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Arterial Stiffness in Children with Chronic Kidney Disease: Prevalence, Risk Factors and Functional Consequences

DOCTORAL DISSERTATION

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VILNIAUS UNIVERSITETAS

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Vaikų, sergančių lėtine inkstų liga, arterijų standumo padidėjimas: paplitimas, rizikos veiksniai ir funkcinės pasekmės

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ABBREVIATIONS

ABPM, ambulatory blood pressure monitoring
AC, arterial compliance
AHA, American Heart Association
AIx, augmentation index
AKI, acute kidney injury
ANZDATA, Australia and New Zealand Dialysis and Transplant Registry
AP, augmented pressure
baPWV, brachio-ankle pulse wave velocity
bfPWV, brachio-femoral pulse wave velocity
BMI, body mass index
BP, blood pressure
CAC, coronary artery calcification
CAKUT, congenital anomalies of kidney and urinary tract
CAPD, continuous ambulatory peritoneal dialysis
Car, carotid artery
CAVI, cardio-anke vascular index
cfPWV, carotid-femoral pulse wave velocity
CI, confidence interval
cIMT, carotid intima-media thickness
CKD, chronic kidney disease
CO, cardiac output
CRIC, Chronic Renal Insufficiency Cohort
CRP, C-reactive protein
CV, cardiovascular
CVD, cardiovascular diseases
ECG, electrocardiography
eGFR, estimated glomerular filtration rate
E_{inc}, incremental Young's elastic modulus
ESH, European Society for Hypertension
ESPN, European Society for Pediatric Nephrology
ERA-EDTA, European Renal Association-European Dialysis Transplantation Association
ESA, erythropoiesis stimulating agents
Fem, femoral artery
FGF23, fibroblast growth factor-23
FFTI, fat-free tissue index
FMD, flow-mediated dilation
FTI, fat tissue index

ftPWV, finger-toe pulse wave velocity
HD, hemodialysis
HDF, hemodiafiltration
HDL, high-density lipoprotein
HF, heart failure
HR, heart rate / hazard ratio
HSMC, human smooth muscle cells
HUS, hemolytic uremic syndrome
IDWG, interdialytic weight gain
IHD, ischemic heart disease
IMT, intima-media thickness
IVS, interventricular septum
KDIGO, Kidney Diseases Improving Global Outcomes initiative
KRT, kidney replacement therapy
KTx, kidney transplantation
LDL, low-density lipoprotein
LoD, limit of detection
LV, left ventricle
LVH, left ventricular hypertrophy
LVID, left ventricle internal diameter
LVM, left ventricular mass
LVMI, left ventricular mass index
LVPW, left ventricle posterior wall
MBD, mineral bone disease
MRI, magnetic resonance imaging
NAPRTC, North American Pediatric Renal Trials and Collaborative Studies
NO, nitric oxide
OR, odds ratio
PD, peritoneal dialysis
pmarp, per million age related population
PP, pulse pressure
PPA, pulse pressure amplification
PR, peripheral resistance
PTH, parathormone
PW, pulse wave
PWA, pulse wave analysis
PWf, pulse waveform
PWV, pulse wave velocity
RAS, renin-angiotensin system
RWT, relative wall thickness

SDS, standard deviation score
SGA, small-for-gestational age
SMC, smooth muscle cells
SONG, Standardize Outcomes in Nephrology Initiative
SSN, suprasternal notch
TT, transit time
uACR, urinary albumin-to-creatinine ratio
ULN, upper limit of normal
Umb, umbilicus
USRDS, United States Renal Data System
VSMC, vascular smooth muscle cells
WHO, World Health Organization

1. INTRODUCTION

1.1. Brief introduction

Chronic kidney disease (CKD) is a relatively infrequent condition in the pediatric population but carries a significant health burden due to a wide spectrum of CKD-related complications. Early cardiovascular (CV) comorbidity is a particularly challenging issue due to its profound effects on premature mortality. CV disease (CVD) remains among the leading causes of death in children with advanced CKD. (1) Moreover, children on kidney replacement therapy (KRT) have significantly reduced life-expectancy which is largely attributed to premature CV mortality. (2) Early CV morbidity becomes particularly evident in young adults who experience several fold increased risk of ischemic heart disease (IHD). (3)

The pathophysiology of CVD in CKD is complex and the mechanisms of its development in childhood are not yet fully elucidated. Children with CKD are particularly suitable to study the mechanisms and risk factors of CVD development in the state of uremia due to the lack of long-standing, age-linked comorbidities (e.g. diabetes, smoking and concomitant chronic diseases). Several pathophysiologic pathways that contribute to the development of CKD-associated CVD are recognized and include: (i) accelerated atherosclerosis, (ii) arteriosclerosis and vascular calcifications, and (iii) left ventricular (LV) hypertrophy along with systolic and diastolic LV dysfunction. Although all of these pathways are mechanistically interrelated, they differ significantly by respective risk factor profiles and clinical consequences. (4)

Of these different CVD development pathways, arteriosclerosis and vascular calcifications are particularly unique to the CKD population. The development of these vascular abnormalities is closely related to mineral bone disorder (MBD), arterial hypertension and other risk factors. (5) The changes in arterial structure lead to increased arterial stiffness that can be measured using non-invasive techniques suitable for everyday practice. Currently, measurement of pulse wave velocity (PWV) is recognized as the gold standard of arterial stiffness assessment. (6) Although widely used in adults, PWV measurements in the pediatric population are challenging because of technical and growth-related aspects. Development and validation of PWV measurement devices in the past decade allowed for reliable and plausible PWV estimations in children. Nevertheless, studies about arterial stiffness in

children with CKD remain scarce and most of the data about the relevance of PWV in the CKD population come from adult studies.

Adult studies have related the development of arterial stiffness measured by PWV with kidney function decline, CKD-MBD and arterial hypertension. (7) Moreover, studies in adults with CKD have provided reliable evidence that arterial stiffness is not only a marker of CVD but a risk factors itself for target organ damage. Stiffness of the arterial tree in adults has been linked to adverse cognitive, kidney and CV outcomes, and proved to be an independent predictor of mortality. (8–10) In contrast, the few studies in the pediatric population have not explored functional consequences of increased arterial stiffness in CKD. Pediatric studies also failed to provide reliable evidence about the development and risk factors of arterial stiffness due to small sample sizes and heterogenous study populations.

Although PWV measurements are recognized as a marker of target organ damage by the 2016 European Society of Hypertension (ESH) guidelines for the management of high blood pressure (BP) in children and adolescents (11), little remains known about the functional impact of arterial stiffening in the pediatric population and about its relevance in pediatric CKD. The present thesis aims to comprehensively describe the prevalence and risk factors of arterial stiffness in children with moderate-to-advanced CKD, and to unravel potential clinical consequences by studying a large cohort of pediatric CKD patients. The longitudinal and highly standardized nature of this analysis is expected to provide strong and reliable evidence about the development and relevance of arterial stiffness in children with CKD.

1.2. Aim and objectives

The aim of this thesis is to evaluate the prevalence and risk factors of arterial stiffness measured by PWV and its functional consequences on LV geometry and CKD progression in children with pre-dialysis stage 3-5 CKD.

Objectives:

1. To evaluate the prevalence of increased PWV and its longitudinal dynamics in children with pre-dialysis stage 3-5 CKD;
2. To evaluate factors associated with increased PWV in children with pre-dialysis stage 3-5 CKD;
3. To determine the association between PWV and LV geometry in children with pre-dialysis stage 3-5 CKD;
4. To determine the association between PWV and CKD progression in children with pre-dialysis stage 3-5 CKD.

1.3. Defended statements

1. PWV is increased in children with pre-dialysis stage 3-5 CKD;
2. Increased PWV in children with pre-dialysis stage 3-5 CKD is associated with age, sex, body dimensions, primary kidney disease, markers of CKD-MBD, proteinuria, dyslipidemia and BP;
3. PWV in children with pre-dialysis stage 3-5 CKD is associated with concentric LV remodeling;
4. PWV in children with pre-dialysis stage 3-5 CKD is not associated with CKD progression.

2. LITERATURE REVIEW

2.1. Epidemiological aspects of pediatric CKD

2.1.1. Definition and Epidemiology of CKD in Children

According to the Kidney Disease Improving Global Outcomes (KDIGO) 'Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease' CKD is defined as *(i)* the presence of structural or functional kidney abnormalities (e.g. proteinuria, hematuria, imaging or histological abnormalities) or *(ii)* estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2; lasting for more than three months. CKD is further classified based on eGFR into five stages (Table 1). (12)

Table 1. CKD staging according to the KDIGO definitions

CKD stage	eGFR (ml/min/1.73 m^2)
1	≥90
2	60-89
3a	45-59
3b	30-44
4	15-29
5*	<15

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate

Compared to the general adult population, CKD is a much less prevalent condition in children. This is strongly related to the different etiologic spectrum of kidney function impairment between adults and the pediatric population. As opposed to adult population where secondary kidney diseases, closely linked to comorbid conditions are prevalent (e.g. diabetic nephropathy), the causes of CKD in children are predominantly congenital. Of those, congenital anomalies of kidney and urinary tract (CAKUT) are the leading causes of CKD in childhood. The prevalence of CAKUT in pediatric CKD varies depending on the geographic region and is reported to be between 34% and 59%. CAKUT is followed by glomerulonephritis (5-29%) and hereditary nephropathies (e.g. Alport syndrome) that comprise approximately 10-22%. Other causes include hemolytic uremic syndrome (HUS; 2-9%), cystic kidney diseases (5-11%), ischemic kidney failure (2-4%) and more rare

causes, such as metabolic kidney diseases, congenital nephrotic syndrome and other. (13) Moreover, the etiologic profile differs by the age of children with secondary causes (e.g. glomerulonephritis) being more common in older children. (14)

A recent analysis of Lithuanian children revealed that glomerulonephritis and HUS were the most common causes of CKD5 (43.1%), followed by hereditary nephropathies (39.7%) and CAKUT (17.2%). The differences between leading causes of CKD in Lithuania and global data are related to the differences in studied populations. Lithuanian data is limited to children with CKD5 only and glomerulonephritis or HUS are more likely to cause severe kidney disease. (15)

Although estimating incidence and prevalence of CKD in children is a difficult task due to the heterogenous definition of CKD by the KDIGO guidelines, several registries were set to collect and report epidemiological data about pediatric CKD. Regional registries, such as the European Society for Pediatric Nephrology/European Renal Association-European Dialysis and Transplant Association (ESPN/ERA-EDTA) registry in Europe or United States Renal Data System (USRDS) and other are aiming to fill the gap in the knowledge about the regional burden of CKD in children. (14) According to the European (ESPN/ERA-EDTA) registry, the incidence of pediatric CKD stage 3-5 is 11-12 per million age related population (pmarp) and the prevalence is around 55-60 pmarp. (13) The incidence of CKD is considerably stable, but the prevalence increased substantially over time and is explained by improved patient care and, consequently, improved survival. The reported incidence of CKD5 and KRT in children worldwide is around 9 pmarp, while the prevalence is ~65 pmarp. In Lithuania, there was a gradual increase in peritoneal dialysis (PD) incidence, while the incidence of hemodialysis (HD) and kidney transplantation remained relatively stable during 1994-2015. Reported incidences of different KRT modalities during 2012-2015 in Lithuania were 2.3, 1.8 and 2.5 pmarp for PD, HD and kidney transplantation, respectively. (15)

Most of the data about pediatric CKD outcomes come from registries of children receiving KRT. Overall, the survival of children on KRT has improved significantly over the last decades and it may be attributed to the advances in the care of children with CKD. This is true both for patients receiving chronic dialysis and also for patients after kidney transplantation. The five-year mortality of children on dialysis decreased by 36% between 1980-1984 and 1995-2000. This decrease is even more pronounced in young children (up to four years of age), where mortality decreased by 79% in the same period. The mortality of transplanted children in Europe has decreased

by 42% during the same period. The overall five-year survival of pediatric patients on KRT is relatively good, reaching 94% in Europe and 89% in United States. (1) Nevertheless, children with CKD5 exhibit markedly reduced life expectancy. According to the latest USRDS data, the life expectancy of children on dialysis and after kidney transplantation is reduced by 40-55 and 12-20 years, respectively. (2) As discussed in the next section, CVD is among major contributors of premature mortality and reduced life-span in pediatric patients with CKD.

2.1.2.Burden of CV Comorbidity in Children with CKD

CVD represents an important comorbidity in the spectrum of CKD-related complications. The detrimental consequences of CV morbidity are well reflected by the mortality data from the pediatric CKD population. Along with infections, CVD is the leading cause of death in children on KRT, accounting for up to 30% of deaths in childhood. Causes of mortality differ by KRT modality: infections are predominant in children on PD and after kidney transplantation, while CVD is predominant in children on HD, where CVD comprises almost 60% of deaths. Patients on HD have a nearly five-fold increased risk of mortality from CV causes compared to transplanted patients. (1) Overall, the estimated risk of CV mortality in children on KRT is reported to be up to 1,000 times higher compared to the general pediatric population. (4) Nevertheless, the increased awareness of CVD and preventive measures have led to a consistent decrease in CV mortality rates and a study from the Netherlands reported a reduction of CV mortality by 91% since the 1970s. (16) Studies of young adults with childhood-onset CKD have reported that up to 50% of all deaths by the age of 30 years are related to CV causes that account for 1.8-13.6 deaths for 100 patient years. (17) Furthermore, a recent analysis of over 10,000 individuals from the Australian and New Zealand registry demonstrated up to five-fold increased risk of IHD in young patients with CKD5. (3)

According to the 2018 USRDS Annual Report, one-year adjusted CVD mortality rate in children with CKD5 for the period of 2011-2015 was eight per 1,000 patient years, decreased by 38.5% compared to the period of 2006-2010 and was highest in patients on dialysis and those younger than four years of age. (2) Previous data has shown that the leading causes of CV mortality in patients on dialysis were cardiac arrest and arrhythmias, cerebrovascular disease, congestive heart failure (HF), cardiomyopathy, acute myocardial infarction and pericarditis. (4) For the reasons outlined earlier, children with CKD5 are classified at the highest tier of pediatric CV risk by the American

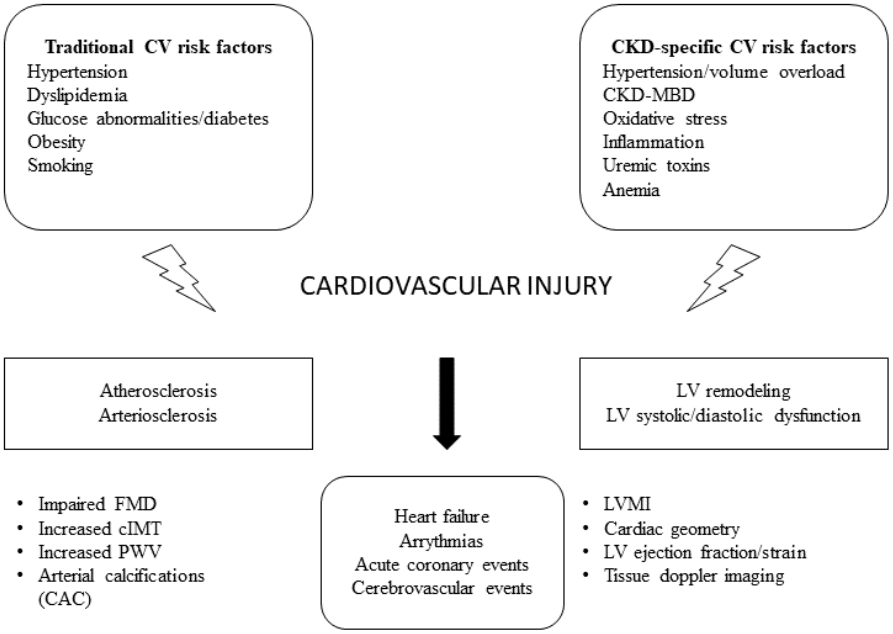
Heart Association (AHA), along with other conditions, such as diabetes, Kawasaki disease, familial hypercholesterolemia and other. (18) Moreover, the importance of CVD has been recently reflected by the Standardized Outcomes in Nephrology (SONG) Initiative where CVD has been ranked among the core outcomes that are considered to be critically important by physicians and patients on dialysis and after kidney transplantation. (19–21)

The premature CV morbidity in the state of CKD is related to substantially increased exposure to a variety of CV risk factors. These could be broadly categorized into (i) traditional (Framingham) and (ii) CKD-specific CV risk factors. Traditional CVD risk factors encompass hypertension, obesity, dyslipidemia, smoking and glucose metabolism disorders. CKD-specific factors are related to common complications associated with advanced kidney function impairment and include, among others, volume overload and severe hypertension, CKD-MBD, oxidative stress, inflammation, uremic toxins, etc. (Fig. 1). (4) The importance of individual risk factors depend on primary kidney disease, severity of kidney dysfunction and the affected target organ in the CV system. Irrespective of the exact mechanisms, the exposure to these risk factors is associated with significant alterations in large vessels and heart, leading to clinically relevant CV comorbidity and the aforementioned consequences.

CV risk factors associated with CKD cause damage in the vessels and the heart leading to premature atherosclerosis and arteriosclerosis and LV dysfunction. Previous studies have reported increased carotid intima-media thickness (cIMT) – a marker of subclinical atherosclerosis – in children with CKD, even in those with mild kidney dysfunction. (22,23) Additionally, children with mild-to-moderate CKD already demonstrate signs of endothelial dysfunction, evident by decreased flow-mediated dilation (FMD). (24) Alterations in the heart manifest by LV remodeling and hypertrophy with progressive diastolic and systolic dysfunction. (25–27) Eventually, these subclinical changes can lead to overt HF, life-threatening arrhythmias, acute coronary and cerebrovascular events. The simplified scheme of CVD development in children with CKD is presented in Figure 1.

Although interrelated and all participating in the common pathway of adverse CV sequalae development, the individual components of this premature CVD in children with CKD have distinct pathological mechanisms and consequences. Arterial stiffness is itself a risk factor and also an intermediate pathophysiologic mechanism affecting overall CV health. Although less studied in children than in adults, it nevertheless represents an important independent axis in the adverse CV sequalae. The further sections of this literature review will focus on the normal physiology and structure of large

arteries in children, the mechanisms leading to arterial alterations in CKD, mechanistic insights into arterial stiffness and its functional consequences, along with the methodological aspects of its monitoring and evidence from clinical studies in CKD across all ages.



Abbreviations: CAC, coronary artery calcifications; cIMT, carotid intima-media thickness; CKD-MBD, chronic kidney disease mineral-bone disorder; CV, cardiovascular; FMD, flow-mediated dilation; LV, left ventricle; LVMI, left ventricle mass index; PWV, pulse wave velocity.

Figure 1. Schematic description of CVD development in children with CKD

2.2. Arterial structure and function in healthy children

Arteries can be classified into large elastic arteries (aorta and its major branches) and smaller muscular (distributing) arteries found distal to aorta (e.g. radial and femoral arteries). All arteries have a similar triple-layered structure (tunica intima, media and adventitia), but with a pronounced variation in the intrinsic composition of the arterial wall. Muscular arteries are smaller in diameter and their medial layer is predominantly composed of vascular smooth muscle cell (VSMC) fibers. In contrast, the medial layer of large elastic arteries is mainly composed of multiple concentric layers of elastin fibers. (28) In healthy children, growth is associated with a gradual

increase in the arterial wall diameter and total wall thickness. The latter increases predominantly due to the increase of the IMT and is directly related to increasing BP, age, body dimensions and is gender-specific with higher arterial dimensions in boys. (29) A recent study of over 4,000 healthy children revealed that the increase in IMT is mainly related to the increase in the medial layer which possibly represents a physiologic adaptation to increasing mean arterial pressure. (30)

The arterial system serves two principal functions: a conduit to deliver blood ejected by the LV to the peripheral tissues and as vascular buffers that provide a "cushioning and dampening" function. Large elastic arteries behave similar to *windkessels;* these are elastic reservoirs, comparable to hydraulic pumps used by firefighters. The aorta and other central arteries store half of the stroke volume and accumulate approximately 10% of the energy generated by the LV during systole. The stored energy is required for effective arterial recoil during diastole which pushes out of the stored blood and thus ensures an uninterrupted blood flow reaching the microvasculature. The attenuation of the pulsatile nature of the blood flow generated by LV contraction also prevents damage that pressure oscillations could cause in the microcirculation. (31)

Elastic properties of the large arteries are essential to ensure the buffering function of the arterial tree. Arteries are non-Hookean materials meaning they exhibit non-linear elasticity that depends on the BP. (32) Arterial elasticity is mainly determined by elastin fibers, however, at higher pressures elasticity is lower and is predominantly maintained by collagen fibers. (31) If arterial elasticity is reduced (e.g. due to elastin fragmentation and loss) the arterial tree becomes stiffer. In physical terms, stiffness can be explained as resistance of an elastic object to strain (relative change in length) imposed by stress (force applied over an area). (33) Increased arterial stiffness results in reduced arterial compliance and distensibility (change in arterial volume for a given change in pressure) and increased PWV. The latter relationship is explained by the Moens-Korteweg equation ($PWV = \sqrt{Eh/2r\rho}$), according to which PWV is directly related to arterial elasticity (E) and wall thickness (h), and inversely to vessel radius (r) and blood density (ρ). (31)

2.2.1.Determinants of arterial stiffness and PWV in healthy children

Age

Although arterial stiffness in the pediatric population is usually viewed as pathological, healthy children demonstrate a gradual progressive increase in arterial stiffness with growth. This physiologic stiffening is accompanied by increasing arterial size and total arterial buffering capacity. (34) Studies of PWV in healthy children revealed similar findings of a gradual increase of PWV during childhood with an approximately 1 m/s absolute rise between the age of six and 18 years. (35–38) Studies in younger prepubertal children suggested that in pre-school children until 6-8 years PWV is in a plateau phase and the age-dependent increase appears only afterwards. (37,39) Elastin has a very long half-life (up to 40 years) and its synthesis is most active during the perinatal period, declines thereafter and becomes virtually negligible once adult dimensions are reached. (40) Thus it seems plausible that at a certain age the gradual loss of elastin fibers due to increasing exposure to stress (e.g. imposed by rising BP) may result in clinically measurable increase in arterial stiffness.

Sex and pubertal status

In a small study of age, body dimensions, cardiac output and BP matched boys and girls, prepubertal girls demonstrated higher PWV compared to prepubertal boys. After puberty PWV decreased in girls and increased in boys, who also became taller, heavier and had larger cardiac output and lower heart rate (HR). (41) Large scale PWV reference studies in children are in line with the findings that puberty may have an effect on arterial stiffness, however, in these studies no differences in PWV between younger boys and girls were found in prepubertal children. In adolescence boys develop higher PWV than girls. (35–38,42) The potential effects of sex hormones on arterial stiffness have also been observed in the adult population with an increase in PWV post-menopausal women (43) and subsequent reduction with the use of estradiol ointments. (44)

BP

The relationship of PWV to BP in children is not uniform. Several studies (35,38,42) have found an independent positive association between BP and PWV, while others involving younger prepubertal children did not reveal any association. (39) Indeed, the relationship between BP and PWV seems to be age-dependent and Hidvegi et al. demonstrated that the trajectories of these

two parameters started to follow same direction at approximately nine years. This phenomenon is presumed to be caused by the gradual loss of elasticity in the aorta, which at certain time point cannot further compensate for the continuously increasing BP. (37)

Body dimensions

Studies in large cohorts of healthy children have consistently shown an independent direct effect of height on higher PWV. (35,36,38,39,42) In addition, a systematic review and meta-analysis has shown that obese children have higher PWV, although an inverse effect was observed in few studies and was considered to be related to increased arterial diameters. (45,46) Body dimensions and height, therefore, appear to have a direct effect on the elastic properties of the arteries (34,38) and have to be taken into consideration when interpreting PWV. Moreover, most of the methods to estimate PWV require a physical measurement of the path length which is directly related to height and, hence, can directly affect the results of the calculated PWV. (47) When comparing age- and height-specific PWV standard deviation scores (SDS), lower age-normalized PWV SDS were found in short-for-age children compared to height-normalized SDS values. This issue could be of particular importance in the pediatric CKD population with high prevalence of growth restriction. (35)

Prenatal and genetic factors

Intrauterine growth retardation could also impact arterial stiffness by the reduced elastin synthesis rates in the perinatal period. (40) Indeed, higher PWV was associated with lower birth weight (small for gestational age, SGA) but not prematurity *per se* in previous studies. (35,48) In addition, paternal hypertension and maternal obesity were also associated with higher PWV in childhood. (35) Race is another well-known determinant of PWV and studies in adult populations have shown higher PWV in the black population compared to Caucasian children. A study of prepubertal Angolan children also demonstrated higher PWV compared to other published pediatric cohorts involving Caucasian children. (39)

HR

In adult studies HR was considered as an important confounder in PWV assessments. In the pediatric studies, however, although inverse relationship of HR with PWV (35,38,42) was shown, the effect disappeared after

adjustments for other potential confounders. Therefore, the effect of HR for PWV measurements in children may be of less importance compared to adults.

2.3. Risk factors for arterial pathology in children with CKD

Children with CKD are exposed to a wide spectrum of potential vascular injuries that can be broadly categorized into the 'traditional' (Framingham) CV risk factors and those that are CKD-specific. The mechanisms of each risk factor development in CKD along with evidence about their effect on vascular health in the pediatric population are reviewed further.

2.3.1. Traditional CV risk factors in children with CKD

Dyslipidemia

CKD is associated with significant changes in lipid composition and development of a pro-atherosclerotic lipid profile, characterized by increased total cholesterol, triglyceride, low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) cholesterol. These changes are accompanied by significant alterations in lipoprotein composition (e.g. increased lipoprotein a and apolipoprotein B), changes in intermediate- and very-low-dense lipoprotein cholesterol and small dense LDL particles. Moreover, in addition to quantitative changes, significant qualitative alterations of serum lipids are observed in the state of uremia, such as oxidative stress induced changes in LDL cholesterol particles. The mechanisms behind the development of dyslipidemia in children with CKD are complex and beyond the scope of this review. However, it is important to note that the lipid profile in patients with CKD as well depends on the primary kidney disease (e.g. patients with nephrotic syndrome) and KRT modality. (49) Single large scale cohort study in children with CKD analyzing the prevalence of dyslipidemia in pediatric CKD demonstrated that 45% of children had dyslipidemia (predominantly increase in triglyceride levels), that was associated with decreasing eGFR and higher proteinuria. (50) In children with CKD, dyslipidemia has been related to increased cIMT and endothelial dysfunction. (51) Similarly, higher arterial stiffness parameters have been reported in children with familial hypercholesterolemia. (52)

Obesity

Although CKD is not directly linked to the development of obesity, children with CKD may develop obesity due to high-risk lifestyle and habits,

similar to healthy children. In addition, patients with immune-mediated kidney disease and after kidney transplantation are frequently treated with steroids, that are closely associated with increased risk of developing obesity. A review of 22 cross-sectional studies in children showed that children with increased adiposity are at increased risk of increased cIMT in adolescence. (53) Moreover, obesity has also been consistently identified as an independent risk factor of increased arterial stiffness in childhood. (46)

Glucose metabolism and diabetes

Studies in adults have revealed that patients with CKD have impaired glucose and insulin homeostasis, which has been demonstrated in up to 65% of patients. (54) The risk of glucose metabolism abnormalities and even diabetes mellitus becomes particularly increased after kidney transplantation and when under treatment with steroids. (55) In a study of 66 pediatric patients with pre-dialysis and dialysis-dependent CKD glucose intolerance was observed in half of the studied patients, four patients met criteria for diabetes mellitus and seven had insulin resistance. (56) These abnormalities themselves have been related to premature vascular ageing in children without CKD. Accelerated arterial stiffening was also reported in children with type 1 and type 2 diabetes and correlated with glycemic control and exposure to other traditional CV risk factors. (57,58) In children with CKD, however, cIMT and LVH did not correlate with the presence of glucose metabolism abnormalities. (56)

Smoking

Tobacco smoking is a well-known CVD risk factor and has been extensively linked to vascular dysfunction in adults. (59) Second-hand smoke exposure in childhood has been linked to higher risk of developing other CV risk factors, such as obesity. (60) Active and passive smoking also represent important risk factors for increased arterial stiffening. (61) Although studies in pediatric patients are scarce, tobacco smoking has been reported to have a synergistic effect with long-term childhood exposure to increased BP on arterial stiffness in young adults. (62)

Hypertension

Hypertension is very prevalent among children with CKD. The mechanisms leading to increase of BP in the state of CKD are complex but activation of the renin-angiotensin-aldosterone system (RAS) is believed to have a crucial role in hypertension development. Moreover, sodium retention

and fluid overload become significantly important to the development of high BP with advancing CKD and particularly in children on dialysis with low residual kidney function. In addition, increased sympathetic tone, hyperparathyroidism, endothelial dysfunction and iatrogenic factors (use of erythropoietin, steroids, growth hormone) also contribute to the development of hypertension. (63) Increased arterial stiffness has been reported in children with increased BP and childhood BP was associated with increased arterial stiffness in adult life. (64,65) Studies in young adults even suggested a possible bidirectional relationship between arterial stiffness and hypertension, hypothesizing that increased stiffness may precede the development of hypertension. (66)

Long-term impact of childhood exposure to traditional CV risk factors

Several large cohort studies followed children into their adulthood to investigate the potential effects of childhood exposure to CV risk factors on adult CV health. These landmark studies, such as the Bogalusa Heart Study or the Young Finns Study, have linked the development of adult arterial disease to CV risk factor exposure in childhood. Analysis of over 800 young adults with an average follow-up of 26.5 years revealed that childhood BP along with cumulative exposure of smoking in later life are independent predictors of arterial stiffness in adult age. (64) In addition, increased BP, dyslipidemia and smoking since childhood were associated with worse mid-life cognitive performance. (67) BP and obesity in childhood were as well linked to increased risk of CKD5 in adult life even in patients without CKD in their young age. (68) The Young Finns Study initially enrolled 4,320 children, of whom 2,060 underwent 30 years follow-up to investigate CV health. In this study childhood dyslipidemia, BMI and adolescent smoking were linked to subclinical atherosclerosis in adult years. (69)

It is hoped that results of ongoing collaborative initiatives, such as the International Childhood Cardiovascular Cohort (i3C), will further describe the effects of childhood risk exposure on CV events in adulthood. (70)

2.3.2. Uremic CV risk factors

Besides the traditional CV risk factors, uremia has a profound effect on vascular health. As discussed further, several studies in children with CKD have shown that uremic CV risk factors significantly affect vascular structure and function. The mechanisms leading to the development of uremia-related complications are briefly reviewed below.

CKD-MBD

CKD is associated with significant alterations in bone and mineral metabolism, collectively termed as CKD-MBD. This condition can be described by the presence of hyperphosphatemia, secondary hyperparathyroidism, increased levels of fibroblast growth factor-23 (FGF23), decreased klotho levels and vitamin D deficiency, along with altered serum calcium levels. The mechanisms leading to CKD-MBD are complex and involve different body systems. When eGFR decreases, phosphate starts to accumulate in the body due to decreased filtration capacity. Phosphate retention leads to slowly progressing hyperphosphatemia, which in turn stimulates production of phosphatonin hormone FGF23, which is synthesized by bone osteocytes and osteoclasts. Increased FGF23 decreases tubular phosphate reabsorption and contributes to absolute and functional vitamin D deficiency by increased catabolism and reduced activation. The increase in levels of FGF23 are also a result of decrease in klotho – an important co-receptor of FGF23, which is produced by the kidney. Klotho has a variety of biologic roles and has been considered to be an anti-ageing hormone. Klotho exists in a membrane bound and circulating forms and has several biological roles, including regulation of calcium, phosphorus, FGF23, parathormone (PTH) and vitamin D levels. CKD is associated with klotho deficiency, which leads to FGF23 resistance and further increase in FGF23 levels, although this is later overcome by the ability of FGF23 to stimulate its receptors in klotho-independent fashion. Increased phosphate also directly stimulates PTH production in the parathyroid glands. Moreover, its production is further exacerbated by decrease in klotho and vitamin D deficiency due to loss of negative feedback loop. Kidney being the organ involved in the last step of vitamin D activation, CKD also significantly affects vitamin D levels and vitamin D activation leading to absolute and functional vitamin D deficiency, which in turn promotes secondary hyperparathyroidism and leads to hypocalcemia. These processes eventually lead to increased bone resorption and the development of renal osteodystrophy and heterotopic mineralization which could also affect arterial walls. (71)

Oxidative stress

CKD is a condition with increased oxidative stress which is characterized by disturbed balance between the pro- and anti-oxidative systems and consequently increased formation of reactive oxygen and nitrogen species. The exact pathophysiology of pro-oxidative state in CKD are not clear and involves different mechanisms, such as uremic-toxin induced generation of

oxidative substances, but also depletion of antioxidants due to changes in nutrition or medication use. The resultant increase in reactive oxygen species can accelerate the development of atherosclerosis and hypertension, promote CKD progression and augment inflammatory response. (72)

Inflammation and uremic toxins

CKD is also recognized as a pro-inflammatory state, severity of which increases with decline in kidney function. Inflammatory response is particularly pronounced in patients with CKD5 and on dialysis and can be determined by measuring simple biomarkers, such as C-reactive protein (CRP) or interleukin 6. Oxidative stress, hypoxia, fluid and sodium overload, changes in gut microbiota and accumulation of gut-derived uremic toxins, all have significant role in the activation of inflammatory response which in turn significantly contributes to premature ageing and also adverse CV outcomes. (73) Uremic toxins, such as indoxyl sulphate or p-cresyl sulfate, originate in gut and are produced by altered gut microbiota which is caused by impaired intestinal transit time, altered dietary patterns and medication use (e.g. antibiotics or iron supplements). Alterations in gut microbiota are increasingly recognized as a significant CV risk factor within and beyond CKD. (74)

The prevalence and extent to which individual traditional or CKD-specific factors contribute to CVD development depend on different aspects such as primary kidney disease or degree of kidney dysfunction (75), but their interplay in the causal pathway of vascular damage in the pediatric CKD population are not always clear. The individual effects of different uremic risk factors on vascular health along with the data from clinical studies in children with CKD are discussed further.

2.4. Arterial changes in CKD

CKD is associated with significant structural changes in the arterial wall characterized by abnormal vascular remodeling and progressive arterial calcifications. The typical phenotype of uremic arteriopathy consists of arterial wall hypertrophy (increased wall-to-lumen ratio and medial cross-sectional area), reduced elastin content (decreased elastin/collagen ratio) and arterial calcifications. (76) Vascular calcifications are considered to be the hallmark of CKD-associated arterial disease and may occur in either intimal or medial layers of the arterial wall. Intimal calcifications are typically observed in older patients when classical atherosclerotic plaques become evident (77). The development of classic plaques is strongly linked to

increasing exposure to Framingham risk factors associated with aging and are further accelerated by CKD. In contrast, progressive calcification of the tunica media of large arteries, a distinct form of CKD-associated vasculopathy termed Monckeberg sclerosis, develops even in childhood (77,78).

The changes in large artery structure are evident even in young patients and arteries from children with pre-dialysis or dialysis-dependent CKD reveal increased calcium load and hydroxyapatite deposition within the arterial wall. (79) Arterial calcification was once presumed to be a passive process involving precipitation of calcium and phosphate in a supersaturated medium. However, in vitro studies investigating arteries from patients with CKD show that arterial calcification is a complex, active and highly regulated process closely related to CKD-MBD, systemic inflammation and oxidative stress. (5,80)

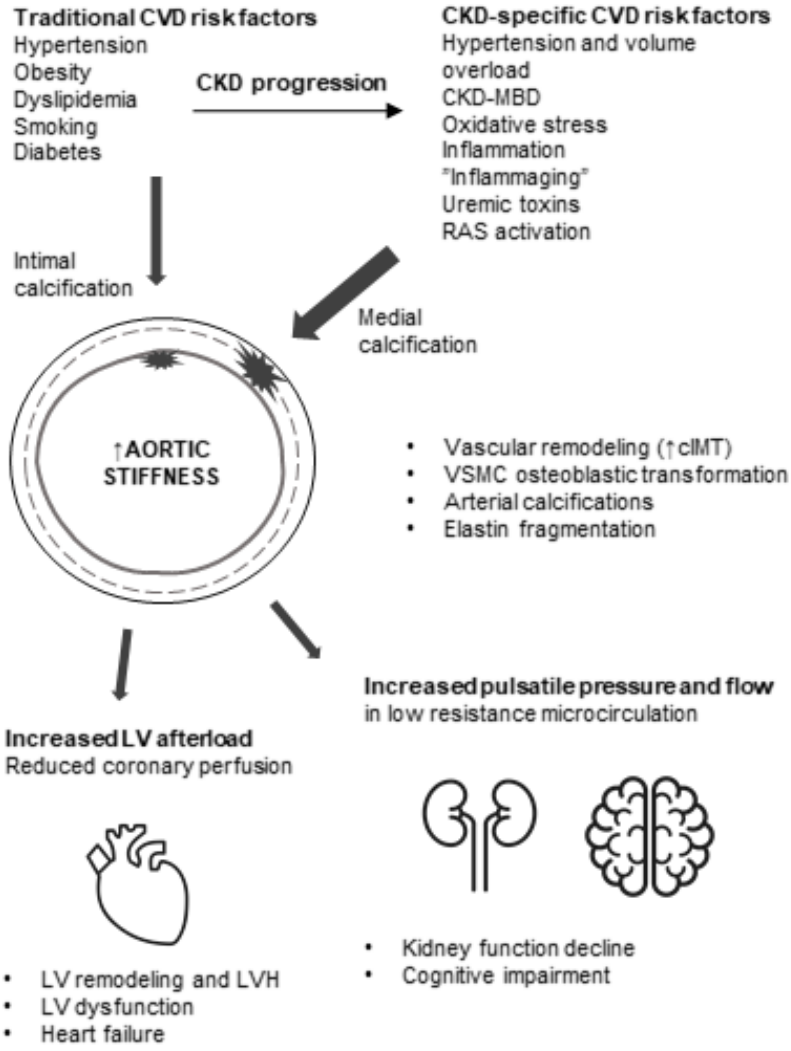
Incubating vessel rings from healthy children with medium high in calcium and phosphate does not result in calcification. On contrary, vessels from children with CKD demonstrate calcium load-dependent progressive vascular calcification in the presence of high phosphate. (78) These differences between the heathy and uremic states are explained by the deranged balance between pro-calcifying factors and calcification inhibitors. (81) The calcification process is realized through the release of mineralizing lipid vesicles by VSMC. These vesicles normally contain mineralization inhibitors (e.g. fetuin A, matrix GLA protein, osteoprotegerin) that are significantly reduced in CKD. (81,82) In addition, the development of overt calcification is preceded by hyperphosphatemia induced apoptosis of VSMC and the deposition of mineralizing apoptotic bodies in the sites of arterial calcification. (5,78)

Another key and distinct characteristic of arterial walls from patients with CKD is the osteoblastic transformation of VSMC induced by the exposition to high phosphate. (78) This transformation is accompanied by ossifying changes in the arterial wall, characterized by the development of bone-like structure: formation of trabeculae, increased osteoclastic activity and expression of bone biomarkers (alkaline phosphate, osteopontin, etc.). (5) Although phosphate is deemed to be the predominant vascular toxin in CKD, other components of CKD-MBD, such as klotho and vitamin D deficiency, increased circulating FGF23 and secondary hyperparathyroidism, have been shown to have independent effects on vascular calcification. (83) In addition, iatrogenic factors, in particular the use of calcium-based phosphate binders and excessive vitamin D intake, have also been associated with increased arterial calcification. (80)

Apart from the microstructural changes observed in arterial biopsies from children in CKD, clinically detectable arterial pathology has been reported even in young children. Measurement of the cIMT by high-resolution ultrasound of the carotid artery is a well-established marker of atherosclerotic burden. Increased cIMT has been observed in children with mild-to-advanced CKD, on dialysis and after kidney transplantation. (22,23,79,84) In early CKD dyslipidemia has been associated with increased cIMT (22) whereas in CKD stages 4-5 (23), and most markedly in patients on dialysis (79), CKD-specific risk factors are causally associated with vascular changes. Direct evidence of calcification, predominantly of the coronary arteries, has been also shown on computed tomography in a smaller proportion of children with CKD5 and on dialysis. (79,85)

Although dysregulated mineral homeostasis and CKD-MBD related factors likely play a central role in arterial structural alterations observed in CKD, other factors, such as hypertension, oxidative stress, chronic inflammation and aging may also contribute to vascular damage. Arterial hypertension – a widely prevalent complication of CKD – increases pulsatile wall stress in major arteries and contribute to progressive elastin fragmentation. (66) Activated inflammatory cells in uremia produce cytokines that stimulate osteoblastic transformation of VSMC and release matrix metalloproteinases, cathepsins and other proteolytic enzymes that induce elastin degradation and promote calcification. (5) Recently, accelerated vascular senescence, oxidative DNA damage and inflammation, collectively termed “inflammaging”, has been shown in children with CKD5 and was closely correlated with vascular pathology. (86) Other factors, including endothelial dysfunction, RAS activation, uremic toxins and advanced glycation-end products may also affect vascular morphology to varying extents. (5)

Changes in the arterial structure, along with respective CV risk factors and potential clinical consequences, are summarized in Figure 2.



Abbreviations: cIMT, carotid intima-media thickness; CKD, chronic kidney disease; CVD, cardiovascular disease; LV, left ventricle; LVH, left ventricle hypertrophy; MBD, mineral-bone disease; VSMC, vascular smooth muscle cells.

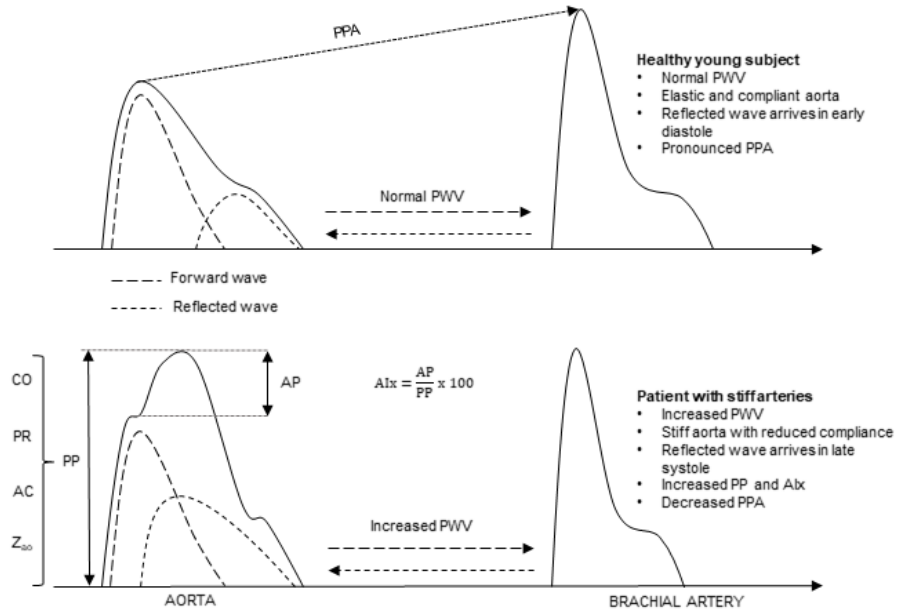
Figure 2. Risk factors, mechanisms and consequences of arterial stiffness

2.5. Arterial stiffness and functional consequences in CKD

Increased exposure to potential vascular injuries and aforementioned structural alterations may alter the function of large arteries and accelerate arterial stiffening in CKD, leading to several important clinical implications.

Arterial stiffness is strongly related to aortic pulse pressure (PP) and is one of the major contributors to LV afterload (Fig. 3). According to the traditional

view, the arterial system was seen as a two-element *windkessel* where pressure-flow relationships are determined by arterial compliance and peripheral vascular resistance. These arterial properties, along with the cardiac output were also considered to be the determinants of PP. (87,88) Later, a three-element *windkessel* model was proposed that additionally incorporates aortic characteristic impedance, which is mainly determined by local aortic stiffness and aortic geometry. (87,89)



Abbreviations: AC, arterial compliance; AIx, augmentation index; AP, augmented pressure; CO, cardiac output; PP, pulse pressure; PPA, pulse pressure amplification; PR, peripheral resistance; PWV, pulse wave velocity; Z_{ao}, characteristic aortic impedance.

Figure 3. Simplified schematic representation of pulse wave forms in healthy subjects and patients with increased arterial stiffness

In addition, arterial stiffness contributes to the shape and magnitude of the aortic pressure waveform (PW) by another indirect mechanism. According to the classical paradigm, the aortic PW is a net result of forward and backward travelling waves. LV ejection creates a forward PW that propagates distally towards peripheral tissues and multiple reflected waves are generated at points of impedance mismatch (e.g. arterial branching or diameter narrowing). The sum of these reflected waves creates a "net" reflected wave which returns to the proximal aorta. The timing when this reflected wave meets the forward-travelling waveform is critical and determined by PWV and left ventricular ejection time (HR) (Fig. 3). At lower PWV and higher HR the PW arrives in

early diastole and allows for increased coronary perfusion during diastole. On the other hand, in case of a high PWV and low HR, the PW arrives in late systole, augmenting systolic BP (and PP) in the aorta. (28,31) This simplified mechanism, however, has been debated and pressure augmentation model that incorporates aortic reservoir pressure and excess pressure related to wave propagations and reflections has been proposed. (87)

Irrespective of the exact underlying mechanism, arterial stiffness represents an important mechanism that may damage major target organs: the heart, brain and kidneys (Fig. 2). First, there is strong mechanistic evidence that increased arterial stiffness contributes to increased LV afterload which can result in LV remodeling and LVH. (90) In addition to the impaired ventricular-vascular coupling, increased arterial stiffness has also been linked to impaired coronary perfusion and myocardial hypoperfusion. (91,92) The combined effect of these sequences can lead to diastolic and systolic LV dysfunction and development of clinically overt HF. These mechanistic notions are supported by a meta-analysis of over 17,000 adults that linked increased PWV to higher risk of IHD, stroke and CVD events independent of the conventional risk factors, such as smoking, diabetes, hypertension or kidney function. (10)

Second important consequence of abnormal arterial stiffening is the reduced "cushioning" capacity of the stiff arterial tree which results in the transfer of pulsatile blood flow generated by intermittent LV contractions into the microvasculature. Organs such as the brain and kidneys that are dependent on continuous perfusion and have a widely spread low-resistance microvascular network are particularly susceptible to damage caused by the exaggerated pulsatile pressure and flow. (93) Arterial stiffness in may result in higher BP within the glomerular capillaries and exacerbation of glomerular hypertension which would then contribute to progressive renal damage. (94) Increased PWV has been associated with a higher risk of incident CKD or kidney function decline in several large communities of adults without CKD of different ethnicities. (95–98) Clinical studies in adults without kidney disease have also reported the adverse effects of increased arterial stiffness on cognition and kidney function. (95,99)

2.6. Measuring arterial stiffness in children with CKD

Measuring early changes in the arterial system is important to quantify the presence and severity of subclinical arterial disease and to evaluate the remote risk of overt CVD development. Measurements of structural changes are important to understand the degree of premature atherosclerosis or arterial

calcifications but are not directly representative of arterial stiffness; patients with CKD show moderate correlation between cIMT and parameters of arterial stiffness, but these may represent differential arterial response to various vascular injuries. (23,100,101) While cIMT reflects the degree of premature atherosclerosis in children, arterial stiffness directly represents a functional risk factor of CKD arteriopathy and may have superior predictive value. (100,102)

2.6.1. Mechanistic insights into measurement of arterial stiffness

Several different parameters may be used to evaluate arterial stiffness in children. In general, they can be categorized into (i) elasticity parameters (e.g. distensibility, Young's elastic modulus, β-stiffness index), (ii) PWV and (iii) pulse wave analysis (PWA)-derived parameters (central BP, augmentation index (AIx)). Arterial stiffness can be described using different physical parameters of stress-strain, pressure-strain and pressure-volume relations. Various indices derived from these physical concepts have been used to estimate arterial stiffness and may be confusing, particularly as they are not all directly representative of arterial stiffness.

In general terms, stiffness can be explained as resistance of an elastic object to deformation (strain, i.e. relative change in length) imposed by stress (force applied over an area). In the simplest form, this could be described by pressure-volume relationship and compliance (C) which represents the change in arterial volume (V) for a given change in pressure: $C = \frac{\Delta V}{\Delta P}$. Distensibility (D) takes into account the initial size of the artery and describes compliance relative to its initial volume: $D = \frac{\Delta V}{\Delta P \times V}$ which allows to compare compliance between arteries with different diameters. However, when calculating C or D arteries are treated as simple hollow structures, not considering the intrinsic elastic properties of the wall. Although C of an artery will be significantly affected by increased stiffness, it can also change due to changes in diameter or wall thickness irrespective of changes in elastic properties. (31,32)

These shortcomings of the compliance parameters to describe arterial stiffness can be overcome by estimating direct stress-strain relationship which is defined as Young's elastic modulus (E). As the initial state of the artery is typically unknown, Young's incremental elastic modulus (E_{inc}) is used more frequently and is calculated from two points of the stress-stain slope. According to the Laplace law, circumferential wall stress is directly related to vessel pressure (P) and radius (r) and inversely to wall thickness (h). Therefore, stress-strain, pressure-strain and pressure-volume relationship

terms are not synonymous and the two latter parameters will also be affected by arterial geometry. Given the non-Hookean properties of the arterial wall, the dependence of distensibility parameters on local BP measurements (e.g. in the carotid artery) and the need to accurately measure arterial wall structure that may not all be easy in young children, the feasibility of these parameters in routine pediatric practice is questionable. (32)

PWV is the velocity of PW transmission throughout the arterial wall and is not synonymous with blood velocity, which is significantly lower and driven by the pressure-gradient created by the PW propagation. (31) PWV according to the Moens-Korteweg equation can be expressed as $PWV=\sqrt{(Eh/2r\rho)}$, where ρ is the density of blood (considered a constant due to its relative stability). Given the previous notions about the components that determine Young's elastic modulus, this equation can be expresses as suggested by Bramwell-Hill: $PWV=\sqrt{(1/\rho D)}$, which describes it as a reciprocal value of distensibility. Due to its close relationship to the factors determining arterial stiffness and independence of local arterial BP measurements PWV is frequently the functional parameter of choice used to estimate arterial stiffness. (31,32)

PWA is another common approach to indirectly estimate arterial stiffness. PWA is typically based on the recording of PW in a peripheral artery (e.g. brachial or radial) by tonometry or oscillometry and estimation of the aortic PW by a mathematical algorithm (transfer function). The amplitude of this PW (corresponding to aortic PP) is closely but not entirely related to arterial stiffness. The percentage change in aortic PP (relative to initial PP) called AIx can be estimated from the PW and is commonly used as a proxy of arterial stiffness (Fig. 3). The simple measurements of PP and more complex PWA along with the AIx and central PP could be seen as attractive parameters to evaluate arterial stiffness. However, brachial PP measurements do not reflect the aortic PP, particularly in young subjects where differences between brachial and aortic PP reach 20 mmHg due to the phenomenon of PP amplification. (87) The amplitude of PP in the aorta although dependent on arterial stiffness, is also dependent on cardiac output, aortic geometry and wave reflection. Wave reflection based parameters, such as AIx are themselves dependent on HR, stature and magnitude of the reflected wave itself.

For the reasons outlined above and the abundance of evidence about its relation to clinical outcomes, PWV is recommended as the gold standard of arterial stiffness evaluation by the ESH (103,104) and AHA (7,105). Furthermore, PWV is also recognized as a proxy of target organ damage in

the US and European adult (106,107) and the European pediatric hypertension guidelines. (11)

2.6.2.Measuring PWV in children

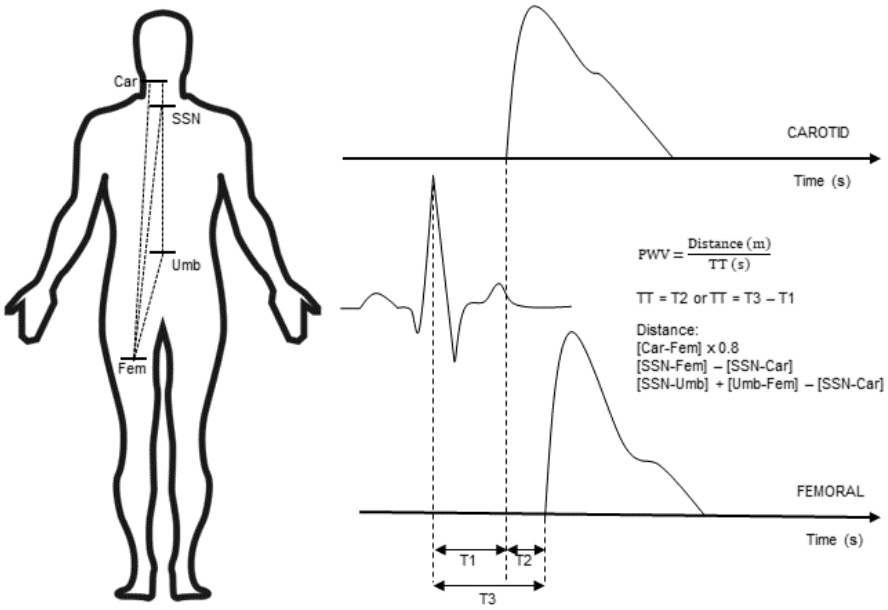
Apart from the invasive measurements, estimation of PWV typically relies on the calculation of the time a PW takes to travel between two points in the arterial system (transit time, TT). When the travel distance (path length) is known, PWV can be calculated as follows: $PWV\ \left(\frac{m}{s}\right) = \frac{Distance}{TT}$. Magnetic resonance imaging (MRI) is the only non-invasive technique that provides most accurate estimation of these two parameters, however, its use is limited by the logistic, cost-efficiency and technical considerations, especially in children. PWV measurement devices for routine practice are based on the non-invasive recording of PW in two predefined sites of the arterial tree, estimation of TT and over-the-skin measurement of the travel distance. Few devices employ techniques that involve single point measurements and statistical algorithms (transfer functions) to estimate presumed PWV. Although most devices share similar approach, major differences in the references sites and methods for PW obtaining and travel distance estimation exist and lead to heterogeneity in PWV estimates.

In general, carotid-femoral (cfPWV) is the most widely used pathway and due to its close approximation to the aortic pathway is recommended as the preferred method for regional PWV calculation. However, alternative arterial sites for PWV estimation have also been used with varying popularity and include: brachio-femoral (bfPWV), brachio-ankle (baPWV), heart-ankle (cardio-ankle vascular index, CAVI) or, a recently introduced, finger-toe (ftPWV) PWV measurements. Of those, ba-PWV and CAVI have been studied in large cohorts and related to clinical outcomes, mainly in the Asian population. Due to the lack of evidence of generalizability to other populations, these approaches have not yet been recommended for clinical use in US and Europe and the review further will focus on cfPWV (further named PWV) or methods providing its approximations. (6)

PW on the selected arteries can be recorded using different techniques (e.g. tonometry, oscillometry, ultrasound) either using two sensors simultaneously or with a single sensor. The TT between the proximal and distal recording sites is then estimated by calculating the time delay between the two systolic upstrokes (“foot-to-foot” method) if PW is recorded simultaneously or using ECG-gating and R wave as an offset (Fig. 4). Distance between the points where PW have been obtained is estimated by over-the-skin path length

measurements. This indirect distance measurement does not take into account the kinking and the non-straight pathway of the major arteries therefore provides only approximate estimates. In adult population, 80% of the direct measurement between the carotid (Car) and femoral (Fem) measuring sites or subtraction of the Car-suprasternal notch (SSN) distance from SSN-Fem distance has been shown be most closely related to the aortic pathway measured by MRI and to provide most accurate PWV results, respectively. (6) A recent study in children has indicated that the subtracted distance estimation is more reliable when compared to 80% of the direct measurement which overestimated the MRI-determined aortic pathway by up to 10%. (108)

Common devices and techniques for PWV assessment are summarized in Table 2 and shortly reviewed below.



Abbreviations: Car, carotid artery; Fem, femoral artery; PWV, pulse wave velocity; SSN, suprasternal notch; TT, transit time; T, time; Umb, umbilicus.

Figure 4. Examples of travel distance and transit time estimations for cfPWV calculation

2.6.3. Overview of the devices for PWV measurements

The devices for cfPWV measurements can be classified based on their approach to recording PWf in respective arteries or use of specific algorithms to derive PWV. These devices can be categorized in to the following:

- *Applanation tonometry*. It is the most frequently used technique for PWV assessment and along with invasive measurements is considered as the gold standard for the validation of other PWV devices. This technique involves the use of tonometric sensors that allow to record PW in carotid and femoral arteries.
- *Piezoelectric mechanotransducers*. These devices are generally very similar to tonometry devices but use piezoelectric mechanotransducers instead.
- *Cuff-based oscilommetric devices*. This group of devices use either a single or two cuffs that oscillometrically derive the PW. The single cuff devices record brachial PW and use mathematical algorithms to transform the readings into PWV values (Mobil-o-Graph and Arteriograph). Vicorder uses two cuffs: neck (carotid) and thigh (femoral) to perform simultaneous oscillometric recordings of the PW with subsequent TT estimation. (109)
- *Other techniques*. A recently introduced device called pOpmetre employs sensors similar to pulse-oximeters that are placed on the finger and toe and ftPWV is estimated. Doppler ultrasound techniques employ recording Doppler waveforms at two different vascular sites (e.g. aortic arch and external iliac artery or carotid and femoral artery). Similarly, MRI techniques, including phase contrast MRI that allows to assess blood flow velocity can be used. (109)

2.6.4. Choosing the suitable device for children

The variety of available methods makes the selection of the most suitable device complicated, particularly given their inherent technical differences and results that cannot be used interchangeably. Several aspects have to be taken into consideration when choosing a device for PWV in children: (i) feasibility (ease of use, operator-dependence and reproducibility), (ii) device validation, (iii) availability of population-specific reference data and (iv) evidence of association with clinical outcomes. The latter is complicated in the pediatric population since hard clinical outcomes would require very long-term follow-up and these associations have not yet been established. Therefore, the clinical value of the device-specific PWV has to be extrapolated from the adult studies and is included in the Table 2 as judged in a previous review by Milan et al. (109)

Feasibility

Feasibility and reproducibility of the measurements are very important aspects in the pediatric population. PWV measurements with tonometry devices require trained operators who manually put the tonometer on the respective arteries. As the angle and position of the sensor are very important to obtain accurate measurements of the PW these measurements may be subject to inter- and intra-observer variations. A feasibility study of SphygmoCor in 40 healthy children showed good reproducibility of PWV measurements. However, reproducibility relied on the strict quality criteria for the obtained PW and this was only possible in smaller fraction of the children (17 out of 40). (110) Moreover, in a validation study for children a more than 20% of the patients who were younger failed to tolerate tonometry measurements. (111) Similar findings were observed in preschool children in whom valid arterial stiffness measurements with Arteriograph could not be obtained in more than 40% of the children. (112) Overall, the reported inter- and/or intra-observer reproducibilities for tested devices (Arteriohraph, SphygmoCor, Vicorder, PulsePen, SphygmoCor Xcel) in children was deemed good. (112–115) Although the reproducibility of measurements is similarly good, operator-dependence, time needed to obtain the measurements and ability for children to tolerate the measurements are important aspects to consider, especially in younger children.

Device validity

As the number of new devices significantly increased over the last decade, there was a clear need for a consensus on how these devices should be validated. To address this issue Artery Society released guidelines for the validation of non-invasive PWV devices that are focused on the preferred measurement – cfPWV. (116) These guidelines define different technical aspects for device validation, including the gold standard (invasive or applanation tonometry) and standard travel distance calculation ([Fem-SSN] – [Car-SSN] or direct distance x 0.8). The accuracy is judged based on the mean difference and standard deviation between the tested device and the gold-standard. In addition, AHA Scientific Statement extended the definition of gold standard to devices that have robust evidence of being linked to clinical outcomes (i.e. Complior or SphygmoCor). (6)

A number of validation studies for different devices have been performed in the adult population using either applanation tonometry or invasive measurements as the gold standard and have been summarized elsewhere. Briefly, SphygmoCor Xcel, PulsePen, Aortic and Complior showed excellent,

while Mobil-o-Graph and Vicorder showed acceptable agreement in adults. (109) It is important to note that validation studies in adults cannot be directly extrapolated to the pediatric population and both statements regarding the validation of PWV devices indicate that devices to be used with children should be validated in children. (6,116) No validation studies against invasive measurements have been performed and SphygmoCor has been used as the gold-standard exclusively.

Kis et al. compared three devices: PulsePen, SphygmoCor and Vicorder in 98 healthy children and young adults using three different pathway calculations for the Vicorder device. PulsePen showed excellent agreement with SphygmoCor, while the agreement between Vicorder and SphygmoCor was dependent on the pathway calculation. The agreement was excellent when direct distance from SSN to the middle of thigh cuff or a subtracted ([SSN-Fem] – [SSN-Car]) distance were used, and acceptable for direct distance from SSN to the top of thigh cuff. (113) Similar findings were found in two other Vicorder validation studies in pediatric population: distance measurement from SSN to the top of thigh cuff showed only acceptable agreement, while the subtracted distance showed excellent agreement with SpygmoCor. (111,114) Moreover, in the study by Kracht et al. distance measurement via umbilicus (Umb) - a pathway that is presumed to better reflect the arterial tree: ([SSN-Umb] + [Umb-Fem]) – [SSN-Car] showed even better agreement. (114) Among other devices, the novel SphygmoCor Xcel and the pOpmetre (using a correction factor) devices have also recently been shown to have excellent agreement with the SphygmoCor device in children. (115,117)

Device-specific pediatric PWV reference values

Given the relatively narrow age- and height-specific reference intervals for children and the inherent differences between PWV estimates by different devices PWV, reference values in children have to be selected based on the device that is being used. Comparison of available reference data revealed differences in the established reference values using different devices with a particularly major difference of measurements obtained with Arteriograph compared to other devices (PulsePen, Vicorder, Mobil-o-Graph). (35,36) This is most likely related to the technical aspects of PWV measurement with Arteriograph.

Therefore, when reference values are being selected several issues have to be taken into account: (i) technical aspects used for reference value estimation, e.g. reference values for the Vicorder have been derived using different pathways, (ii) choosing age or height specific reference values (e.g. age

specific reference values for Vicorder device seem to underestimate PWV in small for age children), (iii) the consistency of the population used for reference values calculation and the target population (i.e. age range, ethnicity, etc.). Height is an important and independent determinant of cfPWV in children, therefore, the choice of height or age-standardized reference values is important (35,36,38,39,42). Age-standardized reference values can underestimate PWV in small-for-age children. (35) This may be of particular importance for accurate stiffness assessment in the pediatric CKD population where short stature is prevalent. (118,119) Available reference values along with the used devices are summarized in Table 2.

Table 2. Techniques and devices for cfPWV measurements and available data from pediatric studies

Device	Method	Pathway[a]	Clinical usability[b]	Validation in children	Device-specific pediatric reference values
SphygmoCor (Atcor Medical)	Applanation tonometry Single sensor ECG-gating required	A, B	+++	Gold standard	Yes (38,120) Age: 6-20
PulsePen (DiaTecne)	Applanation tonometry Single sensor ECG-gating required	A	+++	Yes (113)	Yes (38) Age: 6-20 years
SphygmoCor Xcel (Atcor Medical)	Carotid tonometry Femoral cuff, simultaneous	A	+	Yes (115)	No
PulsePen ETT (DiaTecne)	Applanation tonometry Two sensors, simultaneous	A	-	No	No
Complior (Alam Medical)	Piezoelectric mechanotransducers Two sensors, simultaneous	C	+++	No	Yes (121) Age: 5-17 years
Aortic (Exxer)	Piezoelectric mechanotransducers Two sensors, simultaneous	D	+	No	No
Arteriograph (TensioMed)	Oscillometric Single brachial cuff	E	++	No	Yes (37,122) Age: 3-22 years
Mobil-o-Graph (IEM)	Oscillometric Single brachial cuff	-	++	No	Yes (36) Age: 8-22 years
Vicorder (Skidmore Medical)	Oscillometric Two cuffs (carotid and femoral), simultaneous	A, B, F	++	Yes (111,113,114)	Yes (35,42) Age: 5-19 years

Continued table.

Device	Method	Pathway[a]	Clinical usability[b]	Validation in children	Device-specific pediatric reference values
pOpmetre (Axelife SAS)	Two photodiode sensors (fingers and toes), simultaneous	Height-derived	++	Yes (117)	No
Doppler ultrasound	Doppler probes ECG gating might be required	Height-derived	++	-	Yes [76] Age: 0-20 years
MRI	MRI	Direct estimation	+	-	Yes (124) Age: 2-28 years

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; ECG, electrocardiography; MRI, magnetic resonance imaging.

a) A: [Fem-SSN] – [SSN-Car]; B: [Car-Fem] x 0.8; C: [Car-Fem]; D: [Car-Fem] – [Car-SSN]; E: [SSN-Symphysis]; F: [SSN-Umb] + [Umb-Fem] – [SSN-Car] (SSN = suprasternal notch)

b) According to the evaluation of adult studies by Milan et al. (109)

2.7. Clinical studies in adults with CKD

Arterial stiffness has been extensively studied in adults with CKD and landmark studies, such as the Chronic Renal Insufficiency Cohort (CRIC) study, have clearly established a link between advancing CKD and increasing arterial stiffness. (7) In over 2,500 adults from the CRIC study, PWV increased by 0.23 m/s per each 10 ml/min/1.73 m^2 decrease in eGFR. (7) In CKD5 patients dialysis modality has an important effect on arterial stiffness, with more accelerated stiffening on HD compared to PD. The differences are mainly related to residual kidney function and higher volume overload in patients on HD who also demonstrate cyclical pre-/post HD session variability of PWV. (125) The benefits of kidney transplantation on arterial stiffness and PWV remain unclear and may be influenced by the change in risk factor profile, but patients with a kidney transplant have higher PWV compared to the healthy adult population. (126)

Importantly, arterial stiffness has been correlated with hard outcomes in adults with CKD: all-cause and CV mortality, CV events, CKD progression and cognitive function decline.

2.7.1. PWV and all-cause mortality

In 2,795 adults with CKD from the CRIC study PWV in the highest tertile (>10.3 m/s) was associated with increased risk of overall mortality (adjusted

hazards ratio [HR] 1.72, 95% CI 1.24 to 2.38) over the 7-10 year follow-up. (8) Similarly, other studies of patients with non-dialysis dependent CKD (127,128) and patients on HD (129,130) all showed increased risk of overall mortality with higher PWV values. However, the incremental predictive value of PWV has been recently questioned by Tripepi et al. who compared the performance of PWV and clinical risk scores (Annualized Rate of Occurrence, ARO) in two independent adult CKD5 cohorts. The analysis revealed that PWV resulted in only a modest increase of risk discrimination, risk reclassification over the conventional clinical risk scores (ARO) and, furthermore, impaired model calibration. (131)

2.7.2. PWV and CV outcomes

A meta-analysis of 16 studies performed in different populations (CKD, community-based, hypertensive) that included a total of 17,635 patients revealed that PWV was associated with higher risk of coronary heart disease (CHD), stroke and CVD events independent of the classical risk factors. (10) PWV has also been shown to an independent predictor of fatal and non-fatal CV events in patients with advanced CKD and on HD. (102,127,132–135) In addition, ambulatory PWV have been shown to predict non-fatal MI or stroke in adults on HD. (130) In the CRIC Study PWV in the middle or top tertiles was associated with incident hospitalized HF. (136) In a small study of 44 pre-dialysis CKD patients evaluated by baPWV was the single independent predictor of severe lacunes and severity of carotid/intracranial artery stenosis. (137)

In a cross-sectional study of 96 patients with CKD, PWV was associated with structural remodeling of LV and left atrium. (138) Increased aortic stiffness has also been associated with myocardial fibrosis and LV twist mechanics in adult patients with CKD. (139,140)

2.7.3. PWV and CKD progression

Arterial stiffness in patients with CKD may result in higher BP within the glomerular capillaries and exacerbation of glomerular hypertension which would then contribute to progressive renal damage. (94) Increased cfPWV has been associated with a higher risk of incident CKD or kidney function decline in several large communities of adults without CKD of different ethnicities. (95–98) In the aforementioned CRIC Study patients with PWV in the highest tertile had higher risk of CKD5. (8) The association with CKD progression

and development of kidney endpoints was also demonstrated is several other smaller scale studies. (141–143) However, several studies also failed to find an independent association of PWV with kidney outcomes. (144–146)

2.7.4. PWV and cognitive dysfunction

Brain microcirculation due to its low microvascular resistance can be particularly susceptible to the detrimental effects of the unattenuated pulsatile stress resulting in microvascular brain injury which could contribute to the development of cognitive impairment and vascular dementia. (147) The potential effects of arterial stiffness and PWV on cognitive impairment and even development of dementia have already been shown in elderly adults without CKD. (148–150) In a subset of patients from the CRIC Study, higher PWV was also found to be an independent predictor of cognitive impairment. (7) Similar associations were also demonstrated in smaller cohorts of patients with mild-to-moderate CKD (9) and patients on HD. (151)

Importantly, the predictive value of PWV appears to outweigh that of conventional BP measurements. (7) The associations of increased PWV with adverse outcomes, including all-cause mortality and CV events, are not ameliorated by kidney transplantation and associations with graft dysfunction have also been reported. (126)

2.8. Clinical studies in children with CKD

Data about PWV in the pediatric CKD population remains relatively scarce and largely comes from small case-control studies (summarized in Table 3). In contrast to the adult population, studies in children with CKD do not reveal direct associations between eGFR and PWV. (23,152–155) Available evidence suggests that in earlier CKD stages, when uremia-related risk factors are less pronounced, arterial elastic properties in children remain relatively unchanged. Two large studies involving children with mild-to-moderate CKD reported similar PWV compared to healthy children. (152,156) In fact, the determinants of PWV in children with early CKD stages were age, BP and black race (152) – same as reported in healthy children. (35–39,42) Studies of children with more advanced CKD, however, have reported increased PWV, especially in children on dialysis. (23,157–162)

In contrast to the findings in those with early kidney dysfunction, the determinants of PWV in children with advanced CKD and on dialysis are in line with the findings of arterial biopsy studies. Several studies have reported

strong associations of PWV with markers of CKD-MBD, including serum fetuin-A or fetuin-A/Ca x P ratio, PTH, bone alkaline phosphatase and lower levels of vitamin D. (23,79,82,159,163) In addition, a dose-dependent effect of treatment with active vitamin D on increased PWV was observed in children on dialysis and after kidney transplantation. (79,119,163) Furthermore, the gut-derived uremic toxin indoxyl sulphate, but not p-cresyl sulfate, was independently predictive of PWV increase over 12 months in children with CKD. (164) Whether this represents an effect of altered gut microbiota remains unclear but microbiota composition was not correlated with PWV in children with mild CKD. (154)

BP appears to be the only factor independently and strongly associated with PWV in children across all CKD stages. (23,152,155,161,165,166) Higher PWV has been reported in patients with different ABPM profile abnormalities (155) and a recent study reported night-time and sustained hypertension to be independent predictors of PWV in children with CKD. Interestingly, lower PWV was observed in patients with a normal ABPM profile taking antihypertensive medications compared to untreated patients. (165) Whether this is related to the possible beneficial effect of frequently prescribed RAS inhibitors on arterial stiffness in pediatric CKD is intriguing. (66)

Studies in pediatric CKD did not demonstrate direct associations between BMI and increased arterial stiffness. (23,152) However, recently both underweight and overweight children with CKD and on PD have been reported to be at increased risk of higher PWV. In addition, lower adipose tissue mass was independently associated with lower odds of increased PWV. (158) The U-shaped relationship between PWV and BMI may reflect the importance of the malnutrition-inflammation-arteriopathy axis (167) and resemble the associations between cholesterol and mortality in adults with CKD. (168)

Similar to BMI, direct independent associations of PWV with dyslipidemia were not identified in children with pre-dialysis CKD. (23,152) Instead, current evidence suggests that the effects of traditional CV risk factors cannot be directly transferred to the pediatric CKD population and may be significantly confounded by the uremic milieu. For instance, it has been shown that qualitative changes in HDL, but not quantitative lipid abnormalities may induce vascular injury and associate with arterial stiffness in pediatric CKD. Nitric oxide (NO) release induced by HDL cholesterol, but not HDL levels were significantly correlated with PWV. (169) Two other studies also reported correlations between markers of the NO pathway and PWV in children with pre-dialysis CKD. (155,170) The relationship between endothelial

dysfunction and PWV was further demonstrated by a study which showed an independent association between PWV and circulating endothelial microparticles. (166)

The effects of KRT on arterial stiffness in the pediatric population have not been studied extensively. Increased PWV has been reported in children on PD, HD and after kidney transplantation with the highest values in children on HD. (118,119,157,157–160,163,166,171) Although patients on HD demonstrate highest arterial stiffness, a recent study investigating the effects of hemodiafiltration (HDF) on heart and height (3H Study) reported a significant decrease of PWV over time in patients on HD and HDF. In the 3H Study HDF attenuated progression of cIMT when compared to HD, but the annualized change of PWV did not differ between patients on conventional HD and HDF. The determinants of PWV in this large multicenter cohort were inter-dialytic weight gain (IDWG), BP, lower hemoglobin, and higher PTH. (172)

A longitudinal study of 15 children who underwent kidney transplantation after previous HD did not reveal a significant change in PWV within six months after the transplantation. (173) However, a study investigating the effects of preemptive KRT initiation in children with CKD5 showed that preemptive kidney transplantation was associated with a decrease of PWV compared to initiation of dialysis. (174) Irrespective of the dynamic changes, arterial stiffness appears to remain increased in the pediatric kidney transplant population (162,163,173) and may relate to a novel spectrum of risk factors. Studies in children, however, did not report a significant effect of calcineurin inhibitors or obesity on arterial stiffness. (173,175) Instead, cumulative calcitriol dose, dialysis for more than one year and impaired kidney function have been linked to PWV augmentation after transplantation. (119,163) Of note, patients with a decrease in PWV after kidney transplantation had better graft function. (173)

Although available evidence suggests that arterial stiffness is prevalent in children with advanced CKD, studies of its functional consequences and long-term effects on CV outcomes in adult life are still lacking. PWV was not associated with LVMI in a small study of children on HD but correlated in children with autosomal dominant polycystic kidney disease. (171,176) In contrast, in the adult CKD population increased PWV was associated with structural remodeling of the LV and left atrium, myocardial fibrosis and LV twist mechanics. (138–140) Whether other CKD related risk factors of LV remodeling surpass the effect of arterial stiffness in children requires further studies. One of the explanations for these differences, however, could be age-dependency of the hemodynamic effects of PWV on LV afterload. A study of adults suggested that increased PWV in younger patients may be a

consequence of increasing velocity of myocardial shortening and may represent increased intraventricular PWV with negligible hemodynamic effect. (177)

Table 3. Summary of studies investigating PWV in children with CKD

Author, year	Population (KRT, age, eGFR)	PWV measurement	Summary of principal findings
Shroff et al., 2019 (172)	N=78 HD and n=55 HDF Age: 5-20 years *Multicenter, non-randomized, parallel-arm intervention study (conventional HD vs post-dilution online HDF)*	Vicorder	PWV decreased similarly after 12 months in both HD and HDF groups Predictors of higher PWV score at 12 months were higher IDWG, higher systolic and diastolic BP SDS, lower hemoglobin, and higher PTH
Hsu et al., 2019 (155)	N=125 (CKD 1-4) Age: 9.6 (5.3–14.4) years eGFR: 107 (87–125) ml/min/1.73 m^2 *Cross-sectional study*	Carotid echo-tracking (e-TRACKING)	Higher PWV in CKD3-4 vs CKD1: 4 (3.6–4.8) m/s vs 3.8 (3.4–4.2) PWV positively correlated with serum arginine levels PWV was higher in abnormal 24-hour, daytime BP, nighttime BP, nighttime dipping, BP load and abnormal ABPM profile groups
Holle et al., 2019 (164)	N=609 (CKD3-5; 4C cohort) Age: 12.1 ± 3.3 years eGFR: 28.1 ± 10.3 ml/min/1.73 m^2 *Longitudinal study*	Vicorder	Change in PWV SDS within 12-months associated with baseline indoxyl sulphate levels in adjusted analysis
Duzova et al., 2019 (165)	N=456 (CKD3-5; 4C cohort) Age: 12.5 ± 3.2 years eGFR: eGFR 29 ± 12 ml/min/1.73m^2 *Cross-sectional study*	Vicorder	Night-time hypertension and sustained hypertension predicted increased PWV (adjusted analysis) Higher PWV SDS in IDH, INH and SH compared to normal ABPM profile Lower PWV in normal ABPM group with antihypertensive medication compared to those without
Skrzypczyk et al. 2019 (153)	N=38 (CKD2-5; n=6 dialysis) Age: 12.23 ± 4.19 years eGFR=25.74 ± 8.94 ml/min/1.73m^2 *Case-control study*	SphygmoCor	Higher PWV in CKD vs healthy children: PWV SDS –0.37 ± 1.27 vs –1.10 ± 0.99 PWV SDS did not correlate with eGFR Renalase was not associated with PWV
Karava et al., 2019 (158)	N=26 (CKD2-5; 9 on APD) Age: 14 (range 7-17) years eGFR=30 (range 7-80) ml/min/1.73m^2	Complior	Increased PWV in 16 (61.5 %) FFTI/FTI ≥ 2.5, adjusted to age and sex, was significantly inversely

Continued table.

Author, year	Population (KRT, age, eGFR)	PWV measurement	Summary of principal findings
	Case-control study		correlated to PWV z-score (R2=0.317, p = 0.003) Multivariable model: serum albumin ≥ 3.8 mg/dl and FFTI/FTI ratio ≥ 2.5 were significantly associated with a lower risk for high PWV (when serum phosphorus added to the serum albumin ≥ 3.8 mg/dl lost its statistical significance) U shaped correlation of BMI SDS with PWV SDS (R^2=0.233, p = .04)
Makulska et al., 2019 (159)	N=76 (20 PD, 20 HD, 36 predialysis CKD) Age: 14.3±2.3 (PD); 15±3.3 (HD); 14.9±3.5 (predialysis CKD) years eGFR: NS *Case-control study*	SphygmoCor	PWV higher in HD (6.07±0.86 m/s), PD (5.51±0.68 m/s) and predialysis CKD (5.53±0.69 m/s) vs controls (4.85±0.62 m/s) PWV inversely correlated with fetuin A in the CKD and dialysis patients (r=-0.47; p<0.0001) and all study population (r=-0.39; p<.0001)
Hsu et al., 2018 (154)	N=86 (n=60 CKD1; n=26 CKD2-3) Age: 9.5 (5.2–13) years (CKD1); 13.7 (7.9–16.2) years (CKD2-3) eGFR: 113 (103–129) ml/min/1.73m^2 (CKD1); 79 (54–85) ml/min/1.73m^2 (CKD2-3) *Cross-sectional study*	Carotid echo-tracking (e-TRACKING)	Higher PWV in CKD2-3 vs CKD1: 4.1 (3.7–4.9) vs 3.8 (3.4–4.2) m/s Gut microbiota composition not associated with PWV
Karava et al., 2018 (157)	N=19 (HD; median 10.4 months duration) Age: 15.2 (range 9.0–19.2) years *Cross-sectional study*	SphygmoCor	Increased PWV in 5/19 (26.3 %) patients Interdialytic weight gain not associated with PWV
Conkar et al., 2018 (178)	N=81 (CKD2-4) Age: 13.21 ± 6.02 years eGFR: NS *Cross-sectional study*	Vicorder	PWV inversely correlated with vitamin D levels (r=-0.57; p<.001) and eGFR (r=-0.35; p=.01) in univariable analysis
Schmidt et al., 2017 (174)	N=166 (CKD; n=76 undergone KTx and n=90 started dialysis) Age at start of KRT: 13.9 ± 3.0 years (dialysis); 13.7 ± 2.9 years (KTx) *Longitudinal study (before and 6-18 months after start of KRT)*	Vicorder	In the adjusted analysis PWV SDS increased by 0.3 (CI = 0.06-0.55) in the dialysis group and decreased by 0.35 (CI = −0.09-(−0.6)) in transplantation group (p<.001)
Savant et al., 2017 (152)	N=95 (CKD1-4) Age: 15.1 years eGFR: 63 (42.9-78.5) ml/min/1.73m^2 *Cross-sectional study*	SphygmoCor	Mean PWV SDS −0.1 ± 1.1 Multivariable analysis: PWV associated with older age, higher MAP and black race

Continued table.

Author, year	Population (KRT, age, eGFR)	PWV measurement	Summary of principal findings
Tasdemir et al., 2016 (161)	N=25 (CKD2) Age: 11.8 ± 3.4 years eGFR: 80.6 ± 10.8 ml/min/1.73m^2 *Case-control study*	Vicorder	Higher PWV in CKD vs controls: 5.01 ± 0.75 vs 4.43 ± 1.01 PWV predicted by daytime systolic BP load on ABPM (R^2 = 0.262, p=.009)
Lin et al., 2016 (170)	N=55 (CKD1-3) Age: 8.6 (6.6–14) (CKD1); 14.9 (11.4–16.8) (CKD2-3) years *Cross-sectional study*	Carotid echo-tracking (e-TRACKING)	PWV inversely correlated with citrulline/arginine ratio (r=-0.38, p=.005) in univariable analysis
Sinha et al., 2015 (156)	N=226 (CKD1-5) Age: 11.9±3.7 years eGFR: 64±33.8 ml/min/1.73m^2 *Case-control study*	Vicorder	Similar PWV in normotensive controls, CKD with BP <75th percentile and CKD with BP ≥75th percentile: 5.34±0.82, 5.24±0.83, and 5.50±1.11 m/s
Degi et al., 2014 (175)	N=41 (after KTx) Age: 15.7±3.5 years eGFR: 93.2±25.5 ml/min/1.73m^2 Time on dialysis: 16 (0-60 months) Time since KTx: 39 (3-183) months *Cross-sectional study*	PulsePen	Mean PWV SDS 0.99±1.52 PWV similar in obese and non-obese: 1.1±1.29 vs 0.96±1.6
Shroff et al., 2014 (169)	N=82 (CKD2-5, dialysis, KTx) Age: 12.7±2.1 (CKD2); 11.3±3.3 (CKD3); 12.4±0.9 (CKD4-5); 14.2±3.9 (dialysis); 14.4±4.0 (transplant) years eGFR: 68±6.1 (CKD2); 41±10.7 (CKD3); 14±8.7 (CKD4-5); 47±14.2 (transplant) ml/min/1.73m^2 *Case-control study*	SphygmoCor	HDL from patients with CKD-induced endothelial NO release significantly correlated with PWV (R^2=0.25; P=.002) PWV not associated with circulating levels of HDL cholesterol or with endothelial SO or VCAM-1 production or cholesterol efflux
Alghamdi et al., 2013 (179)	N=24 (CKD2-5) Age: 13.9 (5.9–17.5) years eGFR: NS *Case-control study*	Echo-Doppler of aorta	PWV similar between CKD and controls: 358 (248–560) vs 344 (260–580) cm/s
Makulska et al., 2012 (160)	N=76 (n=20 HD, n=20 PD, n=36 predialysis) Age: 14.3±2.3 (PD); 15±3.3 (HD); 14.9±3.5 (predialysis) years eGFR: NS *Case-control study*	Sphygmocor	Higher PWV in all CKD groups vs controls (higher in HD than PD or predialysis): 6.07±0.86 vs 5.51±0.68 vs 5.53±0.69 vs 4.85±0.62 m/s
Tawadrous et al., 2012 (162)	N= 29 (n=15 dialysis, n=14 KTx) Age: 14.8±3.9 years (dialysis); 16±2.4 years (KTx) eGFR: NS *Case-control study*	Sphygmocor	Higher PWV in patients on dialysis compared to KTx, PWV similar in KTx vs controls: 9.7±3 vs 8.1±1.0 vs 7.7±0.8 m/s PWV not associated with dialysis vintage or time after KTx, P, PTH, Ca x P, vitamin D levels

Continued table.

Author, year	Population (KRT, age, eGFR)	PWV measurement	Summary of principal findings
Aoun et al., 2010 (173)	N=15 (patients on HD who underwent KTx) Age: 10.0±3.8 years (HD onset); 11.1±4.8 years (KTx) Dialysis vintage 12.9±7.4 months *Longitudinal study*	Sphygmocor	No change in PWV from HD to KTx Pre-transplant PWV correlated with systolic BP No correlation on HD of PWV with LVMI, PTH, URR, Hb Better graft function in patients with decrease of PWV post-KTx No correlation of CNI or steroid dose with PWV post-KTx No correlation of birth weight with PWV
Kis et al., 2009 (163)	N=28 (n=11 dialysis: 7 CAPD, 4 HD; n=17 KTx) Age: 13.8±4.3 (dialysis); 15.0±4.2 (KTx) Time on dialysis: 11 (3–78) months; 12 (0–36) (KTx) Time since KTx: 36 (1–166) months *Case-control study*	Pulsepen	PWV SDS higher in dialysis vs after KTx: 1.89±1.36 vs 0.93±0.92 In dialysis patients PWV correlated with Ca × P/fetuin-A (r=0.8, p=.005; significant in multivariable also) and bone alkaline phosphatase (r=0.6, p=.05) In KTx patients PWV correlated with cumulative dose of calcitriol (r=0.5, p=.04)
Shroff et al., 2008 (78)	N=34 (n=10 dialysis, n=24 predialysis) Age: NS eGFR: NS *Case-control study*	NS	PWV did not correlate with calcium load PWV increased in 2 patients with CAC
Dursun et al., 2009 (166)	N=70 (n=37 dialysis: 12 HD, 25 PD, 33 predialysis) Age: 14.6 ± 3.6 (dialysis); 10.8 ± 5.0 (predialysis) Duration of dialysis: 16 (3–140) months eGFR: *Case-control study*	Echo-doppler	PWV higher in dialysis vs predialysis; predialysis did not differ from control: 9.41 ± 3.01 m/s vs 7.69 ± 2.31 m/s vs 6.29 ± 1.39 m/s PWV higher in HD vs PD: 11.7 ± 3.7 m/s vs 8.3 ± 1.8 m/s CD144+ endotheliam microparticles and MBP were independent predictors of PWV (R2 = 0.460, p=.001 and p=0.03, respectively)
Cseprekal et al., 2008 (119)	N=25 (KTx) Age: 15.1 (13.5–16.7) years eGFR: 87.6 (75.35–99.86) ml/min/1.73m^2 Time on dialysis: 9 (0–60) months Time since KTx: 27.0 (0–165.6) months *Case-control study (healthy and ESRD)*	PulsePen	Increased PWV SDS in children after KTx vs controls: 1.01 (0.51–1.52) vs 0.07 (−0.168–0.31) In multivariable analysis: PWV only associated with cumulative calcitriol dose KTx patients with dialysis <1 year had lower PWV compared to ESRD

Continued table.

Author, year	Population (KRT, age, eGFR)	PWV measurement	Summary of principal findings
			KTx patients with eGFR <90 ml/min/1.73m^2 had higher PWV than those with >90 ml/min/1.73m^2
Shroff et al., 2008 (82)	N=61 (dialysis) Age: 13.4 ± 4.1 years eGFR: 8.9 ± 8.0 ml/min/1.73m^2 Time in CKD4: 4.0 ± 2.2 years Time on dialysis: 1.1 (0.25–8.7) years *Case-control study*	SphygmoCor	In multivariable analysis only fetuin A independently predicted PWV
Kis et al., 2008 (118)	N=11 (dialysis: 7 HD, 4 CAPD) Age: 14.3 ± 4.1 years *Case-control study*	PulsePen	Higher PWV in dialysis patients vs controls: 5.72 ± 0.94 m/s vs 4.56 ± 0.50 m/s Higher uremic burden score (high calcium, high phosphate, high PTH and time on dialysis >12 months) correlated with PWV
Shroff et al., 2007 (79)	N=85 (dialysis: 64 PD, 21 HD) Age: 5–18 years eGFR: NS *Case-control study*	Bracioradial PWV and aortic PWV	Higher brachioradial PWV in dialysis vs controls: 8.89 ± 1.9 m/s vs 5.1 ± 1.0 m/s Higher PWV in patients with iPTH >2 times ULN: 8.63 ± 2.3 m/s vs 5.81 ± 1.2 m/s PWV correlated with phosphate (r=0.39, p=.03), Ca x P product (r=0.37, p=.018), vitamin D dosage (r=0.17, p=.03)
Covic et al., 2006 (171)	N=14 (HD) Age: 14.1±2.6 years *Case-control study*	Sphygmocor	Higher PWV in HD vs controls: 6.6±1.0 m/s vs 5.4±0.6 m/s In the multivariable analysis only serum phosphate associated with PWV LVMI not related to PWV

Abbreviations: BMI, body mass index; BP, blood pressure; CAPD, continuous ambulatory peritoneal dialysis; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FFTI, fat-free tissue index; FTI, fat tissue index; HD, hemodialysis; HDF, hemodiafiltration; IDWG, interdialytic weight gain; KTx, kidney transplantation; LVMI, left ventricular mass index; MAP, mean arterial pressure; NO, nitric oxide; NS, not stated; PD, peritoneal dialysis; PTH, parathyroid hormone; PWV, pulse wave velocity; SDS, standard deviation score; ULN, upper limit of normal.

3. METHODS

3.1. Study design and setting

All of the analyses of the present thesis were performed using the data from the *Cardiovascular Comorbidity in Children with Chronic Kidney Disease* (4C) Study (ClinicalTrials.gov Identifier: NCT01046448).

The 4C Study is a multicenter, prospective, observational cohort study investigating the natural course of CVD in children with CKD initiated by Heidelberg University (Heidelberg, Germany; Principal investigator: prof. Franz Schaefer).

The 4C Study prospectively enrolled patients in 55 pediatric nephrology centers across 12 European countries (Austria, Czech Republic, France, Germany, Italy, Lithuania, Poland, Portugal, Serbia, Switzerland, Turkey, United Kingdom) during 2009 and 2010 (full list of participating centers and principal investigators is presented as supplementary material). The patients were followed until dropout (e.g. due to loss-to-follow up, death, transfer to adult centers, etc.) or until the end of the study period in 2019.

The patients were included into the study by local investigators according to the following enrollment criteria:

Inclusion criteria:

- Children aged 6-17 years
- Estimated GFR between 10 and 60 ml/min/1.73 m^2
- Not on KRT

Exclusion criteria:

- Active systemic vasculitis
- Renal vascular anomalies
- Coexisting primary CV anomalies
- Anomalies of the limbs preventing diagnostic procedures

3.1.1. Study organization

The study was coordinated by eight centrally-trained regional coordinators. The doctoral student was a regional coordinator for the region that included participating centers in Italy, France, Austria, Germany and Lithuania since 2014. The responsibilities of the doctoral student included overall

organizational issues of the study process and performing instrumental examinations in patients from the coordinated region. Investigators of the study held biannual study meetings that included the presentation of interim results of the study, along with the discussions of problems encountered during the course of the study.

Study participants were followed in their respective centers with six-monthly assessments that included clinical examinations and data collection, along with the collection of blood and urine samples for centralized analysis. At 12-monthly visits patients additionally underwent comprehensive CV investigation, that also included measurements of PWV and cardiac ultrasound performed by same centrally trained regional coordinators according to predefined standard operating procedures (described below).

3.1.2. Sample selection for the present analysis

The present analysis included patients from the 4C Study with at least one PWV measurement while not on KRT and with eGFR >60 ml/min/1.73 m^2. If the inclusion criteria were met at a visit that occurred later than the baseline visit of the 4C Study, the visit meeting eligibility criteria was set as the new baseline. In cases when PWV measurements were performed not during a regular study visit (that includes clinical data and laboratory samples collections) the closest visit within 90 days of the measurement was assigned for PWV measurement.

3.1.3. Primary and secondary outcomes

The primary outcome of this study was:

- PWV standard deviation score standardized to height (PWV SDS_{height}) (time-fixed at baseline and time-varying)

Secondary outcomes included:

- Abnormal PWV SDS_{height}
- LV geometry
- CKD progression

3.1.4. Ethical statements

The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany; study approval No. S-032/2009 on 2009-05-26) and local institutional review boards of each participating center. Study protocol corresponds to the declaration of Helsinki and all patients and/or their legal representatives, as appropriate, gave written informed consent for the participation in the study.

3.2. Collected data

The following data that was collected at different time points of the study was used for the present analysis:

At baseline only:

- Date of birth;
- Sex;
- Primary kidney disease (classification described below);
- Ethnicity;
- Gestational age and birth weight;
- 24,25(OH)-vitamin D and 25(OH)-vitamin D levels;

6-monthly visits (including first visit):

- Initiation of KRT and modality;
- Anthropometric data (height and weight);
- BP;
- Laboratory data: serum creatinine, serum calcium, serum phosphate, serum albumin, serum uric acid, total cholesterol, triglycerides, HDL and LDL cholesterol, hemoglobin, serum ferritin, serum PTH, high-sensitivity CRP and urinary albumin and creatinine levels;

12-monthly visits (including first visit):

- Echocardiography (described below)
- PWV measurements (described below)

All information required for the study was entered in the study website (www.4c-study.org) with an authorized password-restricted investigator area that allowed to enter patient-level data.

3.2.1. Data sources and measurements

Anthropometric data

Height and weight were measured by local investigators using calibrated scales and meters.

BP measurements

Office systolic and diastolic BP were measured by local investigators using locally available validated and calibrated oscillometric devices with appropriately sized brachial cuffs. Mean value of three consecutive BP measurements taken at one minute intervals after a period of rest in a sitting position was used for the analysis.

PWV measurements

Carotid-femoral PWV was measured using the Vicorder oscillometric device (Skidmore Medical, Germany) which uses two cuffs to capture PW in the carotid and femoral arteries.

Carotid-femoral PWV measurement procedure:

- The patients were investigated in a quiet room, avoiding any outside disturbances.
- A period of 15 minutes of rest was required before the measurements were performed.
- Measurements were performed in a supine position with head and shoulders tilted by approximately 30^{o}.
- Neck cuff was placed on the neck with the neck pad over the right carotid artery and tightened collar-tight.
- Thigh cuff was placed on patient's upper right thigh and tightened. Removing pants or trousers was not required, unless proper tightening of the cuff was impossible.
- No talking or moving was allowed during the measurements.
- Three pathway measurements were taken: SSN to umbilicus (SSN-Umb), umbilicus to mid-thigh cuff (Umb-Fem) and SSN to mid-neck cuff (SSN-Car).
- Travel distance was calculated as (SSN-Umb + Umb-Fem) – SSN-Car. BP measurements were taken on the right arm using a locally available calibrated and validated oscillometric BP device.

- Three measurements each capturing at least 10 beats with good quality waves in the screen were performed. The mean value of these three measurements was used for further analysis.

Echocardiography

Transthoracic echocardiography was performed according to the recommendations for pediatric echocardiogram by the American Society of Echocardiography. (180)

LV dimensions (interventricular septum [IVS] and LV posterior wall [LVPW] thickness and LV internal diameter [LVID]) were measured in the long parasternal axis using M-mode images in end-diastole, at the height of papillary muscles.

Echocardiography was performed using the Acuson P50 (Siemens Healthcare) device. Digitally stored DICOM images were then analyzed using Syngo (Syngo US Workplace, Siemens Medical Solutions, USA Inc) as digital image analysis software.

Laboratory assessments

Laboratory samples were collected at the time of the visit and were frozen at -80^{o}C immediately for further storage.

Serum creatinine, calcium, serum albumin, serum uric acid, total cholesterol, triglycerides, HDL and LDL cholesterol, high-sensitivity C-reactive protein (hsCRP), 25(OH)-vitamin D, 24,25(OH)-vitamin D levels and urinary albumin and creatinine were measured centrally in a validated laboratory. Serum creatinine was measured using an isotope dilution-mass spectroscopy (ID/MS) traceable enzymatic method.

Hemoglobin, PTH, serum bicarbonate and ferritin were measured in local laboratories of the respective centers and results were entered into the system by local investigators.

3.2.2.Data definitions and transformations

Primary kidney diseases were classified as CAKUT, glomerulopathies, CKD post-acute kidney injury (AKI), tubulointerstitial diseases and other diseases as follows:

- CAKUT: Vesico-ureteral reflux; Bladder anomalies; Urethral anomalies; Reflux nephropathy. Renal hypo/dysplasia; Renal dystopia; Renal aplasia; Renal hypoplasia; Renal dysplasia; Multicystic dysplasia; Segmental

dysplasia; Oligomeganephronia; Renal duplication; Ureter of upper renal pelvis inserting caudally; Pyelo-ureteral junction stenosis; Ureter fissus or duplex; Megaureter; Ectopic ureter; Ureterocele; Other; Syndromal malformation; Autosomal dominant polycystic kidney disease; Autosomal recessive polycystic kidney disease.

- Glomerulopathies: IgA nephropathy; Wegener's glomerulonephritis; Henoch-Schoenlein purpura associated glomerulonephritis; Lupus nephritis; Congenital nephrotic syndrome; Infantile nephrotic syndrome; Syndromal nephrotic syndrome; Minimal change glomerulopathy; Focal segmental glomerulosclerosis; Membranous glomerulopathy; Mesangioproliferative nephropathy; Membranoproliferative glomerulonephritis; Rapidly progressing glomerulonephritis; Postinfectious glomerulonephritis; Alport syndrome.
- CKD post-AKI: Post-ischemic CKD; Hemolytic uremic syndrome.
- Tubulointerstitial: Tubulopathy; Interstitial nephropathy; Cystinosis; Oxalosis; Nephrocalcinosis; Nephronophtisis; Metabolic nephropathies.
- Other: Diabetic glomerulopathy; Renovascular disease; Other; Unknown.

Patients were considered to be born small-for-gestational age (SGA) if their birth weight was below 10th percentile.

Height was standardized to calculating age- and sex-specific height SDS by using World Health Organization (WHO) growth reference data. (181) Height SDS values for participants aged 20 years or older (oldest age for which reference data is available) were calculated using sex-specific LMS values for 19-years old individuals.

Short stature was defined as height SDS below the third percentile (below -1.88 SDS).

Body mass index (BMI) was calculated using the following equation: $BMI = \frac{Weight\ (kg)}{Height\ (m)^2}$. BMI values for participants aged 6-19 years were further age and sex standardized to calculating SDS by using WHO growth reference data. (181) Height-age (age corresponding to the 50th percentile height value identical to patient's height) was used to calculate BMI SDS. BMI SDS values for participants aged 20 years or older (oldest age for which reference data is available) were calculated using sex-specific LMS values for 19-years old individuals.

Body composition: obesity, overweight and underweight were defined as BMI SDS >2, >1 and <-2, respectively, according to the definitions of WHO. (181)

Systolic and diastolic BP was standardized to calculating age-, sex- and height SDS-specific SDS using regression equations provided in the "Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents". (182) If participants were older than 17 years (oldest age for which reference data is available), age of 17 years was used in the regression equation. This decision was made based on an exploratory analysis of the study sample (data not shown) that has revealed inappropriately exponentiated systolic and/or diastolic BP SDS values with large sex-disparities by using actual ages for participants older than 17 years old.

Arterial hypertension by office BP measurements was defined according to the 2016 ESH guidelines for the management of high blood pressure in children and adolescents as office systolic and/or diastolic BP values over 95th percentile (>1.645 SDS). (11)

Estimated GFR was calculated using the updated Schwartz equation $eGFR = \frac{K \times height\ (cm)}{Serum\ creatinine\ (\frac{mg}{dL})}$ (where K=0.413). (183) CKD stages were determined according to the KDIGO 'Clinical Practice Guidelines or the Evaluation and Management of Chronic Kidney Disease' guidelines (Table 4).

Table 4. CKD staging by eGFR according to KDIGO guidelines

CKD stage	eGFR (ml/min./1.73 m^2)
1	≥90
2	60-89
3	30-59
4	15-29
5	<15

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Proteinuria was defined as urinary albumin-to-creatinine ratio (uACR) >30 mg/g and nephrotic range proteinuria was defined as uACR >2200 mg/g.

Serum calcium was corrected for serum albumin levels by using the standard correction equation: $Corrected\ serum\ calcium = 0.8 \times (normal\ serum\ albumin - observed\ albumin) + serum\ calcium$

CKD progression was defined as time to a composite event of 50% reduction in eGFR, eGFR < 10 ml/min/1.73 m2 or start of KRT, whichever occurred first. If 50% reduction in eGFR occurred between two study visits, simple linear interpolation was used to determine the time point of the event.

LV mass (LVM) was calculated by using data from 2D echocardiography (diastolic IVS and LVPW thickness, and LVID) and the Devereux equation. (184) LVMI was calculated by a recently introduced equation that allows for age independent LVMI interpretation: $LVMI = \frac{LVM}{height^{2.16}+0.09}$. Left ventricular hypertrophy (LVH) was defined using a single cut-off value of LVMI > 45 g/m$^{2.16}$ irrespective of age. (185)

Relative wall thickness (RWT) was calculated based on diastolic dimensions of IVS, LVPW and LVID, using the following equation: $RWT = \frac{IVS+LVPW}{LVID}$. Additionally, age-normalized RWT was calculated as proposed by de Simone et al. using the following equation: $normalized\ RWT = RWT - 0.005 \times (age - 10)$ and values above 95th percentile (0.38) were considered indicative of concentric LV remodeling. (186)

LV geometry was classified based on LVMI and RWT as outlined in Table 5.

Table 5. Classification of LV geometry according to LVMI and RWT

LV geometry	LVMI	RWT
Normal	≤45	≤0.38
Concentric remodeling	≤45	>0.38
Concentric LVH	>45	>0.38
Eccentric LVH	>45	≤0.38

Abbreviations: LV, left ventricle; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; RWT, relative wall thickness.

PWV values were standardized to calculating height-/age- and sex-specific SDS values using previously published reference data from European children aged 6-18 years using the Vicorder device. (35) PWV SDS values above 95th

percentile (>1.645 SDS) were considered abnormal. In cases when height of study participants was outside the range of published reference data (>190 cm for boys or >180 cm for girls), linear extrapolation was used for the respective LMS values, to calculate SDS values.

All laboratory values that were below the limit of detection (LoD) for respective laboratory tests were set to $\sqrt{LoD}$.

3.3. Statistical analysis

All continuous data was checked for normality by comparing means and medians, visually inspecting histograms and Q-Q plots and assessing skewness and kurtosis parameters. Continuous data is presented as means ± SD (normal distribution) or medians (interquartile range, IQR; non-normal distribution). Non-normally distributed variables were log-transformed for further analysis. Categorical data is presented as frequencies. All data was checked for outliers by investigating extreme values (below and above 0.005 and 0.995 quintiles, respectively), evaluating longitudinal patient-specific trends in the variable of interest and assessing clinical plausibility.

SDS for height, BMI and PWV were calculated using the LMS method (187) and published appropriate reference data (L, M, S values) using the following equation: $SDS = \frac{\frac{Y}{M^L} - 1}{L \times S}$, where Y is the actual measured value and L, M, and S are specific to the published reference values. (35,181) SDS values for systolic and diastolic BP were calculated using previously published regression equations. (182)

Continuous variables between two independent groups were compared by parametric (two-sample Student t test) or non-parametric (Mann-Whitney U test) tests, as appropriate. Continuous variables between more than two independent groups were compared using analysis of variance (ANOVA) or Kruskal-Wallis tests, as appropriate. Chi-squared test was used to compare proportions between two or more groups. Respective post-hoc tests were further applied for group comparisons involving more than two groups for paired group comparisons (Tukey or Dwass, Steel, Critchlow-Fligner tests).

Correlation between two continuous variables was assessed by Pierson or Spearman correlation coefficients for normally and non-normally distributed variables, respectively. Partial correlation coefficients were calculated to assess correlation between two variables adjusted for other covariates.

Univariable linear and logistic regressions models were built to assess the association of covariates at baseline with continuous (PWV SDS_{height}, RWT

and LVMI at baseline) and categorical (abnormal PWV, concentric remodeling, LVH) variables, respectively. Variables with a p value <.20 in the univariable linear regression were further selected for multivariable model building. Multivariable linear mixed effects models with a random center effect were built to determine covariates associated with PWV SDS_{height} at baseline. Further, a generalized multivariable linear model using stepwise forward-backward selection with an entry level of p<.20 and stay level of p<.10 and model selection based on the Akaike Information Criteria (AIC) was built. Additionally, a sensitivity analysis excluding variables with an amount of missing observations that significantly reduce sample size were built to test for potential confounding effect of excluding patient observations, where required.

Longitudinal changes of PWV SDS_{height} over time were fitted using a generalized additive mixed effects model for PWV SDS_{height} with a penalized spline fixed effect for time (years since baseline PWV recording) and patient individual random intercept and slopes. Multivariable linear longitudinal mixed-effects models with patient-individual random intercepts and slopes were fitted to test the associations of each covariate with outcomes of interest (PWV SDS_{height} and absolute PWV). Such modelling allows to partially avoid bias from informative censoring of patients due to the start of KRT (which could be associated with faster eGFR decline). Additionally, a separate model that also included a two-way interaction of covariates with time to test whether the observed effects of covariates over time were stable or changing. Multiple imputation with a MICE (Multiple Imputation by Chained Equations) algorithm was used to impute missing covariate data using appropriate linear mixed effects models and all available covariates as predictors to minimize bias. Model-based inference was conducted using Rubin's Rule to combine the results obtained from different imputed data sets. The follow-up for the longitudinal model with PWV SDS_{height} as the outcome was censored at the age of 17 years due to the lack of reference data for SDS calculation afterwards. Therefore, to avoid information loss and to test model stability, models with absolute PWV values (adjusted for absolute height but not height SDS to account for growth) were built additionally.

Two Cox proportional hazard models (with and without PWV SDS_{height} at baseline) were applied to assess factors (baseline eGFR, age, sex, primary kidney disease, BMI SDS, baseline systolic BP SDS, and baseline log-transformed uACR) associated with the composite endpoint of CKD progression. Kaplan-Meier survival curves stratified by tertiles or the presence of abnormal PWV SDS_{height} were constructed to illustrate and log-rank test

was used to compare CKD progression-free survival between different PWV SDS_{height} strata.

Two-sided P value of <.05 was considered significant. Statistical analysis was performed using SAS Software Version 9.4 (SAS Inc., Cary, NC) or R version 3.2.2.

Data validation

Data was validated by using a standardized data validation plan. Queries were generated each year for missing data or data that falls outside the predefined plausibility ranges (including changes over time, e.g. inappropriate increase or a decrease in height) and sent out to local investigators and regional coordinators for plausibility confirmation and corrections.

4. RESULTS

4.1. Baseline characteristics of study participants

667 patients with a total of 2295 visits out of the 704 patients who were enrolled into the 4C Study satisfied eligibility criteria as described in the methods section and were included into the analysis. Patient selection flowchart is shown in Figure 5.

The population was slightly male predominant (65.5%) and the mean age at baseline was 12.2 ± 3.35 years. Majority of patients were Caucasian (90.8%) and 18.1% were SGA. Majority of children had normal BMI and 8.1%, 18.3% and 5.1% were underweight, overweight and obese, respectively. Mean height SDS was -1.34 ± 1.35 and almost one third (30.3%) of study participants had short stature.

The predominant primary kidney disease was CAKUT (69.6%), followed by tubulointerstitial diseases (12.3%), glomerulopathies (8.6%), CKD post-AKI (5.3%), and other disorders (4.4%). Mean eGFR at baseline was 26.9 ± 11.6 ml/min/1.73 m^2 and 36.7%, 47.8% and 15.4% were in CKD stages 3, 4 and 5, respectively. Majority of children were proteinuric (87.4%) and 13.2% had nephrotic-range proteinuria. Mean systolic and diastolic BP SDS of the study population was 0.83 ± 1.35 and 0.69 ± 1.08, respectively, and 28.2% were hypertensive by office BP measurements.

All clinical and laboratory characteristics of the study population along with the number of patients with available data for each variable are summarized in Table 6.

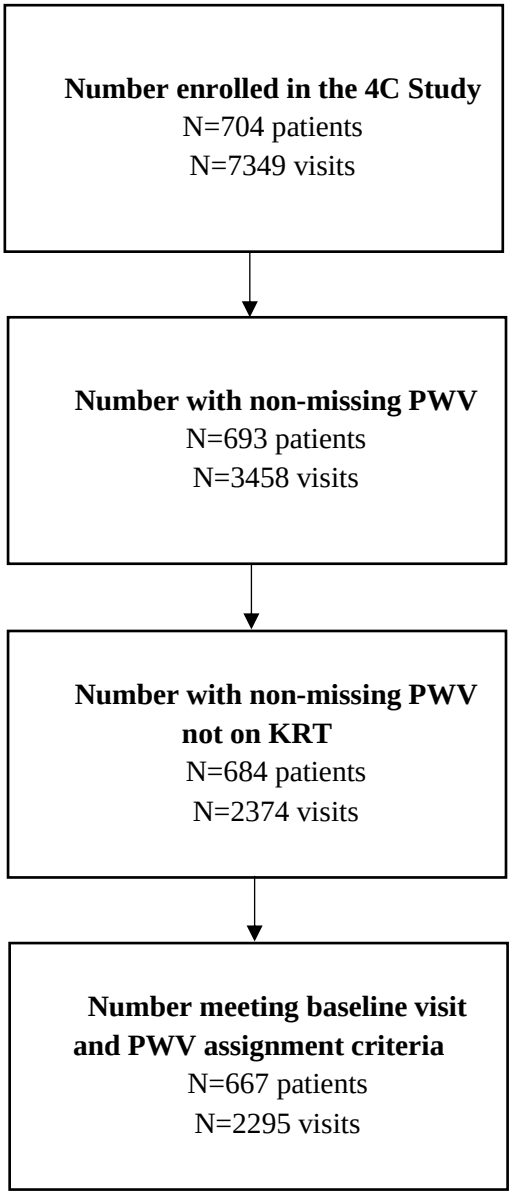


Figure 5. Patient selection flowchart

Table 6. Baseline characteristics of the overall study population and stratified by CKD stages

	All patients (n=667)	N Obs	CKD3 (n=245)	CKD4 (n=319)	CKD5 (n=103)	p value
Age, years	12.2 ± 3.35	667	12.2 ± 3.22	12 ± 3.46	12.7 ± 3.31	.16
Boys, %	437 (65.5)	667	152 (62)	219 (68.7)	66 (64.1)	.25
Ethnicity, %		667				
Caucasian	599 (90.8)		222 (90.6)	284 (89)	93 (90.3)	.81
Non-Caucasian	68 (10.2)		23 (9.4)	35 (11)	10 (9.7)	
Small for gestational age, %	104 (18.1)	574	38 (17.9)	52 (18.9)	14 (16.1)	.83
BMI, kg/m²	18.4 ± 3.84	665	18.6 ± 3.96	18.2 ± 3.74	18.6 ± 3.9	.40
BMI SDS	0.12 ± 1.27	643	0.08 ± 1.27	0.12 ± 1.3	0.18 ± 1.2	.79
Underweight, %	54 (8.1)	643	18 (7.4)	31 (9.7)	5 (4.9)	.25
Overweight, %	122 (18.3)	643	49 (20)	51 (16)	22 (21.4)	.32
Obese, %	34 (5.1)	643	13 (5.3)	16 (5)	5 (4.9)	.98
Height, cm	141 ± 20.1	667	143 ± 19.2	139 ± 20.9	142 ± 19.3	.11
Height SDS	-1.34 ± 1.35	667	-1.07 ± 1.24 [a,b]	-1.45 ± 1.41	-1.6 ± 1.34	.0004
Short stature, %	202 (30.3)	667	53 (21.6)	110 (34.5)	39 (37.9)	.0008
Weight, kg	38.2 ± 15.8	667	39.5 ± 16	37.1 ± 16.2	38.6 ± 14.1	.19
Diagnosis, %		667				
CAKUT	464 (69.6)		169 (69)	231 (72.4)	64 (62.1)	.04
Glomerulopathies	57 (8.6)		18 (7.4)	25 (7.8)	14 (13.6)	
CKD post-AKI	35 (5.3)		19 (7.8)	12 (3.8)	4 (3.9)	
Tubulointerstitial	82 (12.3)		24 (9.8)	43 (13.5)	15 (14.6)	
Other	29 (4.4)		15 (6.1)	8 (2.5)	6 (5.8)	
Systolic BP, mmHg	112 ± 14.9	667	113 ± 14.6	111 ± 15[b]	117 ± 14	.0018
Systolic BP SDS	0.83 ± 1.35	667	0.86 ± 1.33	0.7 ± 1.38[b]	1.14 ± 1.29	.014
Diastolic BP, mmHg	69.1 ± 12.3	667	70.1 ± 11.9[a]	67.4 ± 12.5[b]	72.2 ± 12.2	.0007
Diastolic BP SDS	0.69 ± 1.08	667	0.76 ± 1.05	0.55 ± 1.09[b]	0.93 ± 1.09	.0039
Hypertension by office BP, %	188 (28.2)		70 (28.6)	79 (24.8)	39 (37.9)	.04
HR, bpm	81.9 ± 13.5	657	81.2 ± 13	82.4 ± 13.2	82.3 ± 15.5	.53
eGFR, ml/min/1.73 m²	26.9 ± 11.6	667	39.3 ± 7.59[a,b]	22.2 ± 4.53[b]	12 ± 2.14	<.0001
uACR, mg/g	327 (91.1 – 1231)	660	177 (55.4 – 551)[a,b]	436 (106 – 1514)[b]	975 (336 – 2286)	<.0001
Proteinuric, %	583 (87.4)		202 (82.5)	284 (89.0)	97 (94.2)	.005
Nephrotic range proteinuria, %	88 (13.2)		16 (6.53)	46 (14.4)	26 (25.2)	<.0001
Hemoglobin, g/dL	11.7 ± 1.63	650	12.1 ± 1.56[a,b]	11.5 ± 1.52[b]	11.1 ± 1.82	<.0001
Serum bicarbonate, mmol/L	21.2 ± 3.57	646	21.7 ± 3.26[b]	21.2 ± 3.54	20.3 ± 4.13	.004
Ferritin, µg/l	67.5 (33 – 142)	610	49.7 (25.2 – 107)[a,b]	73.3 (38 – 153)	92.9 (54 – 176)	<.0001
PTH, pmol/L	13.3 (7.53 – 24.1)	645	9.54 (5.83 – 15.2)[a,b]	15.8 (9.06 – 26.4)	20.6 (10.1 – 35.4)	<.0001
CRP, mg/L	0.55 (0.23 – 2.06)	667	0.52 (0.22 – 1.94)	0.55 (0.22 – 2.01)	0.69 (0.27 – 3.47)	.27
Cholesterol, mg/dL	180 ± 51	665	179 ± 52.8	182 ± 48.8	180 ± 53.8	.75

Continued table.

	All patients (n=667)	N Obs	CKD3 (n=245)	CKD4 (n=319)	CKD5 (n=103)	p value
Triglycerides, mg/dL	139 ± 76.4	266	128 ± 72.1	144 ± 71	149 ± 95.7	.22
HDL cholesterol, mg/dL	47.9 ± 14.3	667	49.3 ± 13.6	47.2 ± 14	46.7 ± 16.8	.15
LDL cholesterol, mg/dL	98.5 ± 40.1	663	95.8 ± 38.4	101 ± 40.7	97 ± 42.1	.29
Uric acid, mg/dL	6.51 ± 1.81	665	6.36 ± 1.77	6.61 ± 1.88	6.53 ± 1.65	.26
Serum calcium, mmol/L	2.26 ± 0.18	666	2.27 ± 0.16[b]	2.26 ± 0.17[b]	2.21 ± 0.26	.02
Corrected serum calcium, mmol/L	2.28 ± 0.18	666	2.29 ± 0.18	2.28 ± 0.17	2.25 ± 0.21	.09
Serum phosphate, mmol/L	1.56 ± 0.37	667	1.46 ± 0.34[a,b]	1.56 ± 0.33[b]	1.78 ± 0.48	<.0001
Serum albumin, g/L	38.9 ± 5.68	666	38.8 ± 5.34	39.1 ± 5.71	38.6 ± 6.36	.70
24,25(OH)-vitamin D, ng/mL	0.25 (0.16 – 0.38)	526	0.25 (0.17 – 0.39)	0.26 (0.16 – 0.38)	0.22 (0.12 – 0.33)	.13
25(OH)-vitamin D, ng/mL	10.7 (6.29 – 17.4)	526	12.4 (7.19 – 18.2)	10.3 (5.74 – 16.3)	10.5 (6.31 – 21.5)	.15
Log-uACR	5.70 ± 1.81	659	5.07 ± 1.80[a,b]	5.90 ± 1.76[b]	6.59 ± 1.44	<.0001
Log-CRP	-0.25 ± 1.60	667	-0.30 ± 1.61	-0.30 ± 1.55	0.02 ± 1.74	.17
Log-ferritin	4.24 ± 1.05	610	3.99 ± 1.02[a,b]	4.32 ± 1.06	4.57 ± 0.93	<.0001
Log-PTH	2.58 ± 1.03	645	2.23 ± 0.84[a,b]	2.73 ± 1.06	2.93 ± 1.12	<.0001
Log-24,25(OH)-vitamin D	-1.43 ± 0.83	526	-1.38 ± 0.73	-1.41 ± 0.88	-1.60 ± 0.88	.10
Log-25(OH) vitamin-D	2.30 ± 0.89	526	2.36 ± 0.84	2.23 ± 0.90	2.36 ± 0.99	.29

Abbreviations: AKI, acute kidney injury; BP, blood pressure; BMI, body mass index; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-creatinine ratio.

a) p<.05 compared to CKD4

b) p<.05 compared to CKD5

Baseline characteristics by CKD stage

When stratified by CKD stage (Table 6) height SDS was significantly lower in patients with CKD4 and CKD5 compared to CKD3. Similarly, the proportion of patients with short stature increased with increasing CKD stage. Systolic and diastolic BP, as well as the prevalence of hypertension by office BP measurements, was significantly higher in CKD5 compared to CKD4.

Higher CKD stages were also associated with higher uACR (and proportion of proteinuric children), lower hemoglobin, lower serum bicarbonate, higher serum ferritin, higher LDL cholesterol levels, lower serum calcium, and higher serum phosphate.

Baseline characteristics by sex

When compared by sex (Suppl. Table 1), girls were on average shorter, had higher prevalence of glomerular diseases and lower prevalence of CAKUT, had higher HR, lower hemoglobin, lower serum calcium and lower serum 25(OH)-vitamin D levels compared to boys.

Baseline characteristics by diagnosis group

When comparing patients with CAKUT and the remaining primary kidney diagnoses (Suppl. Table 2), patients with CAKUT were more predominantly male, had higher hemoglobin levels, lower PTH and ferritin levels, as well as lower total, LDL cholesterol and triglyceride levels, and higher serum albumin and 24,25(OH)-vitamin D levels.

4.2. PWV and associated factors at baseline

4.2.1. PWV characteristics at baseline

Mean PWV at baseline was 4.9 ± 0.83 m/s and mean PWV SDS_{height} was 0.31 ± 1.65 (Table 7). Overall, 128 (19.2%) patients had abnormal PWV (>95th percentile for height and sex) at baseline.

Table 7. Description of PWV at baseline

	Mean	**SD**	**Median**	**Min**	**Max**	**Q1**	**Q3**
PWV, m/s	4.9	0.83	4.84	3.04	8.68	4.32	5.36
PWV SDS_{height}	0.31	1.65	0.25	-4.89	8.09	-0.75	1.37
PWV SDS_{age}	0.09	1.51	0.09	-4.31	6.76	-0.94	1.09

PWV SDS_{height} showed a strong direct correlation with PWV SDS_{age} (r=0.921, p<.0001; Fig. 6).

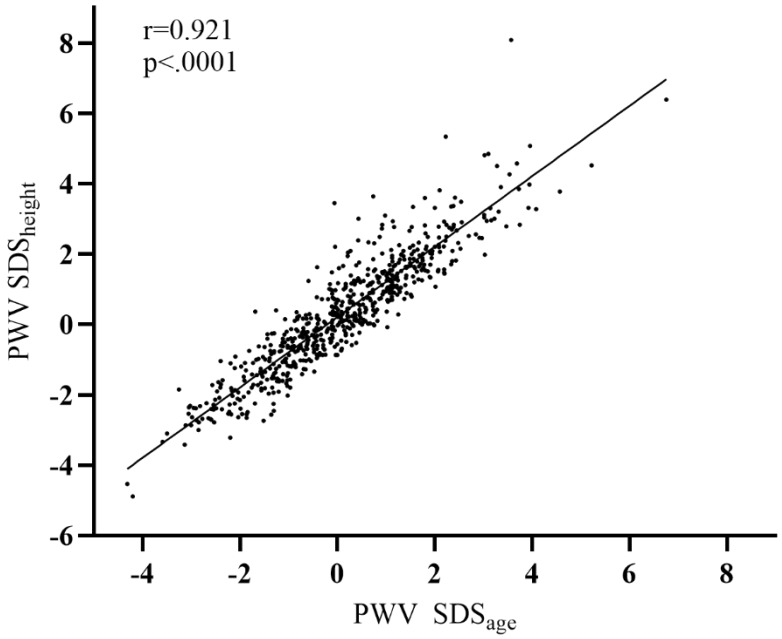


Figure 6. Correlation between PWV SDS_{height} and PWV SDS_{age} at baseline

PWV SDS_{height} was on average 0.21±0.66 higher than PWV SDS_{age} and higher proportion of patients were classified as having abnormal PWV by using height standardized (n=128, 19.2%) vs age standardized (n=95, 14.2%) values (Table 8). 50 and 17 patients were reclassified as having an abnormally elevated PWV when using height- and age-standardized SDS, respectively (Table 8).

Table 8. Distribution of abnormal PWV SDS values by standardization to height or age

PWV SDS_{age}	**PWV SDS_{height}**		
	Normal	**Abnormal**	**Total**
Normal	522	50	572 (85.8%)
Abnormal	17	78	95 (14.2%)
Total	539 (80.8%)	128 (19.2%)	p<.0001

Age was inversely correlated with PWV SDS_{height} (r=-0.131, p=.001) and PWV SDS_{age} (r=-0.093, p=.02) while height SDS was inversely correlated with PWV SDS_{height} (r=-0.258, p<.0001) but not PWV SDS_{age} (r=0.026, p=.50).

Difference between PWV SDS_{height} and PWV SDS_{age} was inversely correlated with age (r=-0.140, p=.0003; Fig. 7) and height SDS (r=-0.771, p<.0001; Fig. 8) but did not differ between boys and girls (0.21±-0.72 vs 0.21±0.52, p=.97).

When comparing absolute PWV values between patients with short and normal stature according to the absolute height of the patient, patients with short stature tended to have higher PWV values (Fig. 9). Absolute PWV values were also higher in short-for-age children when stratifying study population into height quintiles. This significant difference was observed only in the lower height range (highest quintile was excluded from calculations because only three children were short-for-age in that group; Table 9).

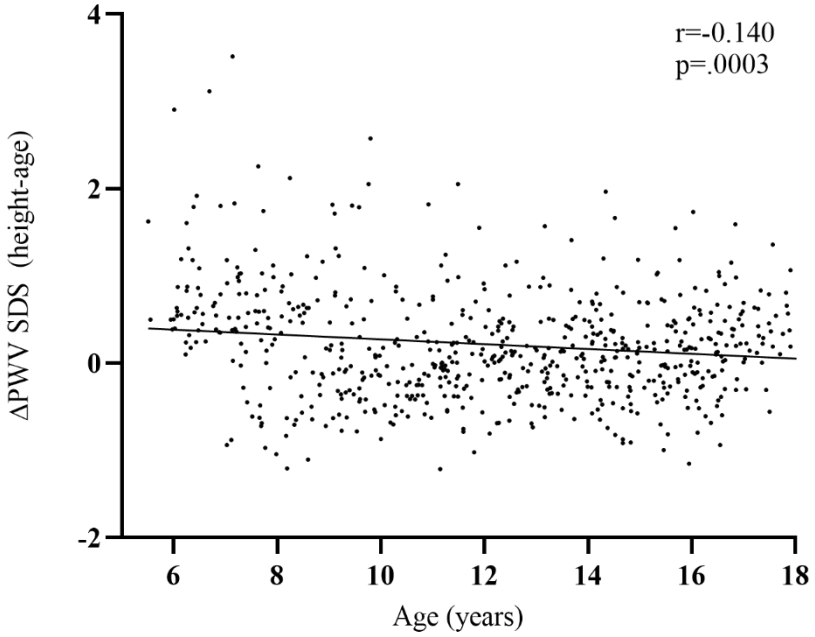


Figure 7. Correlation between PWV SDS difference (height – age) with age

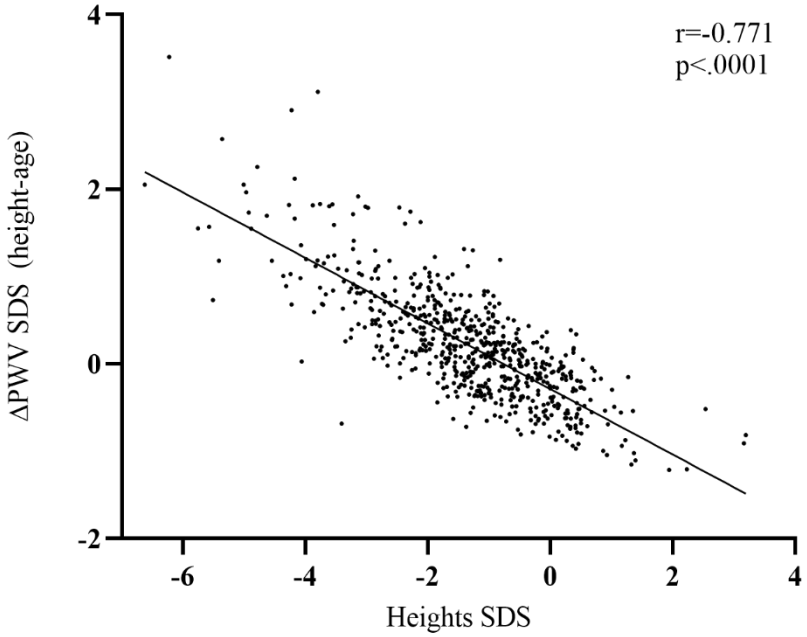


Figure 8. Correlation between PWV SDS difference (height – age) with height SDS

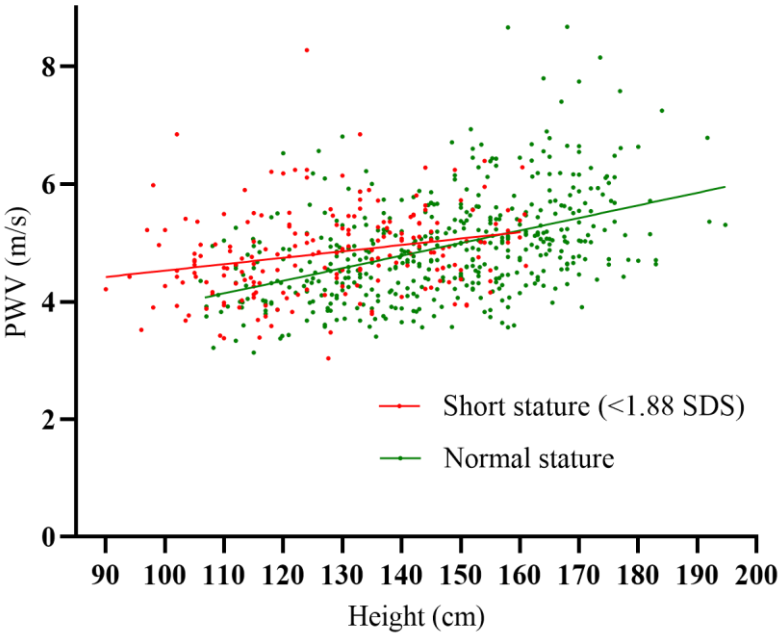


Figure 9. Correlation between absolute PWV and absolute height value stratified by short or normal stature

Table 9. Comparison of absolute PWV values between patients with normal and short stature stratified by height quintiles

Height (cm)	Normal height	Short stature	p value
≤122	4.31±0.67 (n=50)	4.59±0.70 (n=85)	.02
122-135	4.61±0.69 (n=90)	4.98±0.94 (n=44)	.03
135-148	4.69±0.64 (n=88)	5.01±0.46 (n=46)	.001
148-160	5.04±0.85 (n=111)	4.99±0.70 (n=24)	.77

4.2.2. Associations of PWV with clinical and laboratory characteristics at baseline

Kidney function

Mean values of PWV SDS_{height} (p=.26; Fig. 10) and the frequencies of abnormal PWV did not differ by CKD stage (p=.15; Table 10). PWV SDS_{height} also did not correlate with eGFR (r=-0.032, p=.42; Fig. 11) and eGFR was not associated with increased odds of having abnormal PWV (OR 0.992, 95% CI 0.976 to 1.009, p=.38).

Table 10. Distribution of abnormal PWV by CKD stage

	CKD3 (n=245)	CKD4 (n=319)	CKD5 (n=103)	p value
Increased PWV SDS_{height}, %	39 (15.9)	71 (22.3)	85 (17.5)	.15

When compared by primary kidney diagnosis groups, no differences in PWV SDS_{height} were observed (p=.74; Fig. 12).

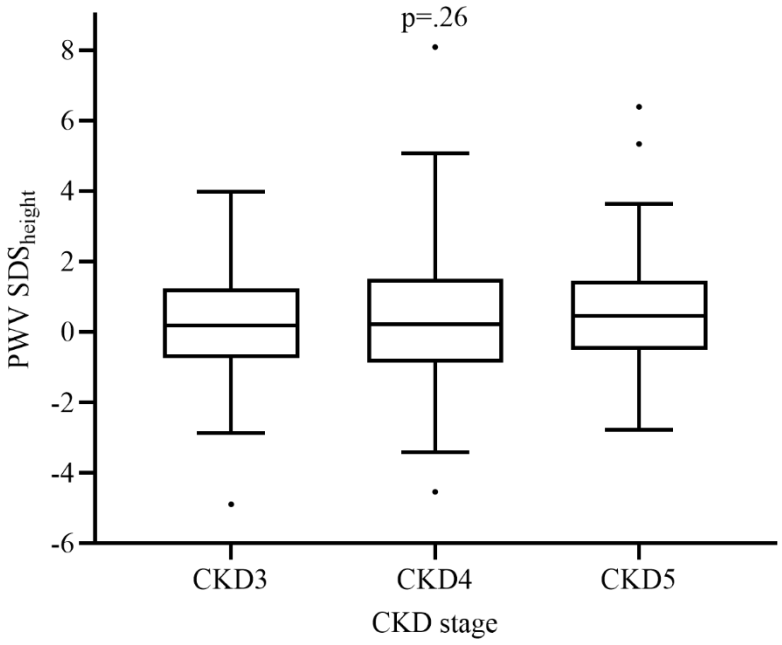


Figure 10. PWV SDS_{height} stratified by CKD stage

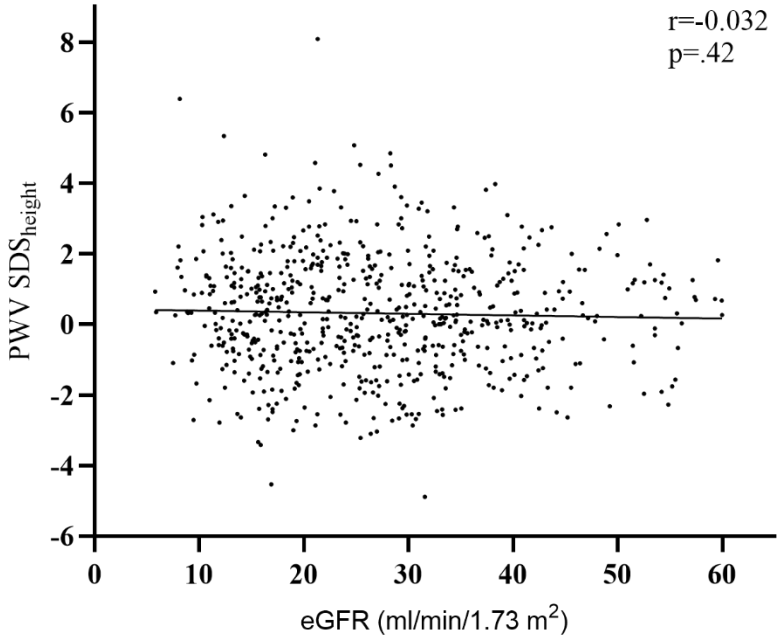


Figure 11. Correlation between PWV SDS_{height} and eGFR

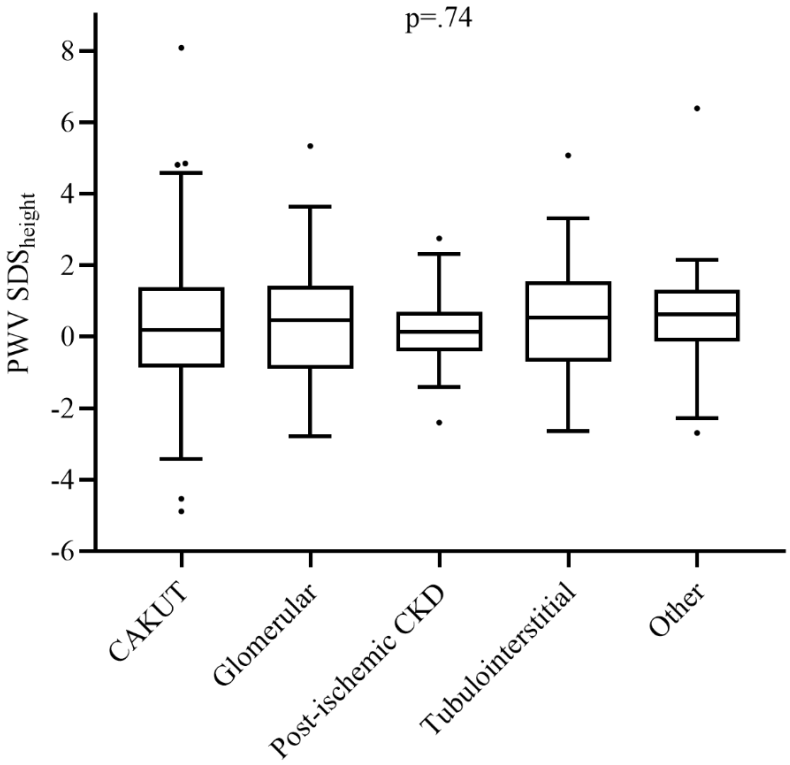


Figure 12. PWV SDS_{height} stratified by primary kidney disease

PWV SDS_{height} was significantly directly correlated with log-transformed uACR (r=0.170, p<.0001; Fig. 13) which was also significantly associated with higher odds of abnormal PWV (OR 1.121, 95% CI 1.003 to 1.253, p=.04). PWV SDS_{height} was significantly higher in patients with nephrotic range proteinuria (-0.08±1.56) compared to patients with non-nephrotic proteinuria (0.27±1.66) and normal uACR (0.92±1.56) (p=.0002; Fig. 14). The association with log-uACR was retained after controlling for age, sex, eGFR, height SDS and systolic and diastolic BP SDS (partial r=0.122, p=.002) but was lost after additional adjustment for log-25(OH)-vitamin D levels (partial r=0.073, p=.10).

Log-25(OH)-vitamin D levels were significantly lower in patients with nephrotic proteinuria (1.69±1.02) compared to patients with non-nephrotic proteinuria (2.38±0.87) and normal uACR (2.53±0.57) (p<.0001) and significantly inversely correlated with log-uACR (r=-0.240, p<.0001).

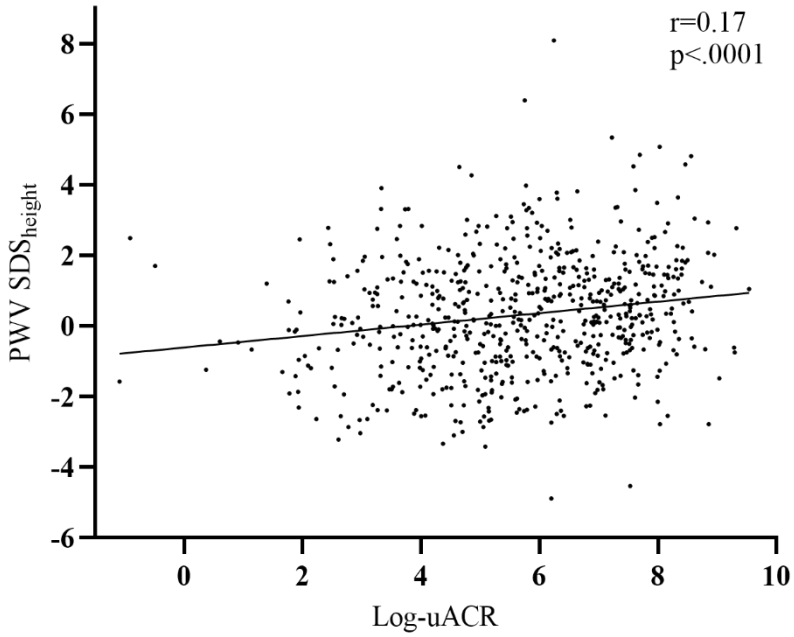


Figure 13. Correlation between PWV SDS_{height} and log-uACR

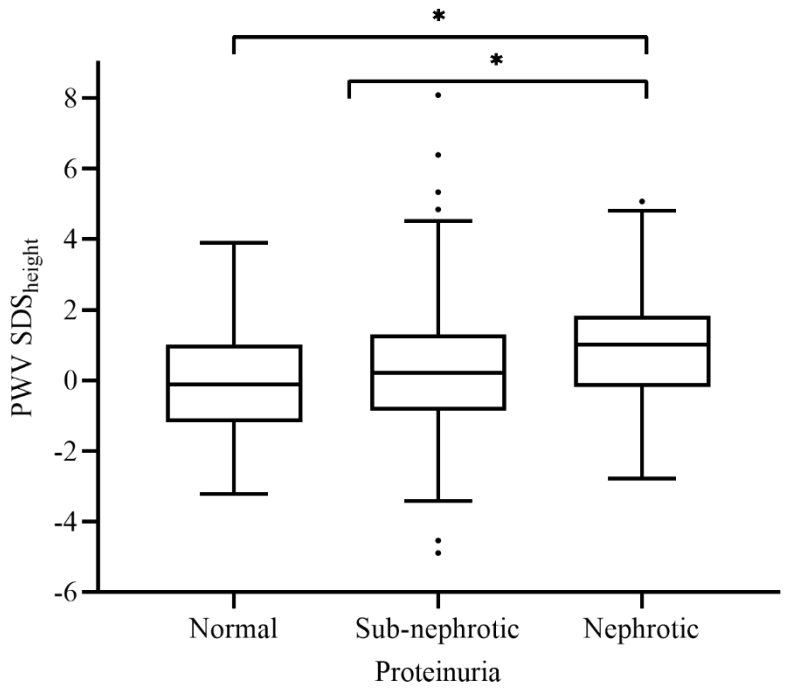


Figure 14. PWV SDS_{height} stratified by proteinuria level

Age, sex and birth weight

PWV SDS_{height} was inversely correlated with age (r=-0.131, p=.0007; Fig. 15) and was comparable by sex (0.23 ± 1.66 vs 0.47 ± 1.62 for boys and girls, p=.06; Fig. 16). The association with age was sex-dependent, boys showed an inverse correlation with age (r=-0.179, p=.0002) but girls did not (r=-0.033, p=.61). Older age was associated with lower odds (OR 0.895, 95% CI 0.844 to 0.949, p=.0002) of abnormal PWV. Sex was not associated with odds of having abnormal PWV (OR for boys 0.782, 95% CI 0.526 to 1.164, p=.23).

PWV SDS_{height} was similar between those born SGA and with normal birth weight (0.38 ± 1.47 vs 0.29 ± 1.69, p=.60; Fig. 17) and being born SGA did not have an effect on abnormal PWV (OR 0.957, 95% CI 0.553 to 1.656, p=.88).

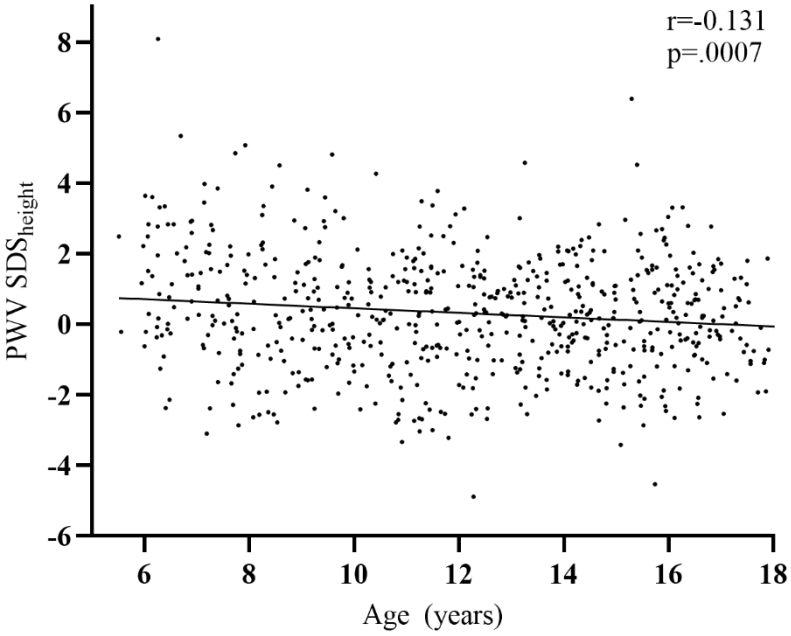


Figure 15. Correlation between PWV SDS_{height} and age

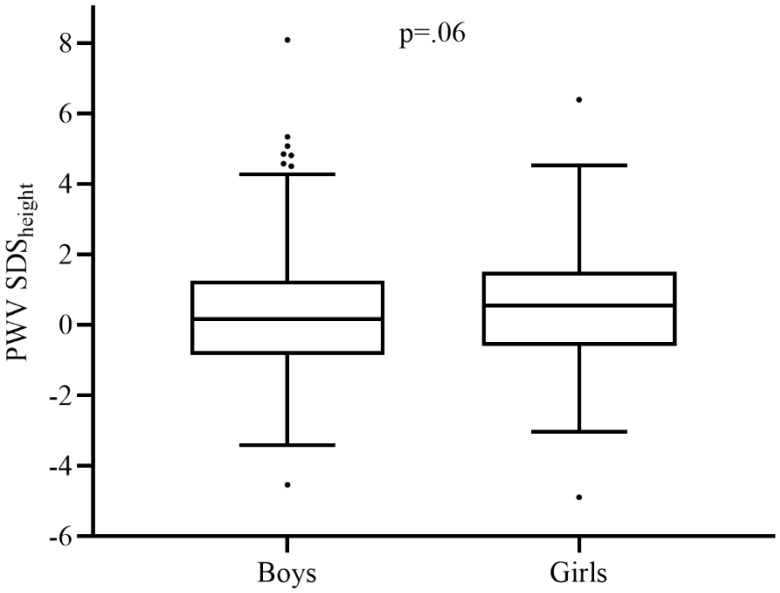


Figure 16. PWV SDS_{height} stratified by sex

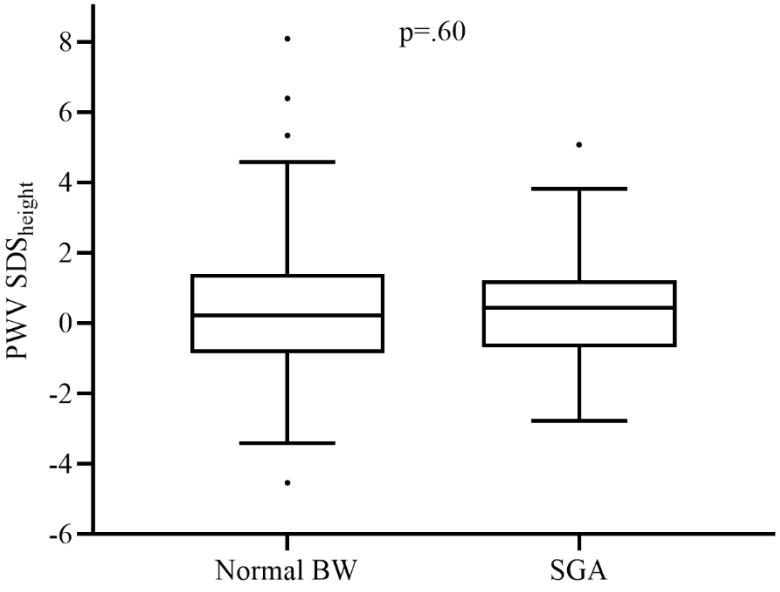


Figure 17. PWV SDS_{height} stratified by birth weight

Body dimensions

BMI SDS did not correlate with PWV SDS_{height} (r=-0.083, p=.90) and was not associated with abnormal PWV (OR 1.094, 95% CI 0.932 to 1.284, p=.27). PWV SDS_{height} was also comparable when stratifying patients by body composition (underweight, normal weight, overweight and obese; p=.75; Fig. 18).

At baseline, PWV SDS_{height} was significantly inversely correlated with height SDS (r=-0.284, p<.0001; Fig. 19) and each increase in one height SDS was associated with lower odds (OR 0.616, 95% CI 0.531 to 0.714, p<.0001) of having abnormal PWV. The association between height SDS and PWV SDS_{height} was retained (partial r=-0.245, p<.0001) after controlling for potential confounders (age, sex, systolic and diastolic BP SDS, HR and eGFR).

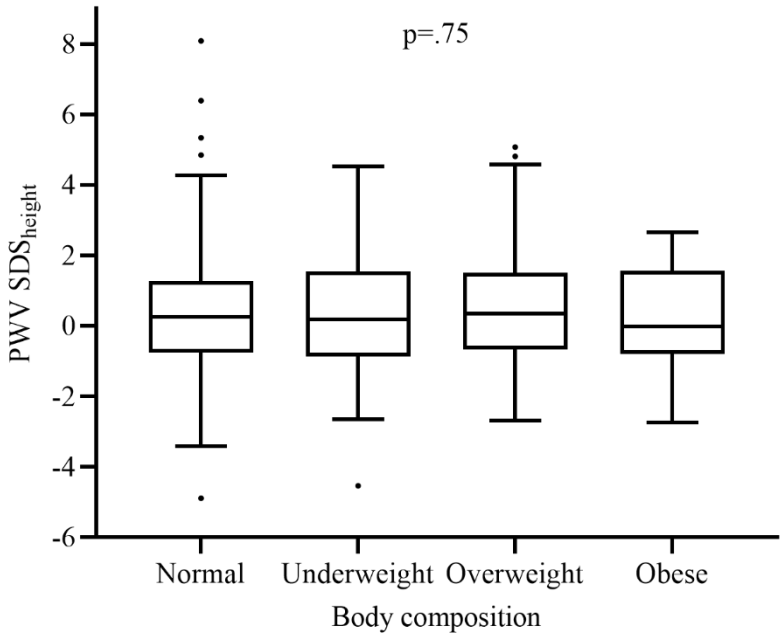


Figure 18. PWV SDS_{height} by body composition

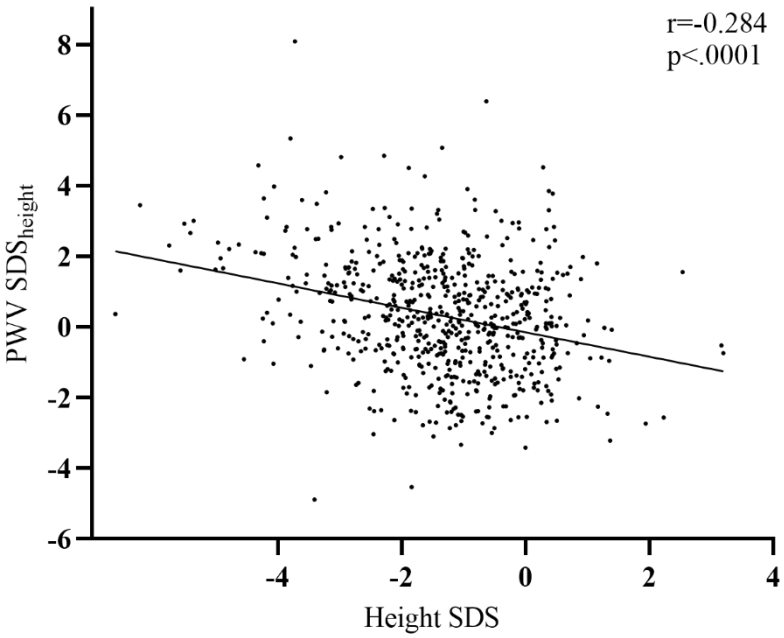


Figure 19. Correlation between PWV SDS_{height} and height SDS

There was a significant interaction between age and height SDS for the effect on PWV SDS_{height}: shorter height was more significantly associated with higher PWV SDS_{height} in younger patients (Table 11; Fig. 20).

Table 11. Model for interaction between age and height SDS on PWV SDS_{height}

Parameter	Estimate	SE	p value
Intercept	-.446	0.342	.19
Age, per year	0.024	0.026	.37
Height, per SDS	-0.950	0.169	<.0001
Age*Height SDS	0.051	0.013	.0002

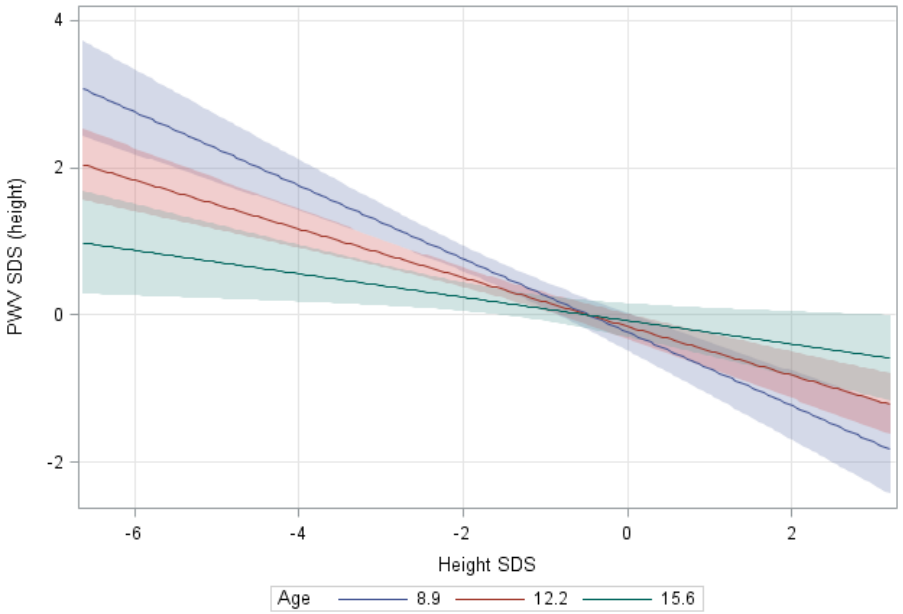


Figure 20. Interaction effect of age and height SDS on PWV SDS_{height}

BP and HR

PWV SDS_{height} significantly correlated with systolic BP and diastolic BP SDS (r=0.347 and r=0.368, respectively; both p<.0001; Fig. 21 and Fig. 22) and was significantly higher in hypertensive vs normotensive by office BP patients (1.10 ± 1.69 vs 0.001 ± 1.53, respectively; p<.0001; Fig. 23). Systolic and diastolic BP increase by one SDS was also associated with increased odds (OR 1.602, 95% CI 1.38 to 1.86 and OR 1.858, 95% CI 1.545 to 2.234, respectively; both p<.0001) of having abnormal PWV. Similarly, hypertension by office BP was associated with 3.347 (95% CI 2.243 to 4.995, p<.0001) times higher odds of having abnormal PWV.

There was significant interaction effect between age and systolic BP on PWV SDS_{height}: the association of PWV SDS_{height} with systolic BP SDS was stronger in younger children compared to older (Table 12; Fig. 24).

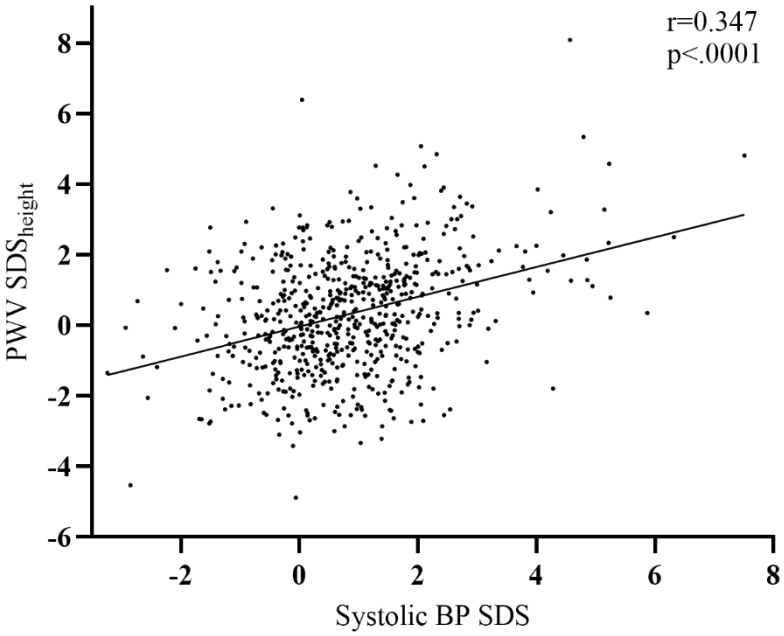


Figure 21. Correlation between PWV SDS_{height} and systolic BP SDS

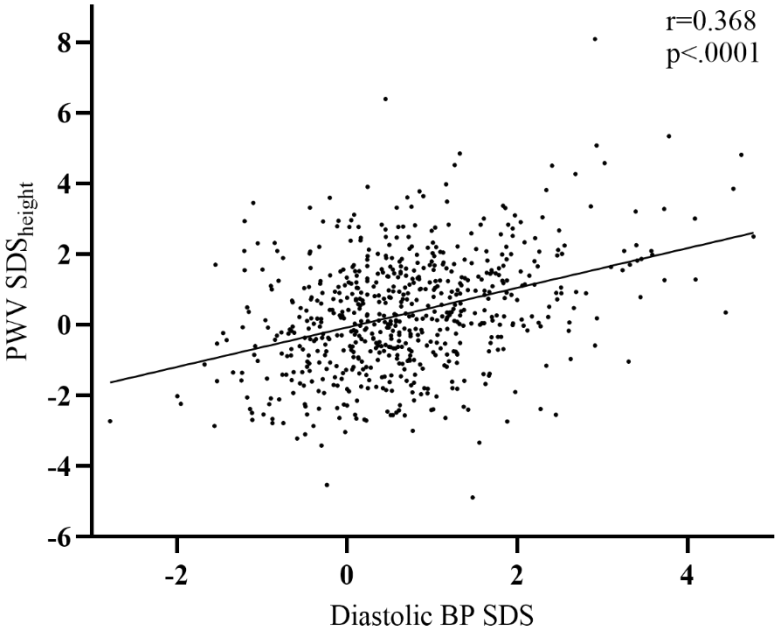


Figure 22. Correlation between PWV SDS_{height} and diastolic BP SDS

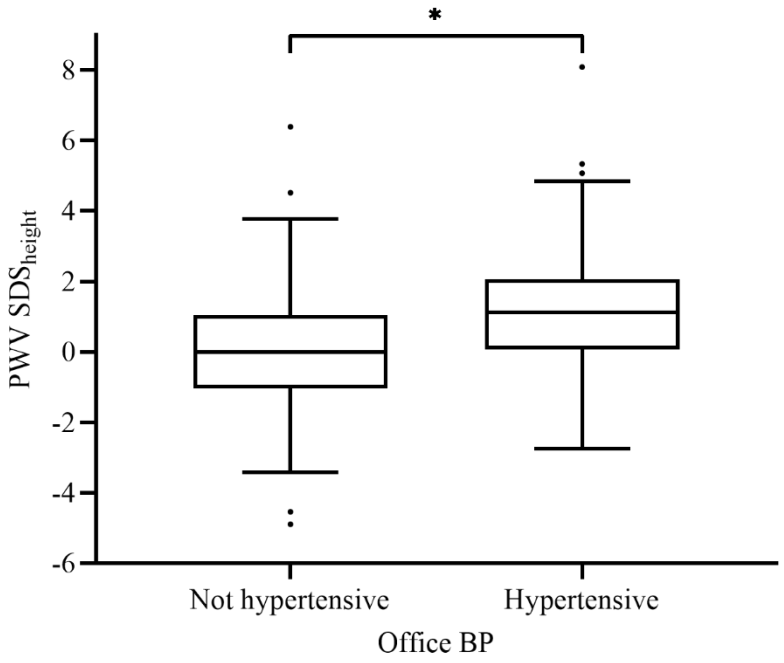


Figure 23. PWV SDS_{height} according to hypertension status by office BP

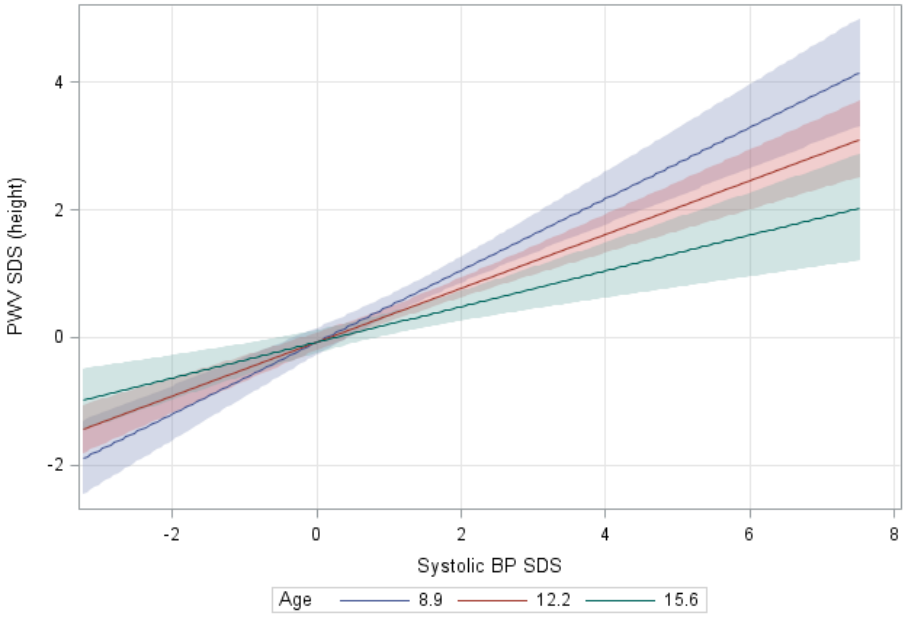


Figure 24. Interaction effect of age and systolic BP SDS on PWV SDS_{height}

Table 12. Model for interaction between age and systolic BP SDS on PWV SDS_{height}

Parameter	Estimate	SE	P value
Intercept	-0.068	0.281	.81
Age, per year	-0.0002	0.021	.99
Systolic BP, per SDS	0.935	0.171	<.0001
Age*Systolic BP SDS	-0.042	0.013	.001

Lipids, uric acid and serum bicarbonate

Serum bicarbonate levels did not correlate with PWV SDS_{height} (r=-0.061, p=.12) and were not associated with altered odds of having abnormal PWV (OR 0.976, 95% CI 0.925 to 1.031, p=.39).

Uric acid levels showed a weak negative correlation with PWV SDS_{height} (r=-0.083, p=.03; Fig. 25) and were associated with a small reduction in odds of having abnormal PWV (OR 0.886, 95% CI 0.793 to 0.991, p=.03). The association, however, was lost after controlling for potential confounders: age and height SDS (partial r=-0.031, p=.39).

PWV SDS_{height} showed a borderline weak correlation with total cholesterol levels (r=0.076, p=.05; Fig. 26) but total cholesterol levels were not associated with abnormal PWV (OR 1.003, 95% CI 0.999 to 1.006, p=.16) and the association became insignificant after adjusting for age (partial r=0.068, p=.08). No correlations with LDL-cholesterol (r=-0.071, p=.07) or HDL-cholesterol (r=-0.03, p=.44) levels were observed.

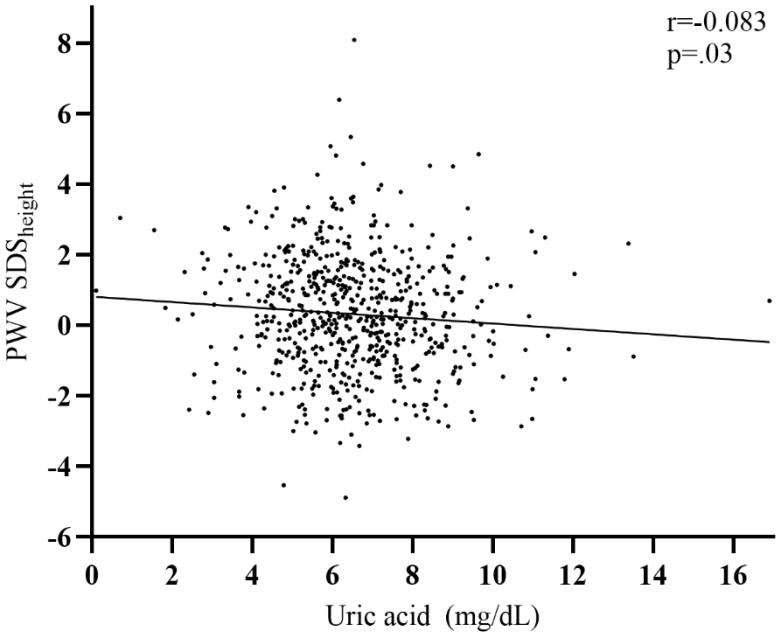


Figure 25. Correlation between PWV SDS_{height} and uric acid

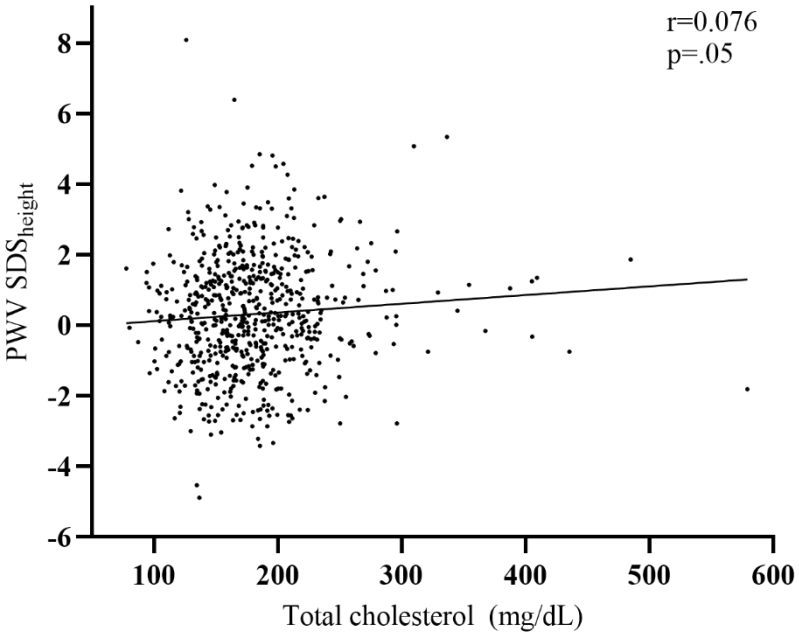


Figure 26. Correlation between PWV SDS_{height} and total cholesterol

PWV SDS_{height} did not correlate with hemoglobin levels (r=-0.027, p=.50; Fig. 27) and hemoglobin was not associated with abnormal PWV (OR 0.960, 95% CI 0.851 to 1.082, p=.50).

Serum log-ferritin levels were inversely correlated with PWV SDS_{height} (r=-0.097, p=.02; Fig. 28) but log-ferritin levels were not associated with abnormal PWV (OR 0.938, 95% CI 0.772 to 1.139, p=.52). The association between log-ferritin and PWV SDS_{height} was retained after adjusting for potential confounders: age, sex, height SDS, eGFR, CRP and hemoglobin levels (partial r=-0.135, p=.0009).

Log-CRP did not correlate with PWV SDS_{height} (r=0.066, p=.09) nor was associated with abnormally increased PWV (OR 1.051, 95% CI 0.934 to 1.183, p=.41).

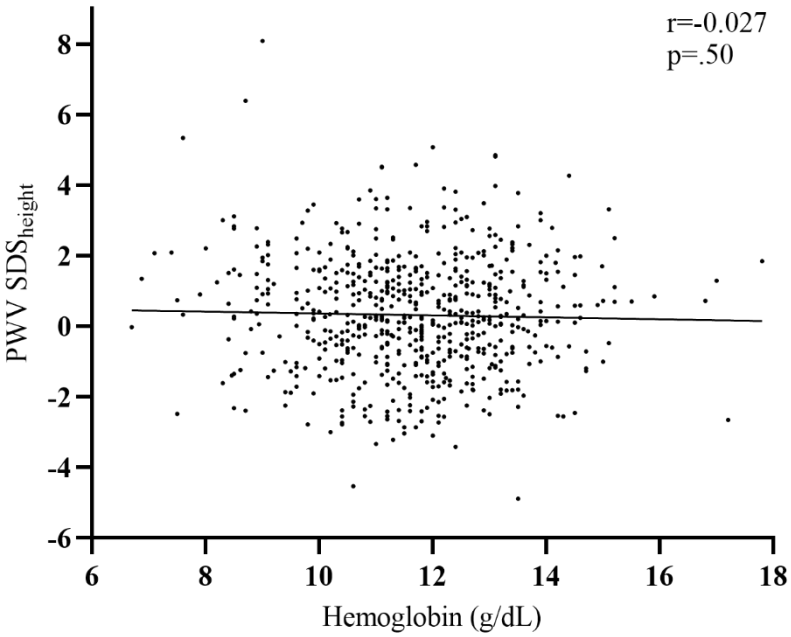


Figure 27. Correlation between PWV SDS_{height} and hemoglobin levels

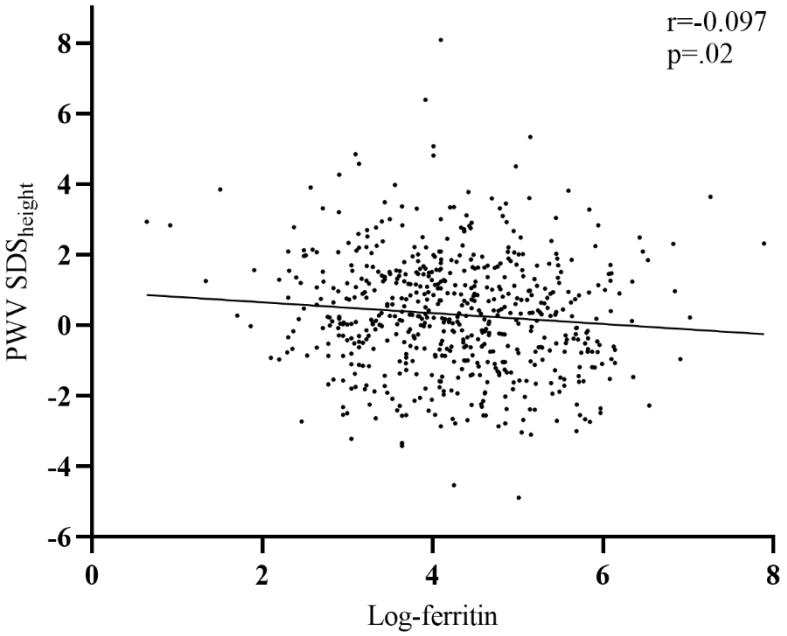


Figure 28. Correlation between PWV SDS_{height} and log-ferritin

CKD-MBD parameters

Serum calcium did not correlate with PWV SDS_{height} (r=-0.041, p=.29) and was not associated with abnormal PWV (OR 0.811, 95% CI 0.285 to 2.308, p=.69). Similar results were obtained after correcting serum calcium levels for serum albumin.

Serum phosphate and log-PTH levels were significantly correlated with (r=0.076, p=.05 and r=0.099, p=.01, respectively; Fig. 29 and 30) with PWV SDS_{height} but not associated with abnormal PWV (OR 1.535, 95% CI 0.943 to 2.497, p=.09 and OR 1.183, 95% CI 0.971 to 1.442, p=.10, respectively). After adjusting for age, sex, height SDS, eGFR, serum calcium, log-PTH/phosphate and log-24,25(OH)-vitamin D and log-25(OH)-vitamin D levels – no association with phosphate (partial r=.002, p=.97) or log-PTH (partial r=0.04, p=.37) was observed.

PWV SDS_{height} was significantly associated with log-25(OH)-vitamin D (r=-0.157, p=.0003; Fig. 31) but not with log-24,25(OH)-vitamin D (r=-0.057, p=.20). Higher log-25(OH)-vitamin D were also associated with lower odds of having abnormal PWV (OR 0.772, 95% CI 0.617 to 0.962, p=.02), but no significant effect of log-24,25(OH)-vitamin D was observed (OR 0.858, 95%

CI 0.664 to 1.108, p=.24). The association with lower log-25(OH)-vitamin D remained (partial r=-0.132, p=.003) after adjusting for age, sex, height SDS, eGFR, serum calcium and phosphate, serum PTH, log-24,25(OH)-vitamin D and log-uACR.

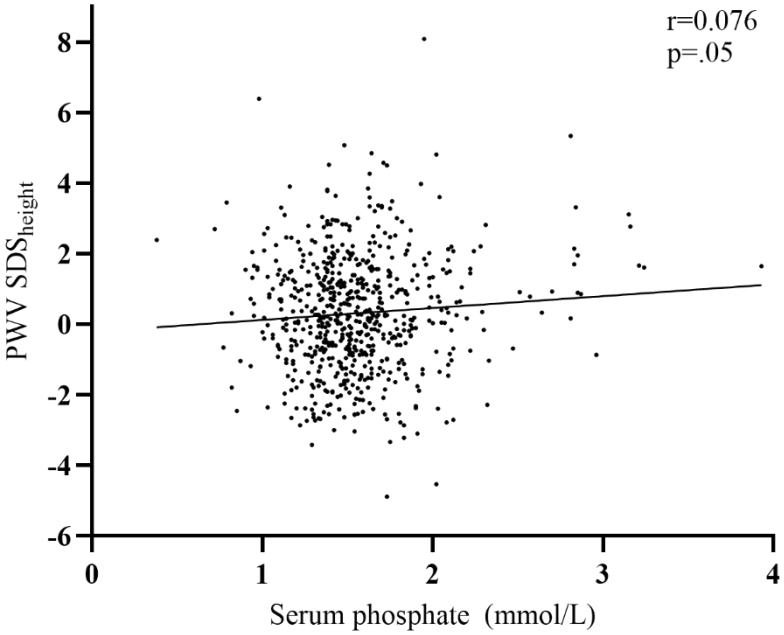


Figure 29. Correlation between PWV SDS_{height} and serum phosphate

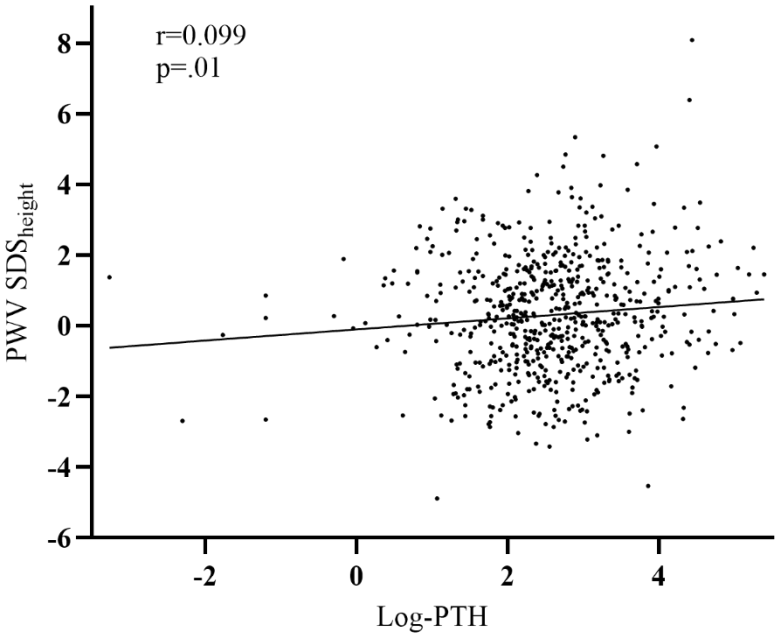


Figure 30. Correlation between PWV SDS_{height} and log-PTH

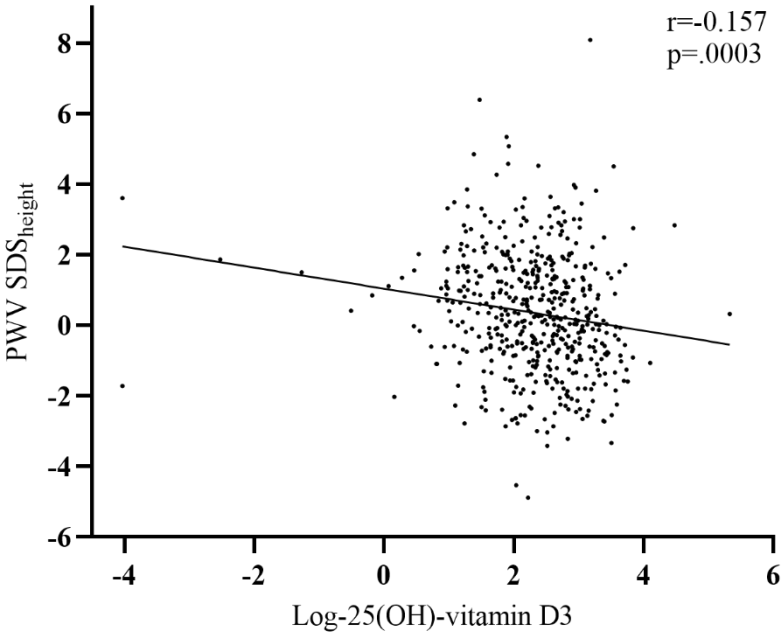


Figure 31. Correlation between PWV SDS_{height} and log-25(OH)-vitamin D

4.2.3. Univariable linear and logistic regression of PWV SDS_{height} with clinical and laboratory characteristics at baseline

Univariable linear and logistic regression models were created to determine baseline covariates that are significantly associated with PWV SDS_{height} at baseline and should be included further in the development of multivariable linear models. Results of the univariable association analysis are presented in Table 13.

Table 13. Univariable linear and logistic regression for PWV SDS_{height} and abnormally high PWV at baseline

	Linear regression (PWV SDS_{height})		**Logistic regression** (PWV SDS_{height} above 95th percentile)		
	Estimate	**p value**	**OR**	**95% CI**	**p value**
Age, year*	-0.064	.0007	0.895	0.844 – 0.949	.0002
Sex (ref: boys)*	0.249	.06	1.279	0.859 – 1.903	.23
Diagnosis (ref: CAKUT)					
Glomerulopathies	-0.315	.32	0.954	0.476 – 1.913	.89
CKD post-AKI	-0.396	.29	0.374	0.112 – 1.248	.11
Tubulointerstitial	-0.456	.27	1.043	0.584 – 1.864	.89
Other	-0.17	.63	0.638	0.217 – 1.879	.42
SGA (ref: no)	0.094	.60	0.957	0.553 – 1.656	.88
BMI, per 1 SDS	0.006	.90	1.094	0.932 – 1.284	.27
Height, per 1 SDS*	-0.346	<.0001	0.616	0.531 – 0.714	<.0001
Systolic BP, per 1 SDS	0.423	<.0001	1.602	1.38 – 1.86	<.0001
Diastolic BP, per 1 SDS*	0.560	<.0001	1.858	1.545 – 2.234	<.0001
HR, per bpm*	0.017	.0005	1.014	0.999 – 1.028	.07
eGFR, per ml/min/1.73 m^2*	-0.005	.42	0.992	0.976 – 1.009	.38
Hemoglobin, per g/dL	-0.027	.50	0.960	0.851 – 1.082	.50

Continued table.

	Linear regression (PWV SDS_{height})		Logistic regression (PWV SDS_{height} above 95th percentile)		
Bicarbonate, per mmol/L*	-0.028	.12	0.976	0.925 – 1.031	.39
Cholesterol, per mg/dL*	0.003	.05	1.003	0.999 – 1.006	.16
LDL cholesterol, per mg/dL	0.003	.07	1.003	0.998 – 1.007	.22
HDL cholesterol, per mg/dL	-0.003	.44	0.988	0.974 – 1.002	.11
Triglycerides, per mg/dL	0.003	.05	1.002	0.999- 1.006	.22
Uric acid, per mg/dL*	-0.076	.03	0.886	0.793 – 0.991	.03
Serum calcium, per mmol/L	-0.37	.29	0.811	0.285 – 2.308	.69
Corrected serum calcium, per mmol/L	0.182	.60	0.987	0.344 – 2.835	.98
Serum hosphate, per mmol/L*	0.337	.05	1.535	0.943 – 2.497	.09
Serum albumin, per g/L*	-0.019	.09	0.944	0.962 – 1.028	.74
Log-uACR*	0.162	<.0001	1.121	1.003 – 1.253	.04
Log-ferritin*	-0.153	.02	0.938	0.772 – 1.139	.52
Log-CRP	0.045	.25	1.051	0.934 – 1.183	.41
Log-PTH*	0.158	.01	1.183	0.971 – 1.442	.10
Log-24,25(OH)-vitamin D*	-0.115	.20	0.858	0.664 – 1.108	.24
Log-25(OH)-vitamin D*	-0.298	.0003	0.772	0.617 – 0.962	.02

Abbreviations: AKI, acute kidney injury; BP, blood pressure; BMI, body mass index; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; PTH, parathormone; SGA, small-for-gestational age; uACR, urinary albumin-creatinine ratio.

**Indicates variables that were selected for multivariable model building*

4.2.4. Multivariable models of PWV SDS_{height} at baseline

All variables with a significance level (p value) of <.20 in univariable linear regression were considered for multivariable model (indicated with an asterisk in the univariable regression table) with eGFR forced into the model.

In case of significant collinearity: systolic and diastolic BP SDS (r=0.651, p<.0001), total cholesterol and LDL cholesterol (r=0.892, p<.0001), variables with a stronger effect (estimate and p value) were selected. Triglycerides were excluded from the multivariable model development due to high number of missing observations (non-fasting values).

In a mixed effects multivariable linear regression model with PWV SDS_{height} as a dependent variable and a random center effect, younger age, lower height SDS, higher diastolic BP SDS, lower log-ferritin and lower log-25(OH)-vitamin D levels were associated with higher PWV SDS_{height} (Table 14). Due to the inclusion of 25(OH)-vitamin D levels, the sample size for the full model was significantly reduced (n=475) and an additional model excluding these variables was built as a sensitivity analysis to test for effect stability. The new model (n=595) revealed similar significant associations (data not shown).

Observed independent associations with PWV SDS_{height} remained after multivariable linear regression with stepwise (forward-backward) selection (Table 15).

Table 14. Multivariable linear mixed effects model with a random center effect for PWV SDS_{height} at baseline

	N=475; AIC 1760		
	Estimate	**SE**	**p value**
Intercept	0.607	1.274	.64
Age, per year	-0.081	0.022	.0002
Male sex	0.038	0.139	.78
Height, per SDS	-0.292	0.052	<.0001
Diastolic BP, per SDS	0.501	0.064	<.0001
HR, per bpm	0.001	0.006	.83
eGFR, per ml/min/1.73 m^2	-0.007	0.007	.35

Continued table.

	N=475; AIC 1760		
	Estimate	**SE**	**p value**
Serum bicarbonate, per mmol/L	0.0102	0.021	.62
Total cholesterol, per mg/dL	0.001	0.002	.44
Uric acid, per mg/dL	0.034	0.038	.38
Serum phosphate, per mmol/L	0.004	0.209	.99
Serum albumin, per g/L	0.008	0.014	.59
Log-ferritin	-0.157	0.067	.02
Log-uACR	0.017	0.046	.71
Log-PTH	-0.023	0.074	.76
Log-24,25(OH)-vitamin D	-0.063	0.089	.48
Log-25(OH)-vitamin D	-0.179	0.087	.04

Abbreviations: BP, blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; PTH, parathormone; uACR, urinary albumin-to-creatinine ratio.

Table 15. Multivariable linear regression model for PWV SDSheight at baseline after stepwise (forward-backward) variable selection

	N=475; AIC=848.3; R^2=0.27		
Parameter	**Estimate**	**SE**	**p value**
Intercept	1.810	0.442	<.0001
Age, per year	-0.079	0.021	.0002
Height, per SDS	-0.332	0.050	<.0001
Diastolic BP, per SDS	0.478	0.061	<.0001
Log-ferritin	-0.158	0.065	.012
Log-25(OH)-vitamin D	-0.287	0.075	.0001

Abbreviations: BP, blood pressure.

4.3. Longitudinal trend and associations of PWV

Median follow-up time of study participants was 1.7 years (IQR 0-3.3 years) with a maximum follow-up of 8.7 years. The longitudinal change of PWV SDS_{height} over time since the first PWV recording was non-linear: PWV SDS_{height} showed a mild decrease until the fourth year and slightly increased thereafter (Fig. 32).

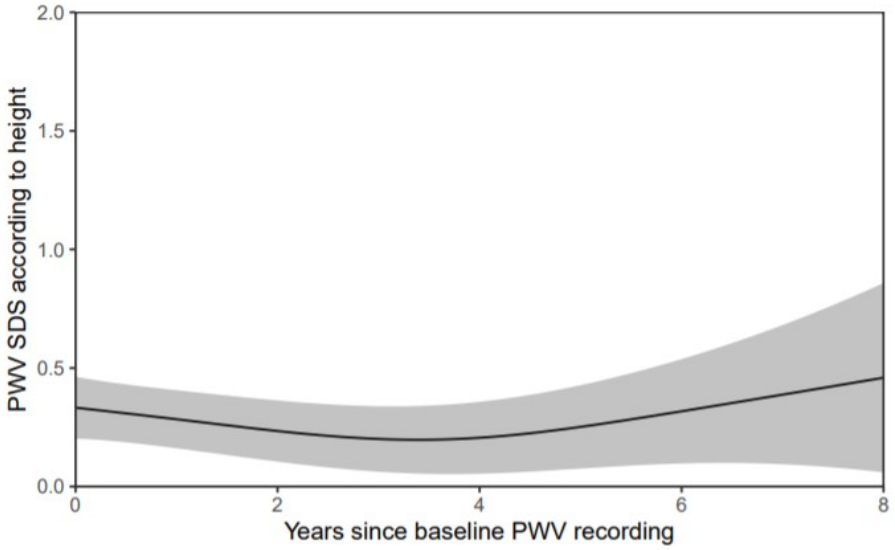


Figure 32. Change of PWV SDS_{height} over time during the observation period (grey area represents 95% CI)

Covariates for the longitudinal mixed effects model to analyze the association with PWV SDS_{height} over time were selected on the basis clinical relevance and taking into account results of baseline analysis. Two separate analyses were performed: one with PWV SDS_{height} as the outcome with patient follow-up censored at 17 years of age and another with absolute PWV values and all observations.

4.3.1. Longitudinal model with PWV SDS_{height}

In the longitudinal model with PWV SDS_{height} time since baseline showed a quadratic non-linear effect (as seen in Figure 32). In addition, younger age at baseline, female sex, diagnosis of tubulointerstitial kidney disease, lower height SDS, higher diastolic BP, higher serum hemoglobin, higher LDL cholesterol, higher log-uACR, and lower log-ferritin were associated with higher PWV SDS_{height} (Table 16).

After inclusion of time-interaction effect the higher PWV SDS_{height} was associated with non-linear time since baseline, younger age at baseline, other kidney diagnoses, lower height SDS, higher diastolic BP SDS, higher LDL cholesterol, higher log-uACR, lower log-ferritin and higher corrected serum calcium. Time-interaction analysis showed that the association of PWV SDS_{height} with female sex and height SDS intensified over time, whereas the effect of other kidney diagnoses decreased (Table 17).

Table 16. Longitudinal linear mixed effects model (PWV SDS_{height}) without time interaction

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time since baseline, years	-0.124	-0.210 – -0.039	.004
Time since baseline^2	0.020	0.005 – 0.034	.009
Age at baseline, year	-0.052	-0.084 – -0.020	.002
Female sex	0.271	0.068 – 0.474	.009
Diagnosis (ref: CAKUT)			
Glomerulopathies	-0.105	-0.519 – 0.308	.62
CKD post-AKI	-0.010	-0.439 – 0.419	.97
Tubulointerstitial	0.322	0.028 – 0.615	.03
Other	0.301	-0.198 – 0.780	.24
BMI, per SDS	-0.051	-0.116 – 0.014	.12
Height, per SDS	-0.295	-0.367 – -0.224	<.0001
Diastolic BP, per SDS	0.295	0.230 – 0.360	<.0001
eGFR, per ml/min/1.73 m^2	0.001	-0.008 – 0.009	.90
Hemoglobin, per g/dL	0.054	0.003 – 0.110	.04
Serum bicarbonate, per mmol/L	0.003	-0.017 – 0.023	.78
HDL cholesterol, mg/dL	-0.004	-0.009 – 0.002	.21
LDL cholesterol, mg/dL	0.003	0.001 – 0.006	.004
Corrected serum calcium, per mmol/L	0.236	-0.158 – 0.629	.24
Serum albumin, per g/L	0.013	-0.006 – 0.031	.18
Log-uACR	0.117	0.065 – 0.169	<.0001
Log-ferritin	-0.095	-0.182 – -0.008	.03
Log-CRP	0.009	-0.031 – 0.050	.65
Log-PTH	0.016	-0.054 – 0.085	.66

Abbreviations: AKI, acute kidney injury; BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-to-creatinine ratio.

Table 17. Longitudinal linear mixed effects model (PWV SDS_{height}) with time interaction

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time since baseline, years	0.398	-0.422 – 1.218	.34
Time since baseline^2	0.024	0.006 – 0.041	.008
Age at baseline, year	-0.055	-0.091 – -0.020	.002
Female sex	0.161	-0.065 – 0.386	.162
Diagnosis (ref: CAKUT)			
Glomerulopathies	-0.031	-0.478 – 0.416	.89
CKD post-AKI	0.045	-0.432 – 0.523	.85
Tubulointerstitial	0.214	-0.115 – 0.543	.20
Other	0.571	0.026 –1.117	.04
BMI, per SDS	-0.075	-0.151 – 0.002	.06
Height, per SDS	-0.329	-0.410 – -0.247	<.0001
Diastolic BP, per SDS	0.332	0.249 – 0.415	<.0001
eGFR, per ml/min/1.73 m^2	0.001	-0.009 – 0.011	.81
Hemoglobin, per g/dL	0.033	-0.031 – 0.098	.31
Serum bicarbonate, per mmol/L	0.015	-0.012 – 0.042	.27
HDL cholesterol, mg/dL	-0.004	-0.011 – 0.004	.22
LDL cholesterol, mg/dL	0.003	0.000 – 0.006	.02
Corrected serum calcium, per mmol/L	0.549	0.011 – 1.086	.05
Serum albumin, per g/L	0.018	-0.004 – 0.041	.11
Log-uACR	0.107	0.044 – 0.171	.001
Log-ferritin	-0.117	-0.209 – -0.024	.01
Log-CRP	0.005	-0.051 – 0.062	.86
Log-PTH	0.066	-0.024 – 0.156	.15
Time*Age at baseline	0.005	-0.011 – 0.022	.53
Time*Female sex	0.096	0.014 – 0.177	.01
Time*Diagnosis (ref: CAKUT)			
Glomerulopathies	-0.044	-0.227 – 0.140	.64
CKD post-AKI	-0.059	-0.205 – 0.008	.64
Tubulointerstitial	0.073	-0.048 – 0.195	.24
Other	-0.250	-0.450 – -0.051	.01
Time*BMI SDS	0.014	-0.013 – 0.040	.33
Time*Height	0.030	0.002 – 0.057	.03
Time*Diastolic BP SDS	-0.022	-0.053 – 0.010	.18
Time*eGFR	0.000	-0.004 – 0.003	.79
Time*Hemoglobin	0.009	-0.018 – 0.023	.51
Time*Bicarbonate	-0.007	-0.018 – 0.004	.23
Time*HDL cholesterol	0.000	-0.003 – 0.003	.96
Time*LDL cholesterol	0.000	-0.001 – 0.001	.66
Time*Corrected serum calcium	-0.177	-0.395 – 0.041	.11
Time*Serum albumin	-0.003	-0.012 – 0.005	.45
Time*Log-uACR	0.007	-0.017 – 0.030	.58

Continued table.

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time*Log-ferritin	0.016	-0.025 – 0.057	.44
Time*Log-CRP	0.003	-0.018 – 0.023	.78
Time*Log-PTH	-0.035	-0.074 – 0.004	.08

Abbreviations: AKI, acute kidney injury; BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-to-creatinine ratio.

4.3.2.Longitudinal model with absolute PWV values

In the longitudinal model with absolute PWV values time since baseline similarly showed a quadratic non-linear effect. However, height and age were directly associated with PWV, as expected physiologically due to growth. The association with gender and hemoglobin was lost, but associations with diagnosis of tubulointerstitial kidney disease, higher diastolic BP, higher LDL cholesterol, higher log-uACR, and lower log-ferritin were retained (Table 18).

After inclusion of time-interaction effect higher PWV was associated with non-linear time since baseline, younger age at baseline, lower height SDS, higher diastolic BP SDS, higher log-uACR and log-ferritin. Time-interaction analysis showed an intensifying effect of tubulointerstitial kidney diseases and decreasing effect of other kidney diagnoses over time (Table 19).

Table 18. Longitudinal linear mixed effects model (absolute PWV) without time interaction

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time since baseline, years	0.039	-0.002 – 0.080	.06
Time since baseline^2	0.011	0.005 – 0.017	.0002
Age at baseline, year	0.070	0.049 – 0.091	<.0001
Female sex	-0.034	-0.127 – 0.059	.47
Diagnosis (ref: CAKUT)			
Glomerulopathies	-0.015	-0.193 – 0.164	.87
CKD post-AKI	0.042	-0.157 – 0.243	.67
Tubulointerstitial	0.163	0.032 – 0.295	.02
Other	0.058	-0.164 – 0.280	.61
BMI, per SDS	-0.024	-0.054 – 0.006	.12
Height, per cm	0.005	0.001 – 0.009	.01
Diastolic BP, per SDS	0.166	0.137 – 0.195	<.0001
eGFR, per ml/min/1.73 m^2	-0.001	-0.004 – 0.003	.68
Hemoglobin, per g/dL	0.019	-0.004 – 0.041	.10
Serum bicarbonate, per mmol/L	0.002	-0.007 – 0.011	.66

Continued table.

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
HDL cholesterol, mg/dL	-0.002	-0.004 – 0.001	.20
LDL cholesterol, mg/dL	0.001	0.000 – 0.002	.03
Corrected serum calcium, per mmol/L	0.099	-0.075 – 0.271	.27
Serum albumin, per g/L	0.004	-0.004 – 0.013	.33
Log-uACR	0.062	0.038 – 0.085	<.0001
Log-ferritin	-0.039	-0.076 – -0.003	.03
Log-CRP	-0.002	-0.022 – 0.017	.80
Log-PTH	0.000	-0.032 – 0.033	.99

Abbreviations: AKI, acute kidney injury; BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract CRP; C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-to-creatinine ratio.

Table 19. Longitudinal linear mixed effects model (absolute PWV) with time interaction

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time since baseline, per year	0.098	-0.256 – 0.453	.59
Time since baseline^2	0.009	0.001 – 0.017	.04
Age at baseline, per year	0.073	0.044 – 0.102	<.0001
Female sex	-0.015	-0.119 – 0.008	.77
Diagnosis (ref: CAKUT)			
Glomerulopathies	0.019	-0.176 – 0.213	.85
CKD post-AKI	0.019	-0.176 – 0.213	.87
Tubulointerstitial	0.083	-0.066 – 0.231	.27
Other	0.173	-0.070 – 0.416	.16
BMI, per SDS	-0.031	-0.067 – 0.004	.08
Height, per cm	0.004	-0.001 – 0.009	.08
Diastolic BP, per SDS	0.178	0.140 – 0.217	<.0001
eGFR, per ml/min/1.73 m^2	-0.001	-0.005 – 0.004	.80
Hemoglobin, per g/dL	0.015	-0.014 – 0.044	.32
Serum icarbonate, per mmol/L	0.008	-0.004 – 0.021	.20
HDL cholesterol, mg/dL	-0.002	-0.006 – 0.001	.14
LDL cholesterol, mg/dL	0.001	-0.001 – 0.002	.42
Corrected serum calcium, per mmol/L	0.206	-0.040 – 0.452	.10
Serum albumin, per g/L	0.004	-0.007 – 0.015	.46
Log-uACR	0.048	0.019 – 0.076	.001
Log-ferritin	-0.060	-0.106 – -0.015	.01
Log-CRP	0.000	-0.028 – 0.027	.99
Log-PTH	0.024	-0.019 – 0.066	.28
Time*Age at baseline	-0.003	-0.010 – 0.005	.47

Continued table.

	Longitudinal linear mixed effects model		
	Estimate	**95% CI**	**p value**
Time*Female sex	-0.012	-0.050 – 0.026	.54
Time*Diagnosis (ref: CAKUT)			
Glomerulopathies	-0.019	-0.099 – 0.060	.64
CKD post-AKI	0.017	-0.051 – 0.086	.62
Tubulointerstitial	0.061	0.009 – 0.112	.02
Other	-0.114	-0.204 – -0.024	.01
Time*BMI SDS	0.004	-0.007 – 0.015	.49
Time*Height	0.000	-0.001 – 0.002	.51
Time*Diastolic BP SDS	-0.007	-0.021 – 0.006	.28
Time*eGFR	0.000	-0.002 – 0.002	.98
Time*Hemoglobin	0.002	-0.009 – 0.013	.77
Time*Serum bicarbonate	-0.003	-0.008 – 0.001	.16
Time*HDL cholesterol	0.000	-0.001 – 0.001	.52
Time*LDL cholesterol	0.000	0.000 – 0.001	.05
Time*Corrected serum calcium	-0.057	-0.149 – 0.035	.22
Time*Serum albumin	0.000	-0.004 – 0.003	.93
Time*Log-uACR	0.008	-0.004 – 0.020	.18
Time*Log-ferritin	0.010	-0.005 – 0.025	.19
Time*Log-CRP	-0.001	-0.011 – 0.009	.82
Time*Log-PTH	-0.015	-0.031 – 0.001	.07

Abbreviations: AKI, acute kidney injury; BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-to-creatinine ratio.

4.4. PWV and LV geometry

A total of 540 patients had LV geometry data available at baseline. Mean LVMI at baseline was 42.2 ± 13.3 g/m^2 and mean RWT (age normalized) was 0.38 ± 0.09. One third of the patients (n=190, 35.2%) had LVH. When compared by LV geometry, 228 (42.2%) had normal LV geometry, 122 (22.6%) had concentric remodeling, 117 (21.7%) had concentric LVH and 73 (13.5%) exhibited eccentric LVH (Table 20). PWV SDS_{height} was higher in patients with concentric remodeling (p=.02, Table 20).

Table 20. Distribution of LV geometry and PWV SDS_{height} with different LV geometry patterns

RWT	LVMI	
	≤45 g/m²	>45 g/m²
	Normal geometry	**Eccentric LVH**
≤0.38	N=228 (42.2%)	N=73 (13.5%)
	PWV SDS_{height}=0.19±1.67	PWV SDS_{height}=0.22±1.66
	Concentric remodeling	**Concentric LVH**
>0.38	N=122 (22.6%)	N=117 (21.7 %)
	PWV SDS_{height}=0.63±1.78	PWV SDS_{height}=0.66±1.55

Abbreviations: LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; RWT, relative wall thickness.

The proportion of patients with abnormal LV geometry was similar when comparing patients with normal or increased PWV (p=.27; Fig. 33).

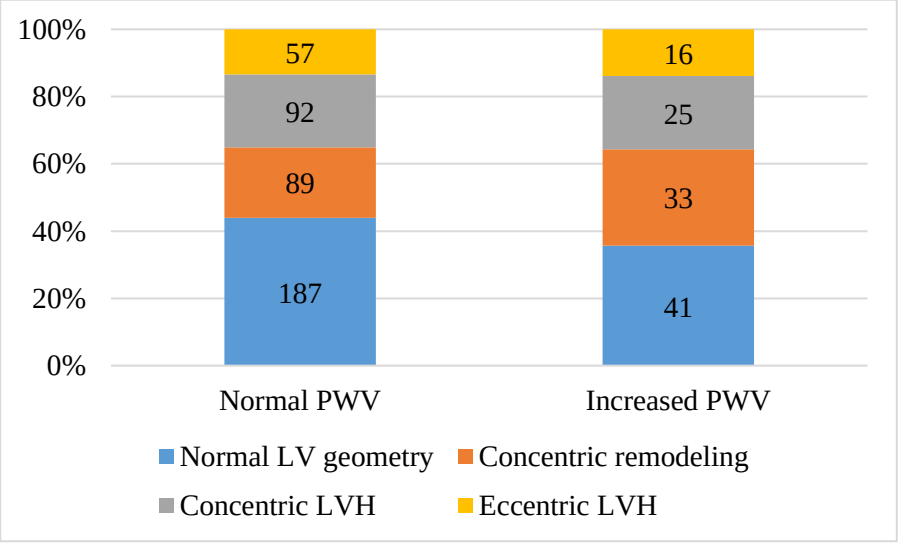


Figure 33. Distribution of different LV geometry by the presence or absence of abnormal PWV

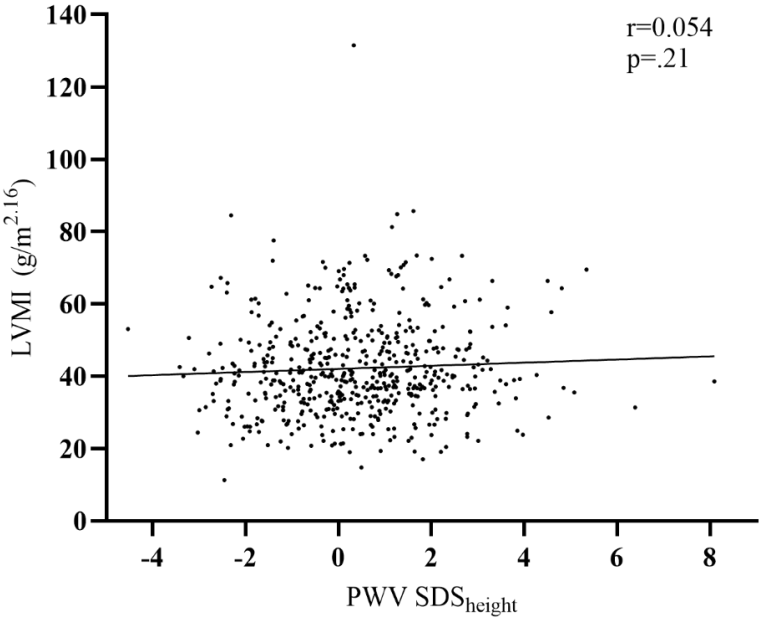


Figure 34. Correlation between PWV SDS_{height} and LVMI

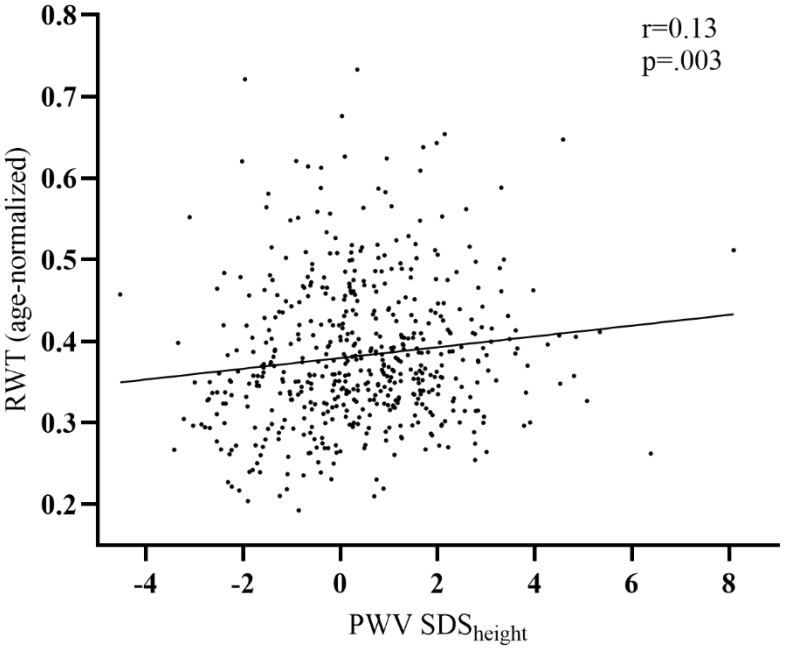


Figure 35. Correlation between PWV SDS_{height} and RWT

PWV SDS_{height} did not correlate with LVMI (r=0.054, p=.21; Fig. 34) but showed weak positive correlation with RWT (r=0.13, p=0.003; Fig. 35). LVMI at baseline was associated with older age, male sex, higher BMI, lower height SDS, higher systolic and diastolic BP SDS, lower eGFR, higher log-uACR, lower hemoglobin, lower serum bicarbonate, higher log-PTH, lower HDL cholesterol, lower serum calcium and lower serum albumin (Table 21). RWT was associated with older age, lower height SDS, higher serum phosphate, higher systolic and diastolic BP SDS, and higher PWV SDS_{height} (Table 21).

In the multivariable regression analysis after stepwise selection LVMI was significantly associated with older age, male sex, higher BMI, lower height SDS, higher systolic BP SDS, lower HR, lower eGFR, lower hemoglobin, lower HDL cholesterol and lower serum calcium but not PWV SDS_{height} (Table 22). RWT was significantly associated with height SDS, serum phosphate and PWV SDS_{height} (Table 23). In the multivariable logistic regression, older age, male sex, higher BMI, lower height SDS, higher systolic BP SDS and lower HDL were associated with higher odds of LVH (Table 24). Younger age and higher PWV SDS_{height} were the only covariates associated with higher odds of concentric remodeling (abnormally increased RWT) (Table 25).

Table 21. Univariable linear (for LVMI and RWT) and logistic regression (for LVH and concentric remodeling) at baseline

	Univariable linear regression N=540				**Univariable logistic regression N=540**			
	LVMI	**p value**	**RWT**	**p value**	**LVH (95% CI)**	**p value**	**Concentric geometry (95% CI)**	**p value**
Age, per year	0.400	.02	-0.002	.05	1.038 (0.984-1.096)	.17	0.931 (0.883-0.982)	.009
Sex, boys	3.318	.006	0.005	.50	1.521 (1.041-2.221)	.03	1.084 (0.755-1.557)	.66
BMI , per SDS	1.899	<.0001	0.001	.71	1.326 (1.134-1.551)	.0004	0.971 (0.843-1.119)	.68

Continued table.

	Univariable linear regression N=540				Univariable logistic regression N=540			
	LVMI	**p value**	**RWT**	**p value**	**LVH (95% CI)**	**p value**	**Concentric geometry (95% CI)**	**p value**
Height, per SDS	-1.620	.0001	-0.008	.004	0.769 (0.674-0.878)	.0001	0.852 (0.750-0.968)	.01
Diagnosis (ref: CAKUT)								
Glomerulopathies	2.711	.19	-0.009	.52	1.634 (0.881-3.032)	.13	0.670 (0.346-1.297)	.20
CKD post-AKI	-3.683	.16	-0.027	.12	0.973 (0.425-2.226)	.77	0.900 (0.401-2.019)	.84
Tubulointerstitial	0.582	.75	0.005	.70	1.216 (0.706-2.093)	.63	1.158 (0.680-1.972)	.45
Other	0.836	.76	0.016	.38	0.757 (0.308-1.858)	.34	1.202 (0.532-2.720)	.52
Systolic BP, per SDS	1.960	<.0001	0.006	.04	1.253 (1.098-1.431)	.0008	1.120 (0.986-1.272)	.08
Diastolic BP, per SDS	1.733	.0009	0.007	.04	1.215 (1.034-1.427)	.02	1.088 (0.929-1.273)	.29
HR, bpm	-0.071	.10	0.000	.21	0.994 (0.981-1.007)	.37	1.009 (0.996-1.023)	.17
eGFR, per ml/min/1.73 m^2	-0.246	<.0001	0.000	.72	0.972 (0.957-0.988)	.0006	1.005 (0.990-1.019)	.54
Log-uACR	0.681	.03	0.001	.71	1.032 (0.936-1.139)	.53	0.984 (0.895-1.083)	.75
Hemoglobin, per g/dL	-1.219	.0005	-0.000	.97	0.853 (0.764-0.953)	.005	0.996 (0.896-1.107)	.94

Continued table.

	Univariable linear regression N=540				Univariable logistic regression N=540			
	LVMI	**p value**	**RWT**	**p value**	**LVH (95% CI)**	**p value**	**Concentric geometry (95% CI)**	**p value**
Serum bicarbonate, mmol/L	-0.593	.0002	-0.000	.96	0.917 (0.872-0.964)	.0008	0.993 (0.947-1.042)	.79
Log-Ferritin	0.465	.41	-0.005	.22	1.110 (0.933-1.321)	.24	0.898 (0.757-1.065)	.22
Log-PTH	1.231	.04	0.006	.15	1.166 (0.970-1.402)	.10	1.132 (0.946-1.354)	.17
Log-CRP, per mg/L	-0.051	.89	0.001	.84	0.932 (0.834-1.042)	.22	0.979 (0.879-1.091)	.71
Cholesterol, per mg/dL	0.001	.92	-0.000	.49	1.000 (0.997-1.004)	.94	1.000 (0.996-1.003)	.78
HDL cholesterol, per mg/dL	-0.129	.001	-0.000	.53	0.984 (0.971-0.997)	.01	0.999 (0.987-1.011)	.88
LDL cholesterol, per mg/dL	-0.003	.86	-0.000	.28	1.000 (0.996-1.005)	.93	0.998 (0.994-1.003)	.48
Uric acid, per mg/dL	-0.210	.52	-0.002	.30	1.021 (0.925-1.126)	.69	0.985 (0.894-1.085)	.76
Serum calcium, per mmol/L	-10.81	.0004	0.006	.78	0.435 (0.170-1.117)	.08	1.071 (0.425-2.702)	.88
Serum phosphate, per mmol/L	0.093	.95	0.031	.002	1.026 (0.652-1.615)	.91	1.570 (1.006-2.451)	.05
Serum albumin, per g/L	-0.231	.02	0.000	.67	0.985 (0.955-1.015)	.31	1.008 (0.978-1.039)	.61
Log-24,25(OH) vitamin D	-0.574	.45	0.002	.66	0.885 (0.692-1.130)	.33	0.963 (0.762-1.217)	.75

Continued table.

	Univariable linear regression N=540				Univariable logistic regression N=540			
	LVMI	**p value**	**RWT**	**p value**	**LVH (95% CI)**	**p value**	**Concentric geometry (95% CI)**	**p value**
Log-25(OH) vitamin D	0.497	.47	-0.002	.68	1.066 (0.852-1.333)	.58	0.946 (0.768-1.165)	.60
PWV, per SDS_{height}	0.431	.21	0.007	.003	1.054 (0.949-1.171)	.33	1.164 (1.048-1.294)	.005

Abbreviations: AKI, acute kidney injury; BP, blood pressure; BMI, body mass index; CAKUT, congenital anomalies of kidney and urinary tract; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathormone; uACR, urinary albumin-creatinine ratio.

Table 22. Multivariable linear regression model for LVMI at baseline after stepwise variable selection

	N=508; R^2=0.18		
	Estimate	**SE**	**p value**
Intercept	71.66	8.985	<.0001
Age, per year	0.494	0.175	.005
Sex, boys	3.549	1.164	.002
BMI, per SDS	1.301	0.449	.004
Height, per SDS	-1.214	0.428	.005
Systolic BP, per SDS	1.888	0.414	<.0001
HR, per BPM	-0.128	0.043	.003
eGFR, per ml/min/1.73 m^2	-0.152	0.049	.002
Hemoglobin, per g/dL	-0.919	0.365	.01
HDL cholesterol, per mg/dL	-0.066	0.039	.09
Serum calcium, per mmol/L	-5.744	3.167	.07

Abbreviations: BMI, body mass index; BP, blood pressure; BPM, beats per minute; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, heart rate.

Table 23. Multivariable linear regression model for RWT at baseline after stepwise variable selection

	N=508; R^2=0.04		
	Estimate	**SE**	**p value**
Intercept	0.302	0.025	<.0001
Height, per SDS	-0.006	0.003	.04
Serum phosphate, per mmol/L	0.030	0.010	.003
PWV, per SDS_{height}	0.005	0.002	.04

Table 24. Multivariable logistic regression model for LVH at baseline after stepwise variable selection

	OR (95% CI)	**p value**
Age, per year	1.078 (1.015-1.145)	.01
Sex, boys	1.634 (1.082-2.468)	.02
BMI, per SDS	1.261 (1.070-1.486)	.006
Height, per SDS	0.800 (0.689-0.929)	.003
Systolic BP, per SDS	1.209 (1.047-1.396)	.01
Hemoglobin, per g/dL	0.874 (0.773-0.987)	.03

Table 25. Multivariable logistic regression model for concentric remodeling (abnormal RWT) at baseline after stepwise variable selection

	OR (95% CI)	**p value**
Age, per year	0.945 (0.895-0.998)	.04
PWV, per SDS_{height}	1.155 (1.037-1.286)	.009

4.5. PWV and CKD progression

A total of 389 patients have reached the composite endpoint of CKD progression with a median time to event of 2.34 years (0.88-4.51). Seventy-five patients (19.3%) reached eGFR <10 ml/min./1.73 m^2, 158 (40.6%) had 50% loss in eGFR and 156 (40.1%) started KRT. Censoring events were defined as in Table 26.

Table 26. Distribution of censoring events

	Frequency (%)
End of study period	7 (3.3)
Patient's wish	31 (14.6)
Lost to follow-up	91 (42.9)
Transition to adult clinic	64 (30.2)
Death	2 (0.9)
Other	17 (8)

CKD progression-free survival did not differ when stratifying study cohort by the presence of abnormal PWV (Fig. 36) or by PWV SDS_{height} tertiles (Fig. 37).

When added to a Cox proportion hazards model that included other predictors of CKD progression (age, sex, primary kidney disease, BMI SDS, systolic BP SDS, eGFR and uACR) PWV SDS_{height} was not associated with the risk of CKD progression (aHR 0.964, 95% CI 0.896 to 1.037) (Table 27).

Table 27. Cox-proportional hazards regression model for CKD-progression free survival

	Estimate	Hazard ratio (95% CI)	p value
Female sex	-0.114	0.891 (0.707-1.124)	.33
Age, per year	0.043	1.044 (1.011-1.078)	.009
Diagnosis (reference: CAKUT)			
Glomerulopathies	0.412	1.510 (1.022-2.232)	.04
CKD post-AKI	0.582	1.790 (1.113-2.879)	.02
Other	0.412	1.510 (0.869-2.625)	.14
Tubulointerstitial	0.718	2.050 (1.504-2.794)	<.0001
BMI, per SDS	-0.006	0.994 (0.910-1.085)	.89
Systolic BP, per SDS	0.096	1.101 (1.017-1.193)	.02
eGFR, per ml/min/1.73 m^2	-0.080	0.923 (0.910-0.936)	<.0001
Log-uACR	0.383	1.467 (1.355-1.588)	<.0001
PWV, per SDS_{height}	-0.037	0.964 (0.896-1.037)	.33

Abbreviations: AKI, acute kidney injury; BP, blood pressure; BMI, body mass index; CAKUT, congenital anomalies of kidney and urinary tract; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-to-creatinine ratio.

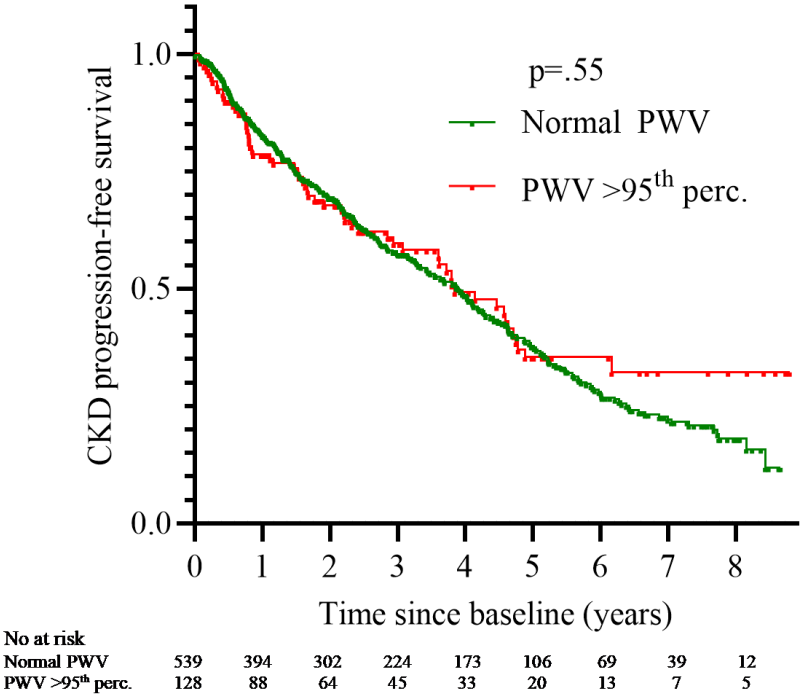


Figure 36. Kaplan-Meier curves for CKD-progression free survival stratified by normal and abnormal PWV SDS_{height}

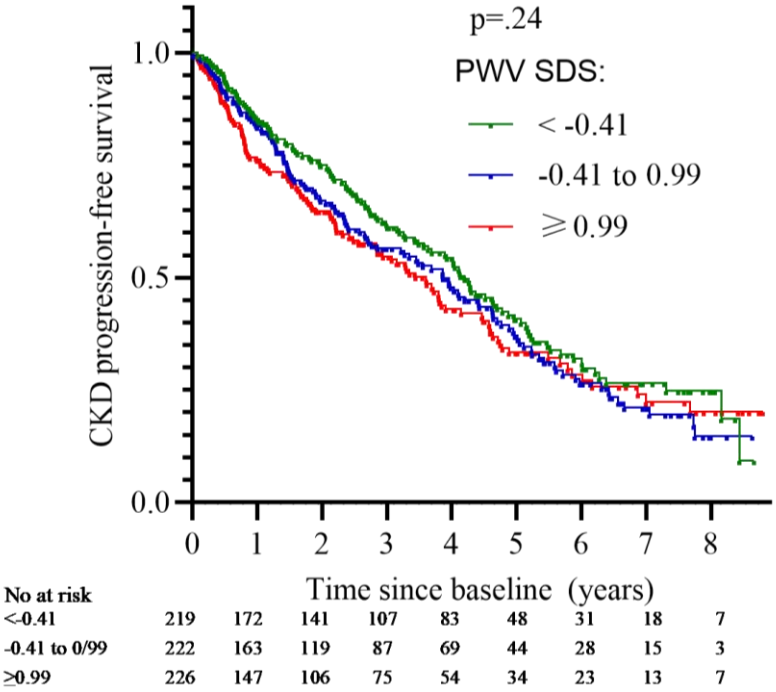


Figure 37. Kaplan-Meier curves for CKD-progression free survival stratified by PWV SDS_{height} tertiles

5. DISCUSSION

This is the first study to date to investigate and describe the characteristics and longitudinal dynamics of arterial stiffness measured by a validated PWV device in a large cohort of children with moderate-to-advanced CKD, as well as to analyze its functional impact on LV geometry and CKD progression. The principal findings of this analysis have shown that children with CKD exhibit increased arterial stiffness compared to healthy peers and abnormally increased PWV is present in one fifth of children. This increase of arterial stiffness associated to a varying extent with younger age, female sex, lower height, tubulointerstitial kidney diseases, higher BP, higher proteinuria, higher LDL levels, higher hemoglobin, lower ferritin levels, lower 25(OH)-vitamin D and higher serum calcium levels. In contrary to the findings of adult studies, increased PWV was not associated with the risk of kidney function decline over time in children. However, this analysis demonstrated an independent association of increased PWV with concentric LV remodeling. These findings are essential to clarify the pathophysiological pathways and risk factors of CV comorbidity in children with CKD and to elucidate the role of arterial stiffness monitoring in the care of these patients.

A number of previous studies have investigated arterial stiffness in children with CKD. In these studies, different parameters have been chosen to evaluate arterial stiffness though majority of studies employed measurements of PWV. PWV is currently recognized as the gold standard of arterial stiffness assessment and is less confounded by other factors than PWA-based parameters and less operator dependent than distensibility based parameters (such as Young's elastic modulus or beta-stiffness index). (6,188) The latter require local measurements of BP (e.g. on the carotid artery) that may be difficult to obtain in hard to reach arteries and technically challenging in younger children. (110,112) Therefore, PWV is the parameter of choice to estimate arterial stiffness in children in routine clinical practice and has been chosen to be used in this study.

The device chosen to be used in the present study was Vicorder (Skidmore Medical) which is an oscillometric device that employs two cuffs (neck and thigh), is easy-to-use and relatively operator-independent. Importantly, Vicorder device was also validated in several studies for use in children and showed excellent agreement with the gold-standard tonometric readings with Sphygmocor. (42,113,114) In addition, reference data from a large cohort of European children aged 6-18 years for PWV measured by Vicorder device is available and has been used to standardize obtained values to age/height in the present analysis. (35) Appropriate device and pathway measurement selection

is essential to obtain reliable estimates of PWV, thus, allowing to draw clinically meaningful conclusions. The pathway calculation chosen for the present study used over-the-skin measurements of pathway via the umbilicus which has been shown to have the best agreement with gold standard PWV measurements in a large validation study and may better reflect the actual pathway of aorta and its branches. (35)

Arterial stiffness in children increases non-linearly with growth and exhibits sex differences that become apparent in adolescence. (35–38,42) Therefore, a single cut-off value cannot be used for differently aged children. Moreover, studies in children have clearly related arterial stiffness in children with body dimensions and height, particularly. (34) For these reasons, PWV has to be normalized to calculating SDS (or using percentiles) to allow for appropriate comparisons in heterogeneous groups of children. Available reference data allows for sex-specific standardization to height or age. (35) Analysis of PWV values normalized to age or height in a large reference cohort of healthy European children showed that PWV values standardized to age underestimated PWV in short-for-age children. (35) This may be of particular importance in the pediatric CKD population where short stature is prevalent. Kis et al. analyzed PWV in 11 children on dialysis and compared them to 133 healthy controls. The analysis revealed that patients on dialysis did not differ from healthy controls when compared to age-matched controls but had higher PWV when compared to height-matched controls. (118) Similar findings were revealed in a study by Cseprekal et al. that investigated 25 children after kidney transplantation. In the latter study, PWV of the transplanted patients differed only when compared to height-age matched controls or when indexed to height. (119)

The importance of height is a known issue when interpreting hemodynamic parameters that may be intrinsically related to body dimensions, e.g. BP in children needs to be standardized to height, age and sex. Previous studies in children have found that arterial tree dimensions are closely related to height. (119) The afore discussed study by Senzaki et al. clearly indicated that age-related decrease in arterial elasticity is accompanied by changes in arterial buffering capacity that are directly related to increase in body dimensions. This suggests that changes in body size with growth may be more important than age-specific changes in arterial elasticity when analyzing large artery functions. (34) This also means that comparing arterial parameters of small-for-age children with age-matched controls may be inappropriate and may lead to underestimation of arterial functional parameters.

In the present cohort, a systematic difference between PWV values normalized to age and height in relationship to height SDS of study patients

was also observed. The difference between PWV standardized to height and age was significantly higher in small-for-age children, corresponding to previous findings. The lack of comparisons to an unconfounded, gold standard method (e.g. invasive PWV estimation) does not allow to reliably conclude that standardization to height provides accurate estimates of factual PWV. Nevertheless, in this cohort of children with CKD absolute PWV values were higher in children with short stature compared to those with normal height. Although abnormally increased PWV was identified in study participants in spite of normalization to height or age, the prevalence of increased PWV was higher after standardization to height (19.2% vs 14.2%). Whether standardization to height is associated with any potential systematic error remains to be answered. It might be possible that standardization of PWV to age, sex and height (as in the case of BP) would be most appropriate in children but is currently impossible due to unavailability of such reference data.

Adult studies have established a clear link between CKD and increased arterial stiffness. (7) Available findings in the population of pediatric patients with CKD, however, were conflicting. In the present study increased PWV was identified in 19.2% of children with moderate-to-advanced CKD. These findings are in contrast to previous larger scale studies in the pediatric population. A study of 95 children from the Chronic Kidney Disease in Children (CKiD) cohort with CKD stages 1-4 revealed similar PWV compared to healthy children (mean PWV SDS -0.1±1.1, standardized to both age and height). (152) Another study by Sinha et al. included 188 children with CKD stages 1-5 and compared them to age matched controls. PWV was similar to healthy controls irrespective of BP level in children with CKD. (156) Both of these studies concluded that PWV is not increased in children with CKD. However, both of these studies also differed significantly from the present study population by degree of kidney dysfunction: mean eGFR was ~63-64 ml/min/1.73 m^2 in both studies, which is more than two-fold higher compared to the 4C population (26.8 ml/min/1.73 m^2). Besides, other differences, including the distribution of primary kidney disease (almost half of children with glomerular diseases in the CKiD Study vs one fifth in the 4C Study) may also have a role in explaining reported differences. Moreover, investigators from the CKiD Study did not report the number of patients with abnormally increased PWV and only reported the mean value of the studied population. This may have limited comprehensive understanding of the studied cohort, considering the wide range of eGFR of study participants (from normal kidney function to advanced CKD) potentially leading to "dilution" of higher PWV values.

On the other hand, a number of small scale case-control studies that included children with advanced CKD or children on dialysis have reported opposite results and reported increased PWV compared to healthy peers. In addition, two studies by Karava et al. that included 26 children CKD stages 2-5 (9 on APD) and 19 children on HD have reported the incidence of abnormally increased PWV to be 61.5% and 26.3%, respectively. (157,158) The results of these studies, however, cannot be directly transferred to the pre-dialysis CKD population and come from small and heterogeneous groups of children. Dialysis may particularly confound the assessment of PWV due to its strong relation to fluid status and residual kidney function. (125) Therefore, the present study is the first to report on the increased PWV in a large cohort of children with moderate-to-advanced CKD not yet on KRT. This finding suggests that arterial stiffness is increased in stages of advanced uremia, where comorbidities that act as potential risk factors for vascular pathology are more pronounced. It may be so that in earlier CKD stages PWV remains relatively unchanged and is similar to healthy children. Indeed, this is further suggested by the fact that determinants of PWV in children with earlier stage CKD (CKiD Cohort) were BP, age and race – same as those found in studies involving healthy children. (35–39,42,152)

Studies in adults have linked decline in kidney function over time with progressive arterial stiffening. In one of the largest cohort studies investigating changes of PWV over time in adults with CKD (CRIC Study) that involved over 2,500 adults with CKD PWV increased by 0.23 m/s per each 10 ml/min/1.73 m^2. (7) However, pediatric studies did not report associations between eGFR and PWV. (23,152–155) Similarly, no association between eGFR and PWV (neither at baseline, nor over time) was found in the present analysis and PWV did not differ by CKD stages. This suggests that kidney function decline, as measured by eGFR, *per se* is not related to arterial stiffening and CKD-related complications may be more important.

Several groups of kidney disorders, such as glomerulonephritis or HUS, could be expected to have a more pronounced increase in arterial stiffness due to the potential systemic effects on the arterial tree. Indeed, increased arterial stiffness was reported in patients with systemic vasculitis and macrovascular complications have been reported in children with atypical HUS. (189,190) However, no systematic differences of PWV were observed when comparing patients by primary kidney disease at baseline. In the longitudinal analysis, however, higher PWV over time was associated with tubulointerstitial diseases and other kidney diagnoses category (with a decreasing effect over time). The latter group included, among others, patients with diabetic CKD and renovascular disease who could be expected to exhibit more severe

vascular phenotype. However, the very small size of this subgroup limits the ability to draw any reliable conclusions.

PWV was significantly associated with proteinuria. After stratifying patients to different degrees of proteinuria, only patients with nephrotic range proteinuria had higher PWV compared to those with sub-nephrotic proteinuria or normal protein excretion at baseline. Several adult studies have investigated the relationship between proteinuria and arterial stiffness. These studies typically focused on arterial stiffness as a potential risk factor for developing proteinuria. Indeed, proteinuria is believed to reflect the generalized vascular disease (191) and is also considered to be a risk factor for both CVD development and CKD progression. (192) Therefore, proteinuria may be considered to be a marker of vascular damage that reflects the effects of increased glomerular pressure with unattenuated pulse pressure reaching kidney microvasculature via the stiff arterial tree. In previous adult studies, aortic stiffness was related to incident proteinuria in patients with type 2 diabetes (193), increased explanation of proteinuria variation in patients with CKD (191) and associated with proteinuria in a screened general adult cohort. (194) Increased PWV has also been reported in adults and children with nephrotic syndrome, however, in these studies increase in arterial stiffness was related to BP and ferritin levels but not proteinuria *per se*. (195,196)

The baseline association of proteinuria with PWV in the present analysis remained after adjusting for BP, age and anthropometric factors but was strongly attenuated and became insignificant after adjustment for 25(OH)-vitamin D levels. Nephrotic range proteinuria has been related to vitamin D deficiency that is frequently observed in patients with nephrotic syndrome and is believed to be multifactorial, including loss of vitamin D binding protein and 25(OH)-vitamin D in urine. (197) In the studied population 25(OH)-vitamin D was lower with increasing uACR levels and was lowest in patients with nephrotic-range proteinuria. On the other hand, in the longitudinal multivariable model PWV SDS_{height} was independently associated with proteinuria over time. The association could be bidirectional – suggesting that long-term exposure to higher arterial stiffness may lead to higher proteinuria, as well as that proteinuria may mirror microvascular damage and/or present a risk factor itself for accelerated arterial stiffening.

In normal physiological conditions arterial stiffness increases with age and this increase becomes evident in school-aged children. Particular differences arise in adolescence when boys start to demonstrate higher arterial stiffness than girls. (35–39,42) In the present study, standardized PWV showed an inverse correlation with age that was more pronounced in boys, i.e. younger boys had higher standardized arterial stiffness, whereas in girls age did not

have major influence on arterial stiffness. Overall, girls are considered to have a general biological survival benefit compared to boys. (198) However, recent data from the USRDS registry suggested that girls on KRT have approximately 36% higher mortality risk compared to boys. (199) Our results add to the existing evidence on sex-disparities in CKD population by demonstrating that girls showed higher and progressively increasing PWV over time compared to boys. This suggests, that in the state of uremic milieu the favorable CV profile of girls is lost. The reasons of this shift in CV risk profile remains unknown and cannot be answered by the design of this study but both intrinsic (biological, e.g. hormonal effects) and external factors (access to care, peculiarities in management) may have a potential role.

The fact that age remained a significant predictor of arterial stiffness in the multivariable model adjusted for various potentially confounding factors, suggests that younger patients are at higher risk of developing arterial stiffness. Previous studies have suggested that the effects of BP on arterial stiffness in healthy children become apparent only in adolescence, when elastic properties of the arteries are unable to further compensate for the increasing pulsatile stress. (37) Present data, however, suggests that younger children may be actually more susceptible to damage caused by a wider spectrum of vascular injuries than BP alone. Younger children at study baseline also demonstrated accelerated arterial stiffening over time, suggesting that early damage to the arterial tree determines a more progressive course of vasculopathy. Additionally, birth weight, which was associated with risk of increased arterial stiffness in healthy children due to impaired perinatal elastin production (35,40), did not influence PWV in the present population, suggesting that CKD-specific vascular injuries may surpass the effects of perinatal risk factors.

Previous studies have shown that body dimensions are important determinants of arterial stiffness in children with CKD. Particular associations are seen with height in healthy children, which increasing height being associated with higher arterial stiffness. (34,38) Shorter children would be expected to have a shorter travel distance and also shorter travel time. Both of which could have an effect on the calculated PWV results. However, this issue was addressed in the present study by using height-standardized PWV values. Even after standardization to height and adjustment for various confounders in a multivariable model, shorter height for age remained an important determinant of PWV, both at baseline and over time. This effect has not been described in previous studies and the explanations behind this association remain unclear. Height, however, could be an integral indicator of severity of CKD and also mirror the quality of its management. (200) On the other hand,

a contribution of a potential statistical confounding arising from the standardization of PWV to height which may not account for age-related changes in arterial elastic properties cannot be ruled out completely.

Previous studies also have identified that obesity is directly linked to increased risk of arterial stiffening. (46) Only a small proportion of patients (5.1%) in the present study were obese and higher fraction of patients (18.3%) were overweight. BMI or abnormally increased body weight, however, were not associated with PWV. A recent study in children without CKD also did not observe differences in PWV between normal weight and overweight or obese children. The authors of this study explained the lack of this association by potentially increased vasodilatory status in children with increased body mass. (201) A study by Karava et al. investigated the relationship between body composition and arterial stiffness in a small group of children with pre-dialysis CKD and on PD. They found a U shaped relationship between BMI and PWV, suggesting both – underweight and overweight may be associated with higher risk of arterial stiffening. (158) The lack of similar associations in this analysis could be explained by the low prevalence of obesity and relatively low prevalence of severe malnutrition-inflammation (8.1% underweight), that are more common in patients on dialysis.

BP is known to be one of the most important determinants of arterial stiffness. Arterial hypertension increases pulsatile stress to large arteries which can in turn promote elastin fragmentation that results in arterial stiffening. BP has been associated with arterial stiffness both in healthy children (35,38,42) and also in previous studies of children with CKD irrespective of the severity of kidney function impairment. (23,152,155,161,165,166) Results of this analysis also clearly indicate that BP is strongly associated with arterial stiffening in children with CKD. Each increase of one SDS in diastolic BP was associated with 80% higher risk of having abnormal PWV and the presence of arterial hypertension by office BP was associated with a three-fold increased risk of abnormal arterial stiffening. Given the significant interactions between age and BP from previous studies in healthy children, (37) we additionally studied whether these effects also exist in children with CKD. In contrary to previous findings from healthy children, younger age but not older age was associated with a stronger contributing effect of BP on arterial stiffening. Thus indicating that in the state of uremia and more severe increases in BP (as compared to physiological changes in BP in healthy children) younger children are more susceptible to detrimental effects of increased pulsatile stress caused by BP.

Uric acid has long been considered a CV risk factor, although the existing evidence remain inconclusive. Even more focus has been put on the

importance of uric acid in the development of hypertension, which may be caused by hyperuricemia induced kidney injury, endothelial dysfunction, vascular inflammation and VSMC proliferation. (202) Contrary to the presumed associations, lower uric acid levels were associated with higher PWV. This association, however, disappeared after adjusting for age and height. Indeed, studies in healthy children have shown direct association of uric acid with age, therefore, the inverse relationship could possibly reflect the effects of younger age on PWV. (203) Serum bicarbonate and metabolic acidosis were linked to higher arterial stiffness in adults on dialysis, and, recently, also in a large group of patients with pre-dialysis CKD. The observed associations were explained by the potential consequences of metabolic acidosis, that include alterations in calcium-phosphorus metabolism, promotion of inflammation and insulin resistance. (204) However, serum bicarbonate was not associated with arterial stiffness in the present cohort.

Dyslipidemia has been associated with arterial stiffness, particularly in patients with familial hypercholesterolemia (205) and these associations have already been described in young children. (52) No previous studies in children with CKD have, however, identified quantitative lipid abnormalities as important predictors of arterial stiffness. (152) Similarly, we did not find independent associations with arterial stiffness in our cross-sectional analysis at baseline. This may be related to the fact that in the state of CKD serum lipids are not related to CV risk in the same manner as in patients with normal kidney function. First, malnutrition-inflammation that becomes more important with progressive CKD may lead to diminished cholesterol levels and therefore affect the potential relationship. (168) Moreover, previous studies in pediatric CKD patients have shown that qualitative but not quantitative lipid abnormalities might be of higher importance on vascular function. Particular focus was put on HDL, which when taken from children with CKD significantly affected NO synthesis and was associated with higher arterial stiffness compared to HDL cholesterol from healthy children. (169) Nevertheless, higher LDL cholesterol levels in the present analysis associated with higher PWV over time, suggesting that long-term exposure to increased LDL may contribute to arterial stiffening. These associations could be explained by different mechanisms (premature atherosclerosis, systemic and local inflammation and effects on endothelial functions) and are supported by studies in hypercholesterolemic children. (52)

Anemia is a well-recognized risk factor of CVD in the CKD population. A term ‘cardio-renal-anemia’ syndrome has been used to define a detrimental association of anemia in the setting of kidney dysfunction to determine poor CV outcomes. The relationship between anemia and adverse CV outcomes

may be explained by different mechanism, including the possible mirroring of metabolic/inflammatory status and reduced oxygen carrying capacity. In addition, the use or erythropoiesis-stimulating agents (ESA) in kidney anemia may aggravate CVD and have been associated with worse CV outcomes. (206) Anemia, however, is more frequently related to LV abnormalities and also was an important independent determinant of LVMI in our analysis. However, a small study in adults on HD has suggested that anemia may also be independently related to arterial stiffness. (207) In contrary to these notions, in the present analysis a small positive association of hemoglobin over time with PWV was observed which was attenuated after inclusions of time-interaction. Due to the inability to account for the use of ESAs and the small and relatively unstable effect, the observed association should be interpreted cautiously.

We did observe a significant relationship between lower ferritin levels and higher arterial stiffness. This association also was observed in the longitudinal analysis, showing a stable association between lower ferritin levels and higher PWV over time. Several studies in adults without CKD have investigated the potential associations between ferritin and arterial stiffness. In these studies, contrary to the findings of this analysis, higher ferritin levels were associated with higher arterial stiffness. (208–210) Ferritin is not only a marker of iron stores in the body, but also a marker of inflammation acting as an acute phase reactant. Indeed, the association between ferritin levels and arterial stiffness is typically analyzed from a point of inflammation and associated effects of oxidative stress. (209) Other authors, however, suggested that iron stores reflected by ferritin but not subclinical inflammation is the reason behind the positive association between ferritin and arterial stiffness. (210) The association of high ferritin levels (>500 ng/mL) over time have also been associated with progressive arterial stiffness in adult patients on HD with a follow-up of three years. (211)

On the other hand, an experimental *in vitro* study reported that the addition of heme to a culture of human SMC (HSMC) inhibited calcium deposition. Heme is major inducer of heme-oxydase and ferritin, therefore, the authors further looked which of these factors have major role in the attenuation of calcification. Calcification was inhibited by addition of ferritin but not heme-oxydase. Furthermore, the authors showed that ferritin also suppressed the osteoblastic transformation of HSMC which was evident by decreased expression of bone biomarkers and downregulation of a gene responsible for osteoblastic transformation. (212) Ferritin is also recognized as a protective factor to endothelium with antioxidant, antiapoptotic and antiproliferative effects. (213) It is therefore possible that the observed counterintuitive

findings in this study reflect the potential protective role of ferritin on vascular remodeling which could result in arterial stiffening.

Indeed, previous studies in the general adult population or the HD population could have explored the different mechanistic roles of ferritin. In the general population where multi-etiologic and ageing driven mechanisms of arterial stiffening could be expected (different to arterial alterations observed in CKD), ferritin could be seen as a marker of inflammation - an important contributor to vascular pathology. In the HD population where frequent parenteral iron supplementation is required, ferritin could also act as a marker of inflammation or severe iron overload. Whereas in the studied population, patients are not expected to receive large-dose parenteral iron therapy and vascular pathology is much more closely related to CKD-MBD. Therefore, the inverse relationship between ferritin and PWV could possibly reflect the protective effect of ferritin. However, these potential explanations are speculative and additional studies focused on the potential effects of ferritin on arterial stiffness in children with pre-dialysis CKD are required.

CKD-MBD is a well-known risk factor of CKD-associated vasculopathy. Previous studies have clearly identified different components of CKD-MBD to be causally associated with arteriosclerosis and vascular calcifications in patients with CKD. Phosphate, in particular, is deemed to be the predominant vascular toxin, capable to induce osteoblastic transformation and calcium load-dependent vascular calcifications in large arteries. (214) Indeed, previous studies in children have identified correlations between phosphate (or Ca x P product) and PWV in children on dialysis. (79,163,171) Similarly to these previous studies, in the univariable regression analysis of the studied population serum phosphate levels were directly associated with higher PWV. However, the association was lost when the model was adjusted for other covariates. Similar findings were observed with PTH that lost its significant univariable effect after adjusting for other covariates. This suggests a more complex interaction of different components of CKD-MBD than individual contributions of serum phosphate or secondary hyperparathyroidism in the pathophysiological pathway of arterial stiffening. These univariable effects could also relate to lower vitamin D levels that are mechanistically related to secondary hyperparathyroidism.

Another important finding of the present analysis is a significant and independent effect of lower vitamin D levels on increased PWV. Large adult cohort studies such as the Framingham Offspring Study or Health Professionals Follow-up Study have found up to two-fold increased risk of CV events in patients with vitamin D deficiency. (215) The negative effects of vitamin D deficiency are explained by increased activation of the RAS

system which has been demonstrated in experimental animal studies, modulation of insulin sensitivity and direct effects on CV remodeling. (215) Studies in adults also suggest that vitamin D may be closely related to vascular pathology and development of arterial stiffness. Large study involving healthy adults demonstrated relationship between vitamin D deficiency and arterial stiffness – lower vitamin D independently correlated with markers of arterial stiffness and endothelial dysfunction. (216) The direct effect on vascular system could be caused by vitamin D mediated decrease in protective endothelial functions, increased calcium influx, activation of the RAS system, stimulation of VSMC proliferation, and alterations in other markers of the CKD-MBD pathway. (216) A study in overweight African Americans even suggested that vitamin D3 supplementation may reduce arterial stiffness. (217) Other studies, however, failed to demonstrate improvements of BP parameters or arterial stiffness with vitamin D supplementation. (218)

To date, only one study investigated the association between vitamin D levels and PWV in children with pre-dialysis CKD and found a similar negative correlation. (178) This study, however, had several drawbacks, including the lack of multivariable analysis and standardization of PWV. In addition, another study found an association between vitamin D levels and AIx in a small heterogenous group of children with pre-dialysis and dialysis-dependent CKD. AIx, however, as discussed previously, is not directly representative of arterial stiffness and is highly influenced by other hemodynamic factors. (219) Of note, we were unable to study the effects of vitamin D deficiency on PWV over time due to the lack of vitamin D testing after baseline visit which could have strengthened or attenuated the evidence of this relationship.

A number of studies in adults with CKD have identified arterial stiffness as a risk factor of CKD progression. The mechanism via which arterial stiffness accelerates kidney function decline is related to the transmission of pulsatile pressure into low microvascular resistance beds due to unattenuated pulsatile flow in the stiff arterial tree. Excess pulsatility and pressure in glomeruli may increase intra-glomerular pressure and lead to faster eGFR decline. (93) In the largest study to date investigating the effects of PWV on various outcomes in adults with CKD – the CRIC Study – PWV was significantly associated CKD progression. Patients with the highest tertile of PWV had higher risk of CKD5 (HR 1.37, 95% CI 1.05 to 1.80) and CKD5 or 50% decline in eGFR (HR 1.25, 95% CI 0.98 to 1.58). (220) Arterial stiffness was also linked to increased risk of developing incident CKD or eGFR decline in several cohorts of adults without CKD of multiethnic origin. (95–98)

The association between arterial stiffness and kidney function decline is, however, equivocal and other studies failed to association between increased PWV and risk of kidney function decline. In a study by Briet et al., involving 180 patients with CKD, carotid artery circumferential wall stress and PP, but not arterial stiffness were associated with CKD progression. (144) Similarly, no associations were detected in an adult cohort of 225 patients with CKD by Chue et al. (145) Another study involving patients with relatively preserved kidney function also failed to show associations between PWV and rapid kidney function decline. (146) No associations with kidney function were also found in the Framingham Heart Study involving a large cohort of adults. (221) The disparities in the findings coming from different studies could be explained by different techniques used to measure PWV and intrinsic differences in studied populations.

So far, no studies have analyzed the potential effect of PWV on kidney function decline in the pediatric population. Association with CKD progression is an important potential consequence of increased arterial stiffness that could provide argument for its monitoring and make it a potential therapeutic target. The present analysis is in line with the studies from the adult population that found no relationship of PWV with CKD progression, neither in univariable analysis, nor after adjustment for important covariates. Whether increased PWV in childhood could have an effect on adult kidney outcomes remains uncertain and additional long-term studies extending patient follow-up into their adulthood would be needed to answer this question. It may be so that the magnitude of PWV increase in childhood is clinically too small to cause substantial target organ damage as compared to adult population with an extended risk factor exposure.

Another important potential consequence of increased arterial stiffness is increased LV afterload as aortic stiffness is one of the major determinants of resistance that LV encounters during systole. (87,89) The resultant increase in LV afterload could in turn lead to LV remodeling. Changes in LV geometry develop as a compensatory mechanism and typically follow a sequence of LV concentric remodeling (evident by increasing RWT, but preserved normal LVMI), followed by concentric LVH and, finally, eccentric LVH. (222) These alterations may further result in systolic and diastolic LV dysfunction which has already been demonstrated to be present in children with CKD (25) and, eventually, clinically overt HF.

Previous studies have reported LVH in nearly half of pediatric CKD patients. (27,223) These findings are in line with the findings from this analysis where half of the studied patients had abnormal LV geometry with eccentric LVH being the predominant pattern, as also reported previously.

(27,223) Concentric LVH typically results from pressure overload (increased afterload), such as in arterial hypertension, whereas eccentric hypertrophy occurs in the setting of volume overload (increased preload) which may be found in children with CKD and progressive loss of residual kidney function. (27,224) Despite the high prevalence of LV geometry abnormalities, the determinants of these early changes remain unclear. A study by Matteuci et al. failed to identify BP as an independent predictor of LVMI or LV geometry in a cohort of children with mild-to-moderate CKD. In this study, male gender, BMI, anemia, eGFR, younger age, serum albumin and CRP levels were among the significant determinants of LV geometry. (27) These findings also suggested that non-hemodynamic risk factors may be more important for the development of LV abnormalities in children with CKD. Other authors, however, have associated BP with LVM in the pediatric CKD population. (223)

In adults with CKD increased PWV was associated with structural remodeling of left ventricle and left atrium, myocardial fibrosis and LV twist mechanics. (138–140) To date, only one small study involving children on HD investigated the relationship between PWV and LVMI and did not find any association. (171) However, such an association was observed in children with ADPKD where high prevalence of arterial hypertension is typically observed even before kidney function decline. (176) In the present study, LVMI or LVH was not associated with PWV but PWV was significantly higher in children with concentric LV remodeling. Importantly, the results of this analysis showed an association between PWV and RWT – an indicator of concentric remodeling. This association persisted after adjustment for other potential confounders and RWT was not associated with neither eGFR, nor BP or other factors. Such association has not been described in children with CKD previously but was suggested in studies involving healthy children. For example, a study involving large group of healthy children and adolescents has shown that global stiffness index (calculated by pooling results of carotid stiffness, augmentation index, brachial distensibility and PWV) was significantly associated with LVMI and RWT. (225) While in another study of pediatric patients with arterial hypertension, RWT was only determined by night-time diastolic BP indices. (224)

The association between PWV and LV concentric remodeling is an important finding providing rationale for PWV measurements in high CV risk pediatric populations, such as children with CKD. Studies in adults have suggested that arterial stiffness measured by pressure-independent methods (such as PWV) is associated with LV concentric remodeling while pressure dependent mechanisms are related to LVM and LVH. This seems to be an

important mechanism in the ventricular-vascular coupling, explaining the relationship between hemodynamics and LV geometry. Arterial stiffening may lead to LV stiffening, which in turn reduces LV filling and leads to LV remodeling. (226) The exact mechanisms behind the observed association between PWV and RWT and whether this is a result of increased aortic impedance, or is related to the timing of the reflected wave, remains uncertain. Nevertheless, studies in adult population provided evidence that concentric remodeling may lead to adverse CV sequalae and worsen CV prognosis. LV concentric remodeling itself is associated with higher risk of CV events even after adjustment for potentially confounding covariates. (227) In addition, concentric remodeling and LVH have also been linked to reduced heart contractility and worse CV prognosis. (228)

The importance of PWV in the development of LV geometry changes has been an object of debate. A study investigating adults with a wide age interval suggested that in younger adults arterial stiffness may actually reflect increased intraventricular PWV and may not be related to increased LV afterload or changes in LV geometry. (177) Present findings, however, suggest that in the state of CKD when arterial pathology is more prevalent when in healthy population, PWV is independently related to cardiac geometry even in young children.

It is important to acknowledge that the present study has several limitations. Missing covariate data in some of the patients did not allow to include the whole enrolled sample for the present analysis. In addition, missing 25(OH)-vitamin D levels over the follow-up precluded the investigation of time-varying association. Incomplete follow-up due to patient dropouts or censoring at the start of KRT decreased sample size over the time, thus reducing statistical power. Informative censoring due to dropouts at the start of KRT may also have an impact of observed associations over time. This study also did not assess cognitive function – an important target of increased arterial stiffness. Importantly, it remains unknown whether standardization to height or age does not introduce systematic error which could lead to risk of observing spurious associations. This possible limitation was addressed by including absolute PWV values into the longitudinal analysis, which made it possible to confirm the stability of some observed associations. In spite of the aforementioned limitations, this study exhibits several important strengths. First, large sample size and longitudinal follow-up of a relatively homogenous group allows to identify significant and reliable associations. Moreover, the use of highly standardized procedures and validated measurement techniques for outcome and covariate assessment along with an extensive statistical adjustment allows to minimize potential information bias.

In summary, this is the first study to demonstrate increased arterial stiffness in a large cohort of well-phenotyped children with moderate-to-severe CKD. The longitudinal nature of this study allowed to confidently identify risk factors of increased arterial stiffness that include younger age, shorter height, female sex, tubulointerstitial kidney diseases, proteinuria, BP, lower vitamin D and ferritin levels, and higher LDL cholesterol levels. The identification of these risk factors have important clinical implications because they allow to identify patients at increased CV risk and select appropriate monitoring strategy. Moreover, several of these risk factors are modifiable (e.g. proteinuria, BP, vitamin D and LDL cholesterol levels) and appropriate therapeutic interventions could possibly attenuate increased CV risk observed in childhood CKD. Finally, this study allowed to elucidate the clinical consequences of increased arterial stiffness in childhood CKD and to show for the first time that it associates with LV geometry but has no effect on the risk of CKD progression.

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CONCLUSIONS

1. Arterial stiffness measured by the gold standard method of pulse wave velocity is increased in one-fifth of children with stage 3-5 pre-dialysis chronic kidney disease.
2. Increased pulse wave velocity in children with stage 3-5 pre-dialysis chronic kidney disease associates with younger age, female sex, lower height, tubulointerstitial kidney diseases, higher blood pressure, lower ferritin levels, higher proteinuria, higher low-density lipoprotein cholesterol and lower vitamin D levels.
3. Increased pulse wave velocity in children with stage 3-5 pre-dialysis chronic kidney disease is associated with concentric left ventricular remodeling but not left ventricular mass index or left ventricular hypertrophy.
4. Pulse wave velocity in children with stage 3-5 pre-dialysis chronic kidney disease is not associated with the risk of chronic kidney disease progression.

CLINICAL AND PRACTICAL IMPLICATIONS

Although it is well-known that CVD carries a significant burden in children with CKD with detrimental effects on patient survival, the strategies to monitor its development remain relatively unknown. ESH pediatric hypertension guidelines recommend monitoring PWV as a marker of target organ damage in hypertensive children. The importance of PWV in the pediatric CKD population with high prevalence of hypertension and additional vascular risk burden, however, was unclear. Recommendations are frequently transferred from the adult population that exhibits large differences compared to children. This study reliably confirmed previous notions about increased PWV in children with moderate-to-advanced CKD. Moreover, the results of this analysis suggest that PWV in children is not a risk factor for CKD progression as suggested by adult studies. However, even in childhood it does associate with surrogate CV outcomes – abnormal LV remodeling. Collectively, these findings provide strong rationale for routine PWV monitoring in children with CKD3-5. Moreover, the identification of non-modifiable risk factors, such as younger age, female sex, tubulointerstitial kidney diseases or shorter height allows for risk stratification when choosing monitoring strategy. Finally, the identification of other modifiable risk factors, such as BP, dyslipidemia, proteinuria and vitamin D deficiency suggests inclusion of PWV as a potential outcome in trials investigating interventions aimed at controlling these factors. Based on the results of this analysis, PWV standardization to height appears to be more appropriate for meaningful interpretation of PWV in children with CKD. Nevertheless, studies with healthy children should aim to develop reference data for PWV accounting for age, sex and height altogether.

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SUPPLEMENTS

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Suppl. Table 1. Baseline characteristics of the study population stratified by sex

Variable	**Boys (n=437)**	**Girls (n=230)**	**p value**
Age, years	12.2 ± 3.41	12.2 ± 3.24	.78
BMI, kg/m^2	18.5 ± 4.01	18.1 ± 3.5	.16
BMI SDS	0.13 ± 1.34	0.09 ± 1.12	.74
Height, cm	142 ± 21.1	139 ± 17.9	.03
Height SDS	-1.25 ± 1.32	-1.51 ± 1.38	.02
Weight, kg	39.2 ± 17	36.2 ± 13	.008
Small for gestational age, %	72 (19.1)	32 (16.2)	.40
Diagnosis			
CAKUT	324 (74.1)	140 (60.9)	.006
Glomerulopathies	30 (6.9)	27 (11.7)	
CKD post-AKI	17 (3.9)	18 (7.8)	
Tubulointerstitial	48 (11)	34 (14.8)	
Other	18 (4.1)	11. (4.8)	
Systolic BP, mmHg	114 ± 15.1	111 ± 14.3	.05
Systolic BP SDS	0.86 ± 1.37	0.78 ± 1.31	.50
Diastolic BP, mmHg	68.9 ± 12.3	69.5 ± 12.4	.57
Diastolic BP, SDS	0.68 ± 1.07	0.69 ± 1.11	.89
HR, bpm	81.1 ± 13.6	83.6 ± 13	.03
eGFR, ml/min/1.73 m^2	26.8 ± 11.5	27.1 ± 11.7	.80
uACR, mg/g	326 (86.4 – 1152)	338 (102 – 1530)	.26
Proteinuric, %	381 (87.2)	202 (87.8)	.81
Neprotic range proteinuria, %	51 (11.7)	37 (16.1)	.11
Hemoglobin, g/dL	11.8 ± 1.63	11.4 ± 1.58	.001
Serum bicarbonate, mmol/L	21.4 ± 3.61	20.9 ± 3.48	.09
Ferritin, µg/l	69.5 (35.8 – 144)	62.5 (29.2 – 141)	.39
PTH, pmol/L	13.1 (7.74 – 23.2)	18.2 (7.28 – 25.5)	.74
CRP, mg/L	0.55 (0.22 – 1.84)	0.56 (0.26 – 3.01)	.23
Cholesterol, mg/dL	178 ± 46.6	186 ± 58.2	.08
Triglycerides, mg/dL	138 ± 76.3	142 ± 76.6	.66
HDL cholesterol, mg/dL	47.1 ± 14.1	49.3 ± 14.7	.06
LDL cholesterol, mg/dL	96.7 ± 38.3	102 ± 43.3	.14
Uric acid, mg/dL	6.57 ± 1.74	6.38 ± 1.92	.19
Serum calcium, mmol/L	2.27 ± 0.18	2.23 ± 0.19	.005
Corrected serum calcium, mmol/L	2.29 ± 0.17	2.26 ± 0.21	.10
Serum phosphate, mmol/L	1.56 ± 0.38	1.55 ± 0.35	.63
Serum albumin, g/L	39.2 ± 5.21	38.3 ± 6.46	.09
24,25(OH)-vitamin D	0.22 (0.16 – 0.38)	0.25 (0.15 – 0.38)	.70
25(OH)-vitamin D	12.7 (6.84 – 19.1)	8.69 (5.05 – 15.5)	<.0001

Suppl. Table 2. Baseline characteristics of the study population stratified by primary kidney diagnosis

Variable	**CAKUT (n=464)**	**Other (n=203)**	**p value**
Age, years	12 ± 3.4	12.7 ± 3.21	.02
Sex, boys	324 (69.8)	113 (55.7)	.0004
BMI, kg/m^2	18.3 ± 3.94	18.5 ± 3.63	.58
BMI SDS	0.14 ± 1.3	0.07 ± 1.21	.54
Height, cm	140 ± 20.5	144 ± 18.8	.01
Height SDS	-1.39 ± 1.42	-1.21 ± 1.18	.10
Weight, kg	37.6 ± 1.62	39.8 ± 15	.09
Small for gestational age, %	70 (17.7)	34 (19.1)	.68
Systolic BP, mmHg	112 ± 14.7	114 ± 15.2	.37
Systolic BP SDS	0.83 ± 1.36	0.81 ± 1.35	.80
Diastolic BP, mmHg	68.8 ± 12.1	69.8 ± 12.9	.32
Diastolic BP, SDS	0.68 ± 1.07	0.71 ± 1.11	.73
HR, bpm	81.5 ± 13.2	83 ± 14.1	.18
eGFR, ml/min/1.73 m^2	27.1 ± 11.5	26.3 ± 11.6	.42
uACR, mg/g	295 (96.2 – 1089)	402 (82.1 – 1846)	.08
Proteinuric, %	408 (87.9)	175 (86.2)	.54
Nephrotic range proteinuria, %	49 (10.6)	39 (19.2)	.002
Hemoglobin, g/dL	11.8 ± 1.56	11.3 ± 1.73	.0008
Serum bicarbonate, mmol/L	21.1 ± 3.53	21.5 ± 3.66	.21
Ferritin, µg/l	61.5 (30.4 – 136)	81.3 (41.8 – 159)	.03
PTH, pmol/L	12.4 (7.53 – 22.6)	15.8 (7.47 – 27.7)	.05
C-reactive protein, mg/L	0.57 (0.23 – 2.26)	0.53 (0.23 – 1.64)	.32
Cholesterol, mg/dL	173 ± 37.8	199 ± 69.6	<.0001
Triglycerides, mg/dL	133 ± 72.0	154 ± 84.6	.04
HDL cholesterol, mg/dL	47.7 ± 14.2	48.3 ± 14.7	.60
LDL cholesterol, mg/dL	93 ± 30.9	111 ± 53.8	<.0001
Uric acid, mg/dL	6.45 ± 1.71	6.64 ± 2.01	.25
Serum calcium, mmol/L	2.26 ± 0.17	2.24 ± 0.21	.22
Corrected serum calcium, mmol/L	2.27 ± 0.17	2.3 ± 0.2	.16
Serum phosphate, mmol/L	1.55 ± 0.37	1.57 ± 0.38	.39
Serum albumin, g/L	39.4 ± 4.38	37.8 ± 7.78	.007
24,25(OH) vitamin D	0.26 (0.17 – 0.39)	0.23 (0.12 – 0.33)	.005
25(OH) vitamin D	10.7 (6.51 – 18.3)	10.6 (5.64 – 16.3)	.17

PUBLICATION LIST

Publications related to the topic of the thesis

1. Azukaitis K, Jankauskiene A, Schaefer F, Shroff R. Pathophysiology and consequences of arterial stiffness in children with chronic kidney disease. Pediatr Nephrol. 2020 Sep 7;
2. Schaefer F, Doyon A, Azukaitis K, Bayazit A, Canpolat N, Duzova A, Niemirska A, Sözeri B, Thurn D, Anarat A, Ranchin B, Litwin M, Caliskan S, Candan C, Baskin E, Yilmaz E, Mir S, Kirchner M, Sander A, Haffner D, Melk A, Wühl E, Shroff R, Querfeld U; 4C Study Consortium. Cardiovascular Phenotypes in Children with CKD: The 4C Study. Clin J Am Soc Nephrol. 2017 Jan 6;12(1):19–28.
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