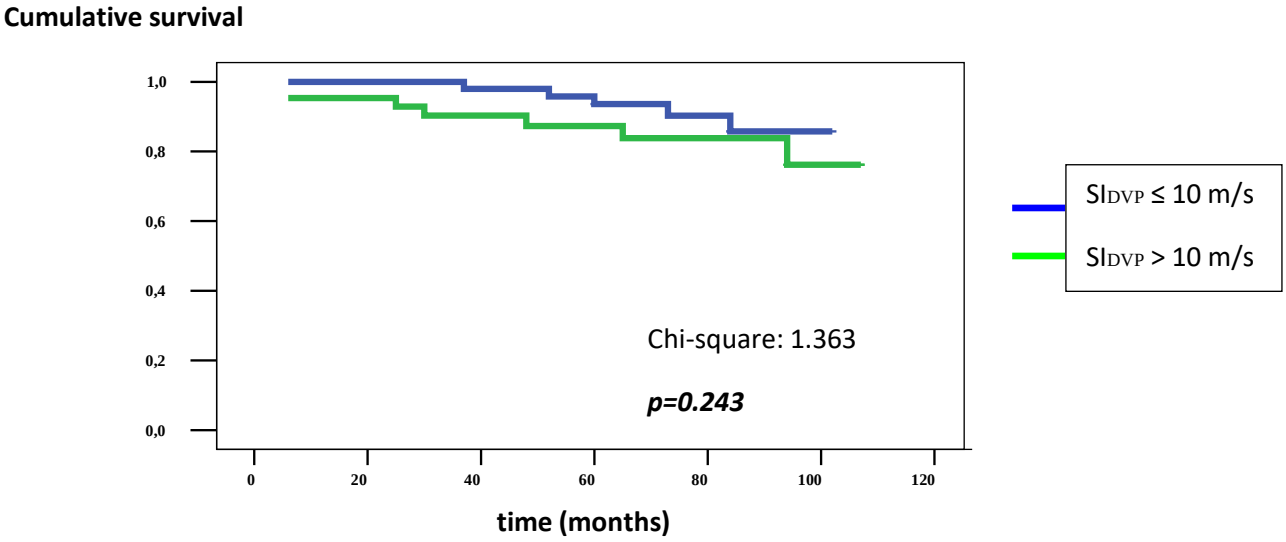


Figure 5. Secondary, cardiovascular end points in IgAN patients with SI_{DVP} > 10 m/s versus SI_{DVP} ≤ 10 m/s.



Cox regression model showed that every 1 m / s increase of the SIDVP value increased the probability of the cumulative endpoint by 17%. In the multivariate analysis, the variables that altered the combined primary endpoints (renal + cardiovascular) were the initial renal function, the carbohydrate metabolism, and the SI_{DVP} value (Table 2).

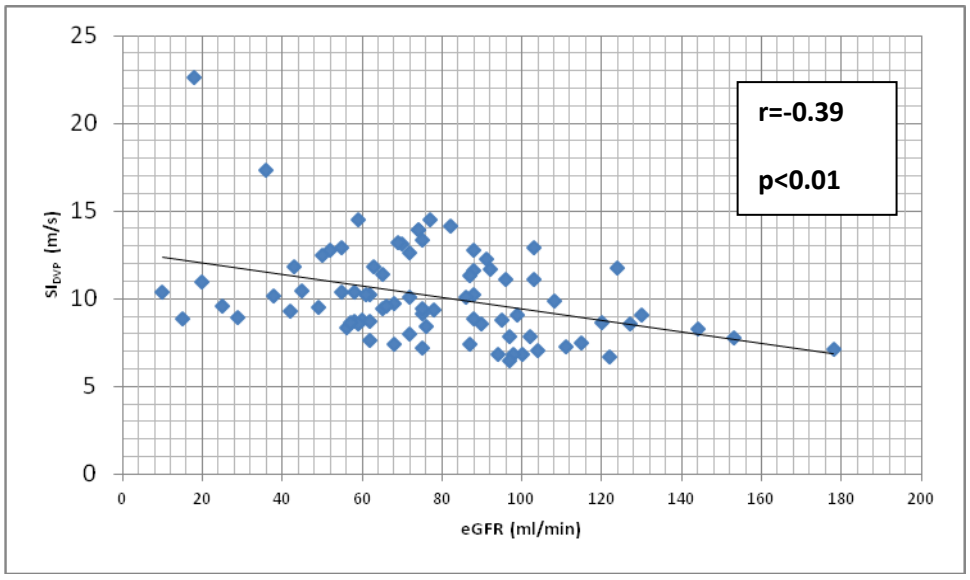
Table 2. Cox regression analysis: Primary endpoint related parameters

Parameters	Relative risk (95% CI)	P value
SI_{DVP}	1.17 (1.01-1.37)	0.039
Age	1.04 (0.98-1.10)	0.232
Dyslipidemia	1.37 (0.56-3.35)	0.496
Obesity	0.73 (0.30-1.79)	0.487
Hypertension	3.15 (0.04-2.68)	0.290
Carbohydrate metabolic disturbance	3.15 (1.27-7.75)	0.013
eGFR (ml/min)	1.03 (1.01-1.05)	0.003

$p<0.05$

In the ROC analysis, the limit value for SI_{DVP} was found as 9.58 m / s that most properly distinguished the two groups for the outcome. In this study, we found significant inverse relationship between the stiffness index and eGFR ($r = -0.39$, $P < 0.01$), as shown in Figure 6.

Figure 6. Correlation between stiffness index and renal function



Discussion

In this follow-up clinical study, we determined the prognostic role of arterial stiffness for cardiovascular and renal endpoints in typical CKD cohorts of patients with IgAN. Our results demonstrated that elevated stiffness index of IgAN renal patients predicts earlier the progression of artery stiffness and the kidney disease progression, as well as the occurrence CV events, prior to the development of ESRD.

Although it is acknowledged that SI_{DVP} is derived from different measurement than PWV which is the gold standard method; however, SI_{DVP} shows significant correlation with PWV in healthy subjects, and also in case of ESRD and coronary heart disease. Advantageous utility of SI_{DVP} by which the arterial stiffness could be assessed is based on its noninvasive manner and has been successfully established in patients with hypertension, diabetes, and in pediatric patients as well. In addition, SI_{DVP} can be applied for risk stratification in hypertensive patients, and also in apparently healthy individuals having CV risk factors, while other authors indicated as the marker of "vascular aging".

In present study, we used 10 m/s cut-off PWV value based on results of several multicentric trials involving large number of patients, and accordingly to the guideline of the European Cardiology and Hypertension Society (ESC/ESH). Here, we found very similar result as the cut-off point from the ROC analysis was 9.58 m/s, although measurements were conducted with photoplethysmography and smaller number of cases.

The prognostic value of arterial stiffness for CV outcome in CKD was first described in ESRD patients in epidemiological longitudinal studies. It is evidenced that CV mortality of patients with chronic renal failure momentously exceeds that of the general population, and that vascular rigidity is an independent predictor of total and CV mortality in patients with hypertension and chronic renal failure. In uremic patients, central PWV closely correlates with both CV and all-cause mortality. Blacher et al. study in this population found that every single increase of PWV by 1 m/s led to 14% increase of CV and total mortality. Our results here were akin, as increased arterial stiffness and 1 m/s increase of SI_{DVP} resulted in higher probability of the cumulative endpoint by 17%. The mechanism leading to increased stiffness in CKD is still unclear. Wang et al. first demonstrated in diverse CKD populations that arterial stiffness increases with the progressive and gradual worsening of the renal function.

Increased early RAAS activity and hypertension are supposed to have effects on the occurrence of vascular events in CKD patients. Others suggested that activation of RAAS is essential in the development of arterial stiffness in kidney diseases. Administration of ACEI or ARB markedly improved stiffness parameters in the general population, in hypertensive and in dialyzed renal patients, as shown by Gusbeth-Tatomir et al. Hypertension is a very frequent complication of IgAN. There are lacking data, however, whether ACEI and / or ARB treatment have any stiffness-lowering effect in IgAN patients. In our study, more than 3/4 of patients received ACEI and / or ARB therapy. The beneficial effects of RAAS inhibition in CKD are well-known in the literature. In this study, patients that exhibited elevated stiffness received RAAS inhibitor treatment in a significantly larger proportion, however, this could not prevent CV and renal endpoints events examined here. It is noteworthy that blood pressure control of the treated study patients was optimal, and despite the increased rate of hypertension in the higher stiffness group, there were no significant differences in the actual blood pressure measurements. In multivariate analysis, hypertension showed no correlation with the combined CV endpoints, which could be explained by the lower number of cases and the intensified reduction of blood pressure. IgAN patients with higher SI_{DVP} developed renal insufficiency earlier, renal impairment also progressed earlier, and not significantly but higher

numbers of CV complications were noted than in the lower SI_{DVP} patients having less rigid vasculature.

Metabolic parameters are important influencing factors for the progression of IgAN, as shown by others and our workgroup previosuly. The presence of metabolic syndrome in CKD worsens the prognosis. The number of metabolic syndrome components and hs-CRP level are potent factors for altering arterial stiffness. In CKD, the serum adiponectin level increases as the renal function decreases and when more metabolic syndrome components are present, which could further affect adversely the vascular stiffness. In this study, patients with stiffer vasculature exhibited multiple metabolic abnormalities that could also have contributions to the higher number of primary endpoints in this group. Conclusively, complex metabolic risk reduction seems important, and thus metabolic disorders should be treated prompt and appropriately in these patients.

We demonstrated that stiffness determined by finger photoplethysmography has prognostic significance, nonetheless there may be occasionally difficulties in registering the digital pulse volume. It may be problematic in some cases, particularly in elder patients to confidently separate systolic and reflective waves, and thus the stiffness index assessment. Atrial fibrillation, frequent atrial and ventricular ectopic activity could also limit the correct pulse curve detection. We estimated and not measured the renal function, however, the use of eGFR is widely accepted in the literature. Evaluation of results may be weakened by the low number of cases. It is conceivable that study follow-up period was somewhat short to be sufficient to prove the difference in the occurrence of CV events. However, limitations of the study may hamper establishing the conclusions, it seems clear that increased arterial stiffness could predict the development of target organ damage in CKD.

5. Arterial stiffness may predict renal and cardiovascular prognosis in autosomal dominant polycystic kidney disease

Methods

Vascular stiffness assessment

We used finger photoplethysmography method by the Pulse Trace System (Micro Medical Ltd., Rochester, UK) to assess pulse-wave velocity, as described earlier. This method enables to determine the SI which can be derived from the DVP, and is reflected as SI_{DVP}. Briefly, DVP includes two distinct waves during the cardiac cycle: the early systolic one that originates from the pressure wave at the time of the left ventricle ejection which could be measured in the finger artery, followed by the second peak due to reflected wave from more peripheral segments, usually the aortic bifurcation. The SI is derived by the body height relative to the time difference between the forward and reflective pulse waves: SI_{DVP} (m / s) = height / Δt. The recorded pulse curve profile is chiefly determined by the PWV of large arteries. Based on the literature data, the method used here does correlate with other methods, such as the central aortic PWV. Higher SI_{DVP} values indicate increased vascular stiffness.

Patients were allowed to take their regular medications. Participants were asked not to smoke and not to drink coffee on the day of the examination. All subjects were examined in supine position after at least 10 min of resting. Single waveform was obtained by averaging the DVP profile for 30 sec. To enhance accuracy of the SI_{DVP} measurements, five period samples were taken, and the upper and lower merits of DVP were deleted. The remaining three merits were averaged and used for further analyses, including the variability test. All measurements were performed in the morning hours between 9 and 11 am, thereby eliminating confounding effects of the circadian variability. The data analyzer individual was unaware of any clinical information of the patients.

Primary and secondary endpoints

As a primary composite endpoint of the study were mortality from any origin, acute coronary event, acute myocardial infarction, stroke as cardiovascular endpoints and end-stage renal disease together. As a secondary endpoints the cardiovascular and renal endpoints were analyzed separately.

Statistical analysis

All results are expressed as mean ± SD for variables with normal distribution, unless otherwise specified. Survival rate was examined by Mantel-Cox log-rank test. Cox regression analysis was used to evaluate the effects of survival factors. ROC analysis was performed to determine which SI was the limit value by our method that separates most appropriately the two groups for the outcome. Multivariate analysis was used to explore the factors that influence CV events and impaired renal function.

Based on the average SI values, patients were divided into two groups by the 11 m/s cut-off point, and outcomes of the two groups were analyzed and compared. The combined primary endpoint included death of any origin, myocardial infarction, stroke and cardiovascular intervention as the CV endpoints, and ESRD (CKD stage 5), start of renal replacement therapy as the renal endpoints. Subsequently, CV and renal endpoints were analyzed separately as

secondary endpoints. Statistical analysis was performed using the SPSS software version 22.0, and P <0.05 was considered as statistically significant.

Results

Fifty-five ADPKD patients (21 male, mean age 45±12 years) were studied. Patients were followed-up for the average of 63±32 months. Clinical characteristics and occurrence of CV risk factors are summarized in Table 3. The mean SI_{DVP} value was 11.11 ± 2.22 m/s by which patients were divided into two groups, as shown in Table 3. Patients with increased SI_{DVP} (SI_{DVP} > 11 m/s) were significantly older (P < 0.05) and their renal function was significantly declined (P < 0.05).

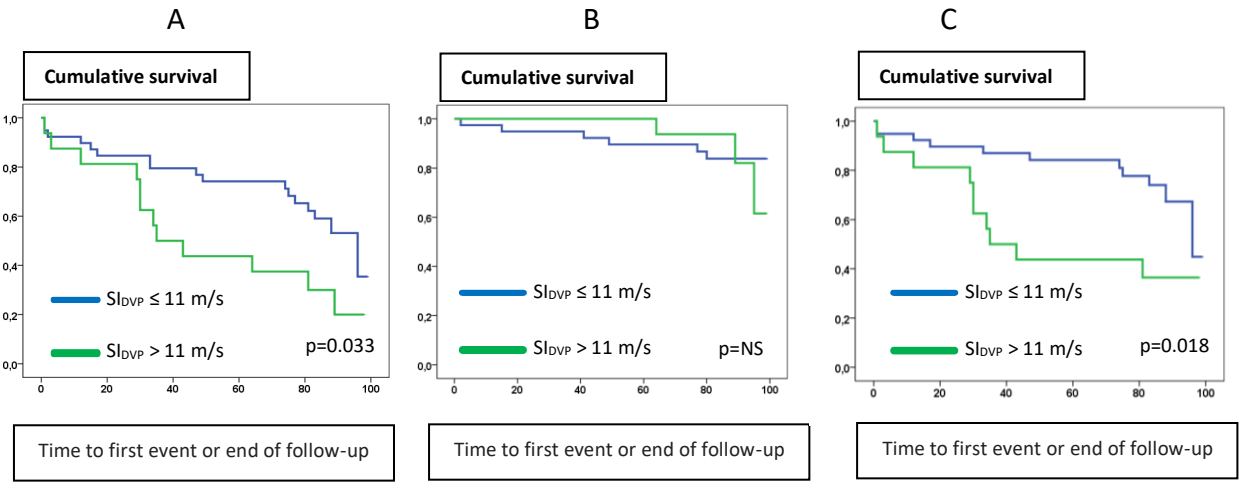
Table 3. Baseline characteristics of ADPKD patients

	ADPKD patients (n=55)	SI ≤11 m/s (n=27)	SI >11 m/s (n=28)	*p* value
Male/female (n=)	21/34	8/19	13/15	0.103
Age (year)	45±12	41±10*	49±11*	0.002
Follow-up time (month)	63±32	67±31	58±33	0.156
Duration of ADPKD (year)	13.2 ± 9.2	12.8 ± 8.5	13.7 ± 9.7	0.364
eGFR (ml/min/1,73m2)	72 ± 36	82 ± 37**	61 ± 31**	0.013
Stiffness index (m/s)	11.11 ± 2.22	9.44 ± 1.41***	12.71 ± 1.56***	0.001
Metabolic syndrome components:				
Obesity(n, %)	22 (40)	11 (37)	11 (39)	0.457
BMI (kg/m^2)	28.5	28.4	28.5	0.453
Carbohydrate metabolic disorders (n, %)	13 (24)	5 (18)	8 (28)	0.194
Dyslipidemia (n, %)	26 (47)	13 (48)	13 (46)	0.450
Hypertension (n, %)	46 (84)	21 (78)	25 (89)	0.128
Metabolic syndrome (n, %)	13 (24)	5 (18)	8 (28)	0.194
Smoking (n,%)	20 (36)	9 (33)	11 (39)	0.326
ACEI/ARB therapy (n,%)	46 (84)	21 (78)	25 (89)	0.128

*p<0.05, **p<0.05, ***p<0.05

The occurrence of primary combined and secondary endpoints on the basis of the SI_{DVP} values are shown in Figures 7A, B and C. Figure 7A shows that probability of combined primary endpoint (CV and renal) was significantly higher in the group with increased stiffness (SI_{DVP} > 11 m/s) compared to the group of SI_{DVP} ≤ 11 m/s with more elastic arteries (Chi square: 4.571; p = 0.033). For the CV endpoint (Figure 7B) there was no significant difference in the outcome (Chi-square: 0.004, P = 0.952). In contrast, probability of the renal endpoint (Figure 7C) was significantly higher with increased arterial stiffness (Chi-square: 5.591, P = 0.018).

Figure 7. Primary combined (A), secondary cardiovascular (B) and renal (C) end points in ADPKD patients with SI_{DVP} > 11 m/s versus SI_{DVP} ≤ 11 m/s.



We performed ROC analysis to determine the value of SI that separates most appropriately the two groups for any outcome by the used finger photoplethysmography method, and this SI_{DVP} value was 10.66 m/s.

The Cox regression model showed that every 1 m/s increase of SI increased the probability of the cumulative endpoint by 18.7% [Odds ratio (OR): 1.187 (CI: 1.001-1.408); P = 0.048].

Multivariate analysis was used to explore those factors that could predict CV events and the renal function decline, independently of other parameters; in this model only baseline renal function (eGFR) was found as an independent prognostic factor for the combined CV and renal outcomes (Table 4, P < 0.001).

In the group of patients with metabolic syndrome there were significantly more CV events than in the group of patients without metabolic syndrome (Chi-square: 6.246; P = 0.012) (Figure 8A and B). SI_{DVP} value of patients with metabolic syndrome was significantly higher than in patients without metabolic syndrome (12.1 ± 2.3 m/s versus 10.8 ± 2.1 m/s; P = 0.036). Cox regression analysis showed that metabolic syndrome was an independent predictor for only CV endpoints (P = 0.022), but not for the combined primary and secondary renal endpoints (Table 5).

Figure 8. Primary combined (A) and secondary cardiovascular (B) end points in ADPKD patients with metabolic syndrome versus without metabolic syndrome.

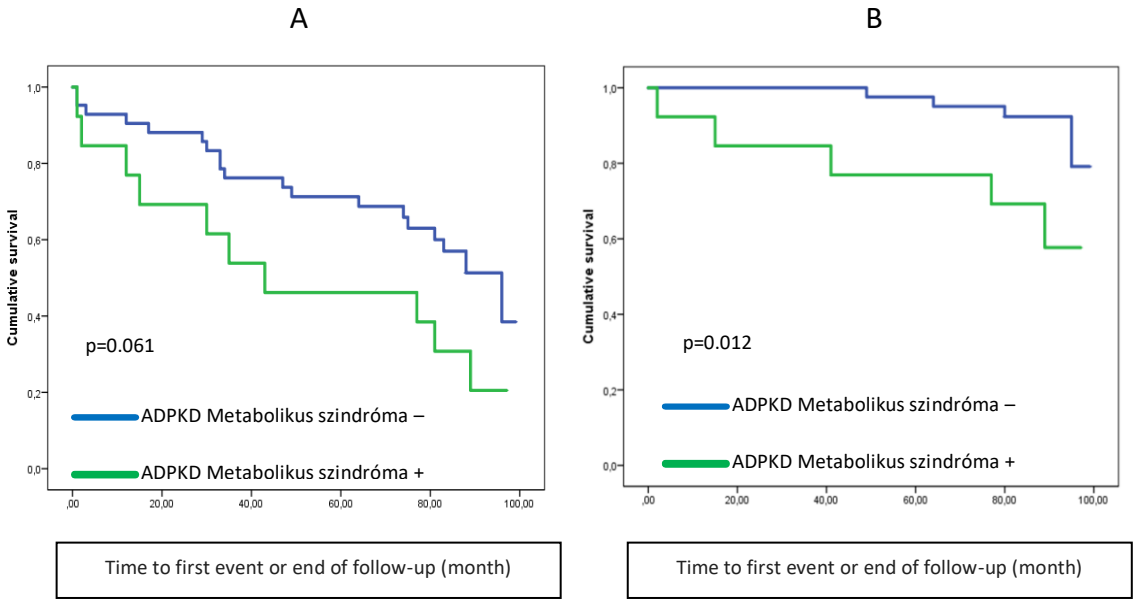


Table 4. The prognostic role of each parameter in association with combined primary endpoints (multivariate analysis)

	B	SE	Wald	df	***p***	Exp(B)
SI_{DVP}	-0.049	0.114	0.182	1	0.670	0.952
Age	-0.017	0.022	0.603	1	0.437	0.983
eGFR	-0.055	0.010	28.208	1	<0.001	0.946
Gender	-0.884	0.455	3.786	1	0.052	0.413
Hypertension	-0.924	0.896	1.063	1	0.303	0.397
Carbohydrate metabolic disorder	0.016	0.507	0.001	1	0.975	1.016
Dyslipidemia	0.810	0.528	2.350	1	0.125	2.247
Obesity	-0.686	0.550	1.558	1	0.212	0.504

p<0.05

Table 5. The prognostic role of metabolic syndrome in association with primary and secondary endpoints in ADPKD (Cox regression analysis)

	Hazard Ratio	**95% CI**		**Coeff.**	**S.E.**	**Z-statistic**	***p* value**
Primary, combined endpoints	2.0463	0.9493	4.4108	0.7160	0.3919	1.8273	0.0677
Cardiovascular endpoints	4.6768	1.2398	17.6418	1.5426	0.6774	2.2773	0.0228*
Renal endpoints	1.0653	0.3892	2.9164	0.0633	0.5138	0.1232	0.9020

p<0.05

Discussion

In this study, we found that increased arterial stiffness was an independent prognostic factor for the combined cardiovascular and renal outcome in ADPKD patients. Arterial stiffness measured by Pulse Trace System appears useful and applicable method for estimating the renal and cardiovascular prognosis in ADPKD.

It was evidenced several years ago that arterial stiffness is markedly increased in ESRD. Recent data suggested that arterial stiffening is increased in CKD, even with mild to moderate loss of renal function or in the presence of microalbuminuria.

The pathophysiological mechanism of increased arterial stiffness observed in CKD is not entirely defined. Several putative mechanisms have been implicated, including chronic hypervolemia, chronic microinflammation, lipid peroxidation, suppression of the nitric oxide system, excessive sympathetic activity, activation of the RAAS, as well as increased mechanical stress due to either hypertension or the arterial wall calcification.

ADPKD patients exhibit vascular dysfunction as increased arterial stiffness develops very early during the course of ADPKD, even in patients with normal renal function. In previous cross-sectional study, we showed that arterial stiffness is increased as renal function starts declining in the homogeneous CKD group of IgAN patients. Moreover, by comparing IgAN patients with ADPKD patients we concluded that etiology of CKD may also affect the degree of arterial stiffness; as our data showed that arterial stiffness develops earlier and the progression is more rapid in ADPKD than in IgAN patients with comparable renal function.

The pathomechanism of premature CV lesions in ADPKD is not fully understood, however, several factors could be involved in the development of CV abnormalities in ADPKD. Nauli et al. implied that vascular dysfunction in ADPKD may be a consequence of inherited disorder of the solitary cilium. Disorder of the primary cilium which covers the internal surface of endothelial cells of the blood vessels may trigger biochemical cascade mechanisms leading to decreased nitric oxide (NO) availability and endothelial dysfunction. As a result, vascular remodeling may develop via cilium dysgenesis due to impaired cell differentiation and altered connective tissue structure, thus leading to increased vascular rigidity. Consistenly, the plasma concentration of NO was found lower in ADPKD.

In another hypothesis, the cause of vascular complications in ADPKD could be explained by the polycystic kidney itself. Briefly, enlarged kidney cysts causing structural damage of the nephrons and distortion of the normal kidney structure leads to intrarenal ischemia that activates the RAAS system, which has important role in CV remodeling. Activation of RAAS occurs very early in ADPKD patients, in both adults and children, often preceeding the onset hypertension and renal failure. Hyperplasia of the renin-producing cells in the juxtaglomerular apparate could be observed in the removed kidneys of ADPKD patients, indicating the involvement of RAAS activity. The vast majority of our ADPKD patients were treated with RAAS inhibitors, and thus we could not draw any conclusion regarding this observation.

Kocyigit et al. reported relationship between early arterial stiffness and inflammatory biomarkers in normotensive ADPKD patients by showing that arterial stiffness was increased prior to the onset of hypertension or renal function decline, and interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) levels were all significant predictors of PWV. These findings indicate the presence of low-grade, systemic inflammatory processes contributing to the early arterial damage, and thus the resultant arterial stiffness in ADPKD.

Epidemiologic longitudinal studies have clearly demonstrated that arterial stiffness has an independent predictive value of CV outcomes in different populations. Blancher et al found that every 1 m/s elevation of PWV increased the CV and total mortality by 14% in ESRD. Recently, Nowak et al. reported that impaired endothelium-dependent vasodilation and enhanced arterial stiffness were independent predictors of CV events and mortality in ADPKD children and young adults with preserved renal function. In our study increased arterial stiffness in ADPKD patients was also an independent prognostic factor for combined cardiovascular and renal outcomes.

Mao et al. reviewed wide range of metabolic abnormalities that have been reported as part of the clinical spectrum of ADPKD. Pietrzak-Nowacka et al. revealed several components of metabolic syndrome in ADPKD, such as hypertension, abdominal obesity, and higher fasting blood glucose level. However, consistent findings in different populations and presice underlying molecular mechanisms and their links to the genetic defects in ADPKD are still remained uncertain.

In the review of Ecder, early occurrence of CV complications in ADPKD is emphasized and multifactorial risk reduction (hypertension, obesity, dyslipidemia, and smoking) is warranted, particularly, the early detection hypertension to prevent vascular complications, and the use of angitensin converting enzyme inhibitor (ACEI) treatment to reduce CV events. In our study, 84% of ADPKD patients received ACEI or angiotensin receptor blocker (ARB) therapy as antihypertensives, and that could also positively alter SI_{DVP} and the CV outcomes.

Our results demonstrated that arterial stiffness determined by finger photoplethysmography has a prognostic value, although occasionally there are difficulties in registering the digital pulse volume. It may be problematic in some cases, mostly in elder patients to confidently separate systolic and reflective waves, and thus assess the stiffness index. Atrial fibrillation, frequent atrial and ventricular ectopic activity could also limit the correct pulse curve detection. We estimated and not measured the renal function, however, the use of eGFR is widely accepted throughout the literature. Data analyses may be weakened by the low number of our cases. It is conceivable that study follow-up period was somewhat short to be sufficient to prove the difference in the occurrence of CV events and renal failure.

However, limitations of the study may hamper establishing the conclusions, it seems clear that increased arterial stiffness could predict the development of target organ damage in CKD including ADPKD.

6. The impact of osteocalcin, osteoprotegerin and osteopontin on arterial stiffness in chronic renal failure patients on hemodialysis

Methods

Blood pressure and pulse wave velocity measurements

Blood pressure was measured using calibrated automated devices with appropriate cuff-size. Results are presented as predialytic measurements. Data of pulse pressure and mean arterial pressure were calculated.

Carotid-femoral PWV and augmentation index (AIx) were measured using applanation tonometry (SphygmoCor system, AtCor Medical Australia). Measurements were performed before hemodialysis sessions in supine position after at least 10 min rest in quiet, stable temperature room. Control assessments were carried out in the mornings under the same cirmcumstances. Pulse wave recordings were consecutively performed at two superficial arterial sites (carotid-femoral segment). All recorded readings merit the manufacturer's quality control standards integrated into the software package. The carotid-femoral PWV was calculated.

Laboratory measurements

Routine biochemical parameters were measured by standard methods. The serum concentrations of osteocalcin (OC), osteoprotegerin (OPG), and osteopontin (OP) were measured by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) (IBL International GmbH, Hamburg, Germany and BioVendor Laboratory Medicine Inc., Brno, Czech Republic). The corresponding intra-assay CVs were ranged between 4.7 - 5.5%; 4.4 - 10.1% and 2.5 - 4.9%, while the inter-assay CVs from 5.7 to 8.3%; 8.6 to 9.3% and 1.7 to 9.0% (Table 6).

Statistical analysis

Statistical analyses were performed using the 21.0 software of the SPSS (SPSS, Inc., Chicago, IL, USA). Normality of data distribution was tested by Kolmogorov-Smirnov test. Non-normally distributed parameters were transformed logarithmically to correct their skewed distributions. Correlations between continuous variables were assessed by linear regression model using Pearson's test. Data were expressed as means ± SD in case of normal distribution, or median (lower/upper quartile) in case of non-normal distribution. Backward multiple regression analyses were performed to determine the relative contribution of selected independent variables using constructed model to the variance of the dependent variable. Values of $P < 0.05$ were considered statistically significant.

Ethical considerations

Ethical considerations

The research was approved by the Regional Ethical Committee. The investigation conforms to the principles outlined in the World Medical Association Declaration of Helsinki. Informed written consent was obtained from all participants.

Table 6. Clinical and laboratory characteristics of 68 chronic hemodialysis patients and 35 controls

Clinical data	Hemodialysis patients	Controls	Biochemical data	Hemodialysis patients	Controls
Age (years)	59.7±13.3	60.3±16.2	Creatinine (µmol/l)	658.7±185.2	101.8±9.2*
Height (cm)	166.6±8.6	171.7±9.1	BUN (mmol/l)	17.5±3.8	5.8±1.8*
Body weight (kg)	73.6±17.7	75.2±19.1	spKt/V	1.7±0.32	NA
Body mass index (kg/m²)	26.3±5.1	27.6±7.2	Hemoglobin (g/l)	113±11.1	140.8±6.2*
Dialysis vintage (months)	50.9±40.5	NA	Albumin (g/l)	42.1±4.6	41.8±5.2
SBP (mmHg)	121.2±21.7	124.1±24.2	Total cholesterol (mmol/l)	4.64±1.1	4.89±1.6
DBP (mmHg)	79.4±8.8	81.8±9.4	LDL cholesterol (mmol/l)	3.0±0.9	2.68±1.1
Pulse pressure (mmHg)	50.2±15.9	49.6±17.2	HDL cholesterol (mmol/l)	1.22±0.46	1.28±0.38
MAP (mmHg)	91.8±12.4	90.1±14.6	Triglyceride (mmol/l)	1.51±1.24	1.42±1.28
AIx	31.6±10.4	28.8±8.1	Sodium (mmol/l)	138.4±2.1	137.6±3.2
PWV (m/s)	11.5±3.4	8.6±2.8*	Potassium (mmol/l)	4.7±0.6	4.1±0.4*
Central augmentation pressure (mmHg)	13.8±7.3	12.7±8.4	Calcium (mmol/l)	2.2±0.2	2.3±0.3
Central SBP (mmHg)	117.3±20.2	121.1±21.4	Phosphate (mmol/l)	1.6±0.4	1.1±0.2*
Central DBP (mmHg)	79.2±9.3	82.1±10.2	Alkaline phosphatase (IU/l) iPTH	114.0±43.1	96.6±18.9
Central pulse pressure (mmHg)	41.5±13.9	40.1±14.8	(pmol/l)	58.6±23.9	2.2±1.8*
			Vitamin D_3 (nmol/l)	7.7 (5.3; 12.79)	34.2(7.8;24.81)*
			Osteocalcin (ng/ml)	370.5±246.7	19.8±5.4*
			Osteopontin (ng/ml)	848.9 (697.5; 979.5)	216.7(27.7-468.2)*
			Osteoprotegerin (pmol/l)	26.2±9.9	5.2±1.3*

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; AIx, augmentation index; PWV, carotid-femoral pulse wave velocity; BUN, blood urea nitrogen; spKt/V, single pool Kt/V; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoporotein cholesterol; iPTH, intact parathyroid hormone, NA: not applicable, *p<0.05.

Results

In CRF patients on hemodialysis, PWV and AIx were significantly elevated compared to contol subjects without major CV, renal, and metabolic co-morbidities (Table 6). Furthermore, the serum levels of bone-related proteins, OC, OPG and OP were all multiple times higher in uremic HD patients than in the controls.

Univariate linear regression analyses were used to reveal associations of vascular stifness (VS) markers with clinical and laboratory parameters (Table 7). We found that PWV correlated positively with age (r=0.411, P<0.000); and negatively with serum creatinine (-0.412, P<0.000), urea nitrogen (r=-0.427, P<0.000), phosphate (r=-0.325, P<0.007), potassium (r=-0.307, P<0.011) and OC (r=-0.247, P<0.049, Figure 9). AIx closely correlated with central pulse pressure (r=0.405, P<0.001), augmentation pressure (r=0.800, P<0.000), systolic blood pressure (r=0.316, P<0.000); while AIx had inverse correlation with body height (r=-0.254, P<0.036), body weight (r=-0.277, P<0.022), heart rate (r=-0.436, P<0.000), and urea nitrogen (r=-0.321, P<0.008). There were no relationshiphs between PWV or AIx with any other examined variable.

Of the bone-associated proteins (Table 7), OC was positively correlated with serum creatinine (r=0.543, P<0.000), urea nitrogen (r=0.358, P<0.004), phosphate (r=0.471, P<0.000) alkaline phosphatase (r=0.375, P<0.002), iPTH (r=0.512, P<0.000), central systolic blood pressure (r=0.348, P<0.005), and HD duration (r=0.255, P<0.042). OC, however, negatively correlated with PWV (r=-0.247, P<0.049). OPG was positively correlated with age (r=0.652, P<0.000), and negatively with BMI (r=-0.313, P<0.011), body weight (r=-0.371, P<0.002) and height (r=-0.261, P<0.03). Importantly, there were no significant correlations between OP and any uremic clinical/laboratory parameters measured routinely in HD. One can assume that OC (r=0.282, P<0.024) and OPG (r=0.256, P<0.040) are related, suggesting that these distinct bone-related proteins may interact in uremic patients receiving regular HD.

Figure 9. Bone related proteins (osteocalcin, osteopontin and osteoprotegerin) association with PWV

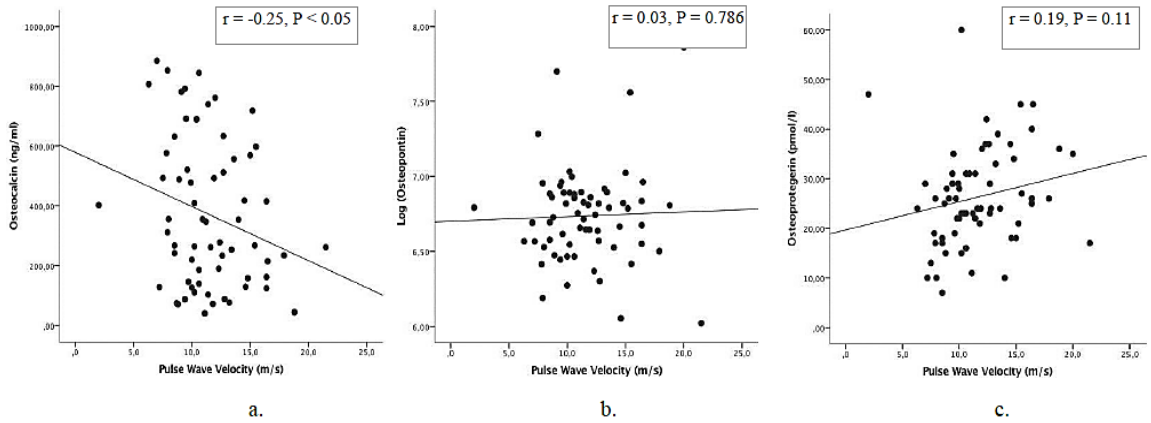


Table 7. Statistically significant correlations in univariate linear regression analysis

Stiffness parameters			Bone-associated proteins		
	r	P		r	P
PWV-age	0.41	<0.001	OC-serum creatinine	0.543	<0.001
PWV-serum creatinine	-0.412	<0.001	OC-BUN	0.358	<0.004
PWV-BUN	-0.427	<0.001	OC-serum phospate	0.471	<0.001
PWV-serum phoshate	-0.325	<0.007	OC-alkaline	0.375	<0.002
PWV-serum potassium	-0.307	<0.011	phosphatase		
PWV-OC	-0.247	<0.049	OC-iPTH	0.512	<0.001
AIx-central PP	0.405	<0.001	OC-central SBP	0.348	<0.005
AIx-augmentation	0.800	<0.001	OC-time spent on HD	0.255	<0.042
pressure			OC-PWV	-0.247	<0.049
AIx-SBP	0.316	<0.001	OPG-age	0.652	<0.001
AIx-body weight	-0.277	<0.022	OPG-BMI	-0.313	<0.011
AIx-body height	-0.254	<0.036	OPG-body weight	-0.371	<0.002
AIx-heart rate	-0.436	<0.001	OPG-body height	-0.261	<0.034
AIx-BUN	-0.321	<0.008	OP-OC	0.282	<0.024
			OP-OPG	0.256	<0.040

PWV, carotid-femoral pulse wave velocity; BUN, blood urea nitrogen; OC, osteocalcin; AIx, augmentation index; PP, pulse pressure; SBP, systolic blood pressure; HD, hemodialysis; OPG, osteoprotegerin; BMI, body mass index. p<0.05

Multiple linear regression models were applied to establish independent variables that have significant impact on bone-related proteins in our clinical setting (Table 8). The variance of OC was negatively influenced by PWV (β=-0.25, P<0.029) and BMI (β=-0.26, P<0.026) and positively by systolic blood pressure (β=0.37, P<0.001) and hsCRP (β=0.23, P<0.049). OPG as dependent variable was found to correlate directly with age (β=0.69, P<0.000) and inversely with BMI (β=-0.31, P<0.001). When the model was conducted with OP as dependent variable, LDL-cholesterol proved to be the only significant factor associated with OP (β=0.25, P<0.044).

Table 8. Statistically significant correlations in multiple linear regression analyses

Osteocalcin as a dependent variable

		Model 1 (R^2 = 0.291)		
Variable	β	t	P	%
PWV	-0.25	-2.24	0.029	6.1
SBP	0.37	3.25	0.001	11.5
BMI	-0.26	-2.28	0.026	7.0
CRP	0.23	2.00	0.049	5.8

Osteoprotegerin as a dependent variable

		Model 1 (R^2 = 0.557)		
Variable	β	t	P	%
Age	0.69	7.60	0.001	43.3
BMI	-0.31	-3.54	0.001	9.6

Osteopontin as a dependent variable

		Model 1 (R^2 = 0.096)		
Variable	β	t	P	%
LDL cholesterol	0.25	2.05	0.044	6.0

β: standardized regression coefficient; %: relative contribution to the variance, respectively; PWV, pulse ware velocity; SBP, systolic blood pressure; BMI, body mass index; CRP, C- reactive protein; LDL cholesterol, low-density lipoprotein cholesterol. $p<0.05$

Further analyses by multiple regression model disclosed that the variance of PWV as dependent variable was only affected by age (β=0.53, P<0.001), bone-related proteins had no significant contributions. AIx, the other measure of VS appeared to be independent of all clinical/biochemical parameters analyzed.

Discussion

In this study, we demonstrated that PWV as a marker of VS, was increased significantly, and serum levels of bone-related proteins (OC, OPG, OP) were also several-

fold higher in CRF patients on regular HD. As these proteins have been implicated in the development of VC, their relationships with PWV were determined, and only OC was found to correlate with PWV.

In patients with impaired renal function plasma OC levels are markedly elevated due to increased bone turnover and decreased renal elimination. Conflicting results are available concerning plasma OC as a predictor of CVD and related mortality. In general, low circulating OC concentrations were found to be associated with an increased risk of CVD. In agreement with our findings, inverse relationship was observed between the risk of CV events and plasma OC levels in patients on regular HD, suggesting vasoprotective role for OC. By contrast, large prospective and cross-sectional studies demonstrated U-shaped association of OC with fatal CV events. One recent study provided convincing evidences by showing that OC may be implicated in the endothelial damage-related VC in CRF patients. Activated endothelial cells in uremia are capable of producing microparticles that induce OC expression in endothelial progenitor cells, VSMCs and fibroblasts. Therefore, imbalance in the process of endothelial damage and repair may contribute to VC via increasing the number of OC-positive endothelial progenitor cells.

Elevated circulating OPG levels have been reported in patients with CV and/or with chronic renal diseases, and it was found to be independently associated with all-cause mortality and atherosclerosis. Similar findings were published in CRF patients on regular HD and in those receiving kidney transplantation. However, there have been studies that failed to document such an association. Our own results are consistent with these findings as PWV was unrelated to OPG, but was positively associated with age and negatively with BMI. One can speculate that age and BMI overwhelmed the effects of OPG, or alternatively, OPG we measured was underphosphorylated but phosphorylated OPG is required to achieve VC inhibition. It is still debated whether the expression of the elements of OPG/RANKL/RANK axis in the vascular lesion and the high levels of circulating OPG are an epiphenomenon or they have counter-regulatory role to attenuate the calcification process.

In CKD patients elevated circulating OP levels were reported, but except for a small group of HD patients no independent association could be detected between OP levels and VC. In experimental uremia, plasma OP was increased and correlated positively with aortic atherosclerosis. In our study we confirmed that plasma OP level is markedly increased in HD patients, but it does not relate to VS parameters. Of note, however, the risk factor LDL-cholesterol had independent influence on PWV.

In conclusion, our study in patients receiving maintenance HD provided additional evidences that PWV as a measure of VS increased significantly and there was several-fold increase in serum levels of interrelated bone-specific proteins (OC, OPG, OP). PWV was found to be independently associated only with OC and age but risk factors for arterial calcification had significant impact on OC (systolic blood pressure, hsCRP, BMI), OPG (age, BMI) and OP (LDL-cholesterol).

The relatively small sample size in this study is a potential limitation. Furthermore, the cross- sectional study design could not confer the causal relationship between arterial stiffness, bone-related proteins and potential confounders. Longitudinal, prospective studies including detailed analysis of the impact of various etiology and medication on clinical and biochemical parameters are to be conducted in HD patients to draw definitive conclusion.

7. Summary

Arterial stiffness can be measured by several methods. In follow-up studies with IgAN and ADPKD patients, we applied the finger photopletismographic technique using the Pulse Trace System to measure SI_{DVP}, in contrast with the 'gold standard' Sphygmocor method. SI_{DVP} is a complex parameter altered by the elasticity of large central arteries and peripheral arteries. The utility of SI_{DVP} has been successfully demonstrated in patients with hypertension, diabetes, coronary heart disease and ESRD, and healthy individuals. In addition, SI_{DVP} was valuable to establish the risk of hypertensive and apparently healthy subjects with different CV risk factors. However, this method has not been tested in IgAN or ADPKD patients to measure vascular stiffness prior to our previous and current studies.

Our results indicate that finger photopletismographic method can be successfully applicable to predict prognosis, and increased vascular stiffness (SI> 10 m / s or> 11 m / s) in both IgANP and ADPKD is an independent prognostic factor in the occurrence of major cardiovascular events and end-stage renal failure. The higher SI value implies poor prognosis in case of any type of kidney disease. Increased arterial stiffness by SI helps to select the group of CKD patients, who are required to be monitored more closely, provided further cardiovascular examinations, and given maximal renal protection therapy, in regard with both CV and renal protection.

We showed further evidence that in ESRD patients receiving HD PWV was significantly higher in vascular stiffness measurements by using the standard Sphygmocor method, and serum levels of related bone-specific proteins (OC, OPG, OP) were increased by several-fold. PWV was found to be independently associated with OC and age but other risk factors for arterial calcification had significant effects on OC (systolic blood pressure, hsCRP, BMI), OPG (age, BMI) and OP (LDL-cholesterol).

8. Conclusions

In CKD, the stiffness index measured by the Puse Trace System is valuable prognostic parameter to predict kidney disease progression, as well as CVD progression.

Determination of bone-specific proteins in CKD may be important parameter for better understanding the process of vascular calcification. Bone-related proteins show significant relationships with vascular stiffness, and thus these proteins could provide molecular basis for therapeutic strategy, or even potenial therapeutic targets in the future.

We belive that assessment of arterial stiffness parameters has justification in the clinical practice of CKD patients in order to identify renal patients with higher CV risk and provide information on prognosis.

9. New scientific results

1. In IgAN and ADPKD, increased arterial stiffness predicts end-stage renal failure, mostly in patients with multiple CV risk factors, and therefore early measurement of arterial stiffness is recommended.

2. Digital pulse volume (arterial SI) measurement using the Pulse Trace System is well applicable and valuable method in CKD to predict renal failure and CV events.

3. In IgAN and ADPKD patients, increased number of components of the metabolic syndrome causes higher CV risk and poor renal prognosis.

4. We established the role for OC in the development of VC, although our results failed to document direct relationship between OP, OPG and vascular lesion; high circulating levels of bone-specific proteins could be an epiphenomenon or they may have counter-regulatory role to attenuate the uremic calcification processes.

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11. Publications of the author

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