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Relation of pre-clinical arterial disease to blood pressure in children with chronic kidney disease

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Relation of pre-clinical arterial disease to blood pressure in children with chronic kidney disease

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Thesis submitted for the Degree of Doctor of Philosophy (PhD) of the University of
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Abstract

Childhood chronic kidney disease (CKD) is a devastating illness requiring life-long medical input, often progressing to end stage kidney disease (ESKD) requiring dialysis and renal transplantation. Despite an increasing number of children now surviving through childhood and early adulthood, heart disease remains one of the major causes of death in individuals with childhood-onset CKD as young adults and it is likely this relates to onset of pre-clinical cardiovascular disease developing during childhood.

Arterial stiffening relates to the severity of CKD, being greatest in those with dialysis dependent CKD, and is thought to be driven, at least in part, by excess body weight, hypertension and metabolic changes associated with CKD but their contribution to arterial disease progression remains poorly understood.

The relationship of blood pressure with arterial disease remains unclear in the paediatric literature. Previous studies performed in children pre-dialysis, those on dialysis and following kidney transplantation have measured pulse wave velocity (PWV) of the carotid-femoral pathway (i.e. mainly the aorta) and/or measures of carotid mechanics have been examined but these studies have been limited by lack of concurrent measures of carotid blood pressure (required to determine functional elasticity of the carotid artery). Furthermore, whilst the potential impact of age and blood pressure (BP) have been adjusted for, when comparing differences between children with and without CKD, this comparison has not been performed between age and blood pressure matched groups.

The objectives of my thesis are to 1) to determine the use of an easy to perform, well tolerated technique to measure PWV in children. 2) to compare estimates of central aortic systolic pressure with that measured directly from catheter placed in the aortic root. 3) to determine typical estimates of systolic blood pressure amplification and 4) to determine the association of arterial function and structure with severity of childhood CKD and to examine the relation of these measures to blood pressure. 5) to design a controlled trial to evaluate effects of aggressive versus standard blood pressure control on cardiovascular target organ damage.

My research findings report novel data relating to my project objectives. We compared two different techniques to measure PWV (volumetric and tonometric) and observed that the volumetric technique is easy to perform, well tolerated and reproducible when measurements are made by the same observer consecutively, but that the results of the two techniques are not inter-changeable. My work for objective 2 and 3 measured central blood pressure at the aortic root at the time of arterial cannulation and confirmed that blood pressure measured in the arm differs from that close to the heart. We validated simple non-invasive methods to measure blood pressure in children and showed that peripheral systolic amplification is substantial, including those with and without hypertension and mild to advanced CKD, with a mean amplification of ~ 20 mmHg and thus may be relatively more important than in adults. In a cohort of children with and without CKD, we performed a comprehensive characterization of arterial biomechanics and observed that the changes in elastic properties of the carotid artery were related to increased blood pressure, and not to decreased glomerular filtration rate. Important limitations to this cross-sectional study include lack of knowledge of

duration of both hypertension and CKD and lack of formal sample size calculation. Despite these limitations the results from my thesis suggest that blood pressure reduction may be an effective means to protect against arterial stiffening and needs to be evaluated using a controlled clinical trial. The design of such a trial is presented.

List of abbreviations

ΔA	Change in cross-sectional area
ΔD	Carotid distension
ΔT	Transit time
ABPM	Ambulatory blood pressure monitoring
ACEi	Angiotensin converting enzyme inhibitor
ADMA	Asymmetric dimethyl arginine
Alx	Aortic augmentation index
ANOVA	Analysis of variance
ANZDATA	Australia & New Zealand National Renal Registry Database
AP	Augmentation pressure
BHS	British Hypertension Society
BMD	Bone mineral density
BMI	Body mass index
bpm	Beats per minute
BPW	Backward pressure wave
CAC	Coronary artery calcification
CAKUT	Congenital anomalies of the kidney and urinary tract
Ca-P	Calcium – phosphate product
CC	Cross-sectional compliance coefficient
CCA	Common carotid artery

CCHD	Complex congenital heart disease
CI	Confidence interval
cIMT	Carotid intima medial thickness
CKD	Chronic kidney disease
CKiD	Chronic Kidney Disease in Children study
cPP	central or carotid pulse pressure
cSBP	Central aortic systolic blood pressure
cSBP_{carotid}	Estimated central systolic blood pressure by carotid wall tracking
cSBP_{inv}	Invasively measured aortic systolic blood pressure
cSBP_{RT}	Radial tononometric estimate of central systolic blood pressure
CV	Cardiovascular
CWS	Circumferential wall stress
CWTR	Carotid wall thickness radius ratio
DBP	Diastolic blood pressure
DBP_{inv}	Invasively measured diastolic blood pressure
DC	Cross-sectional distensibility coefficient
D_d	Diastolic internal lumen diameter
DMC	Data monitoring committee
D_s	Systolic internal lumen diameter
ECG	Electrocardiogram
eGFR	estimated glomerular filtration rate
E_{inc}	Young's incremental Elastic Modulus
ELCH	Evelina London Children's Hospital

eNOS	Endothelial nitric oxide synthase
ERF	Established renal failure
ESCAPE	Effect of Strict Blood Pressure Control and ACE Inhibition on the Progression of CRF in Pediatric Patients study
ESKD	End stage kidney disease
FMD	Flow mediated dilatation
FPW	Forward pressure wave
GFR	Glomerular filtration rate
HD	Haemodialysis
HDL	High density lipoprotein
HOT-KID	Hypertension Optimal Treatment in Children with Chronic Kidney Disease study: The HOT-KID study- a randomised trial to compare effects of aggressive versus standard targets in blood pressure on target organ damage in children with CKD
IMT	Intima medial thickness
iPTH	Intact parathyroid hormone
IQR	Inter-quartile range
ISCRTN	International Standard Randomised Controlled Trial Number
KDOQI	Kidney disease outcomes quality initiative
LC-MSMS	Liquid chromatography mass spectrometry
LDL	Low density lipoprotein
LERIC	Late Effects of Renal Insufficiency in Children study
LOA	Limits of agreement

Lp(a)	Lipoprotein a
LV	Left ventricle
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMI	Indexed left ventricular mass
MAP	Mean arterial pressure
MAP_{inv}	Invasively measured aortic mean blood pressure
NAPRTCS	North American Pediatric Renal Transplant Cooperative Study
NKF-	National Kidney Foundation's Kidney Disease Outcomes Quality
K/DOQI	Initiative
NO	Nitric oxide
NR	Not reported
NRES	National Research Ethics Service
PD	Peritoneal dialysis
PDA	Patent ductus arteriosus
pDBP	Peripheral diastolic blood pressure
PP	Pulse pressure
pSBP	Peripheral systolic blood pressure
PWV	Pulse wave velocity
PWVa	Aortic pulse wave velocity
PWVbf	Brachio-femoral pulse wave velocity
PWVcf	Carotid-femoral pulse wave velocity
RAAS	Renin Angiotensin Aldosterone System

R&D	Research and Development
REC	Research and Ethics committee
RRT	Renal replacement therapy
SBP	Systolic blood pressure
SD	Standard deviation
SDMA	Symmetric dimethyl arginine
SDS	Standard deviation score
SE	Standard error
SEM	Standard error of the mean
t1	Time delay of the proximal pressure wave
t2	Time delay of the distal pressure wave
TG	Triglyceride
UKCRN	UK Clinical Research Network
WCSA	Wall cross-sectional area

Publications from this thesis

Measurement of pulse wave velocity in children: comparison of volumetric and tonometric sensors, brachial-femoral and carotid-femoral pathways. Keehn L, Milne L, McNeill K, Chowienczyk P, Sinha MD. **J Hypertens**. 2014; 32(7):1464-9.

Central aortic blood pressure from ultrasound wall-tracking of the carotid artery in children. Comparison with invasive measurements and radial tonometry. Milne L, Keehn L, Guilcher A, Reidy JF, Karunanithy N, Rosenthal E, Qureshi S, Chowienczyk PJ, Sinha MD. **Hypertension**. 65: 1141-6, 2015.

Response to Validation of Devices and Methods for Noninvasive Estimation of Central Aortic Blood Pressure in Children. Milne L, Keehn L, Guilcher A, Reidy JF, Karunanithy N, Rosenthal E, Qureshi S, Chowienczyk PJ, Sinha MD. **Hypertension**. 66:e8, 2015. doi: 10.1161/HYPERTENSIONAHA.115.05748.

Decreased arterial elasticity in children with nondialysis chronic kidney disease is related to blood pressure and not to glomerular filtration rate. Sinha MD, Keehn L, Milne L, Sofocleous P, Chowienczyk PJ. **Hypertension**. 66: 809-15, 2015.

Statement of conjoint work

The research work of this thesis was done jointly with my supervisor, Professor Phil Chowienczyk under whose guidance I undertook all of the statistical analyses and created the tables and figures for the thesis.

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Miss Paula Sofocleous helped with acquisition of part of data in chapter V and for the recruitment of participants for studies described in chapter III, IV, V and VI.

Miss Jane Boston helped with recruitment of participants for study in chapter IV.

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Chapter 1: Introduction

1.1. Scale of the problem- CKD during childhood

Chronic kidney disease (CKD) is a major public health issue with an estimated 6-16% of the adult populations surveyed in the developed world affected.¹⁻⁴ Both pre-dialysis CKD and established renal failure (ERF) or end stage kidney disease (ESKD) requiring dialysis and transplantation have rapidly increased over the last two decades.²⁻⁵

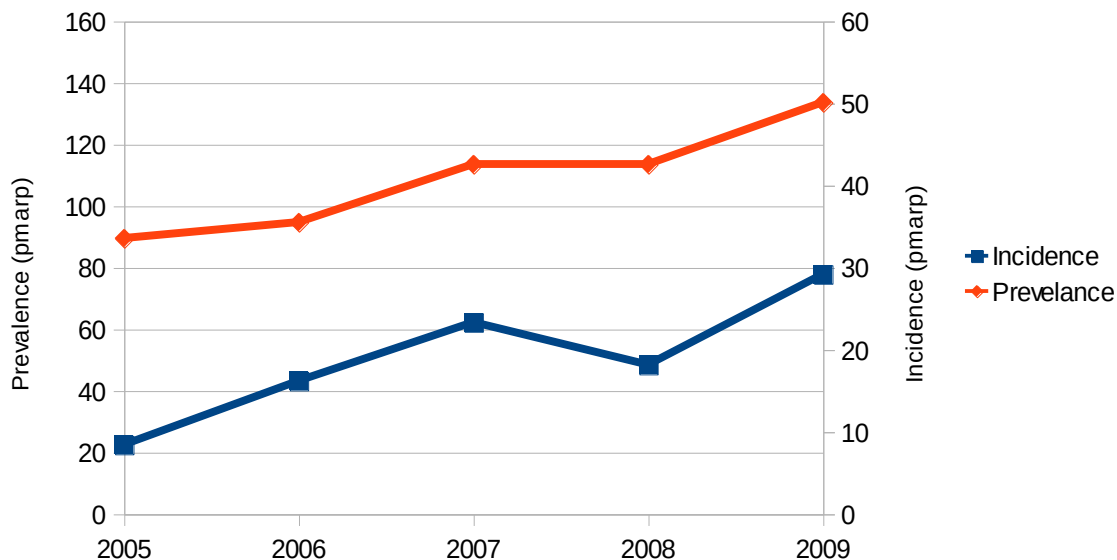
Although children with ERF account for a fraction of the total ERF patient population it is a devastating illness during childhood, with a profound impact on the child and their family.⁶ CKD is one of the commonest chronic illnesses of childhood that leads to lifelong health needs including future need for organ transplantation.⁷

Population based studies in children including both predialysis and dialysis cohorts, report an incidence of 7.5-12.1 and prevalence of 29.4-74.7 cases per year per million in children <18 years of age.^{6,8-10} In the United Kingdom, the incidence and prevalence rates of children with ERF for the UK has remained stable and was 9.3 and 58.2 per million children <16 years of age.¹¹ At any time there are about 150-200 children with ERF requiring regular dialysis in the United Kingdom, with 50-70 new patients commencing dialysis each year.¹¹ The number of children with moderate to severe CKD being treated in specialist paediatric nephrology units in the UK is not known but is estimated to be 5-10 times more than the prevalent ERF population.

We recently reported the incidence and prevalence of children with predialysis stages 3-5 of CKD at our tertiary Paediatric Nephrology centre at the Evelina London Children's Hospital (ELCH) serving the South East England.¹² The mean incidence and prevalence

of children <16 years during the 5-year study period was 17.5 and 90.0 per million age-related population (pmarp), respectively. We reported a marked increase in incidence and prevalence over the 5 year study period (incidence 8.4 to 25.2 pmarp; prevalence 79.5 to 104.7 pmarp) [Figure 1].¹²

Figure 1: Annual incidence and prevalence rate in children with stages 3-5 of CKD over the 5 year period 2005-2009 in the South East of England



In this cohort, there were 288 children (58% males) with median (IQR) age of 6.7 (2.3, 12.1) years and nearly 60% of prevalent cases secondary to congenital anomalies of the kidney and urinary tract (CAKUT), and only 17% secondary to an underlying glomerular disease¹², in keeping with other published paediatric reports.⁶

1.2. Definitions and stages of chronic kidney disease

Renal function or glomerular function rate (GFR) can be measured accurately using a 'formal' measure of renal function or estimated from the plasma creatinine using validated equations and is expressed in 'ml/min/1.73m²'. The most widely accepted method for estimating renal function in children is by the Schwartz formula¹³, with recent modifications to the equation suggested as a result of improvement in the techniques to measure plasma creatinine.^{12,14,15} In children, as in adults CKD is categorised based on increasing severity of renal dysfunction (**Table 1**).^{16,17}

Table 1: Classification of chronic kidney disease in children from the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) stages of chronic kidney disease^{16,17}

CKD Stage	Clinical state	GFR in ml/min/1.73m ²
1	Kidney damage with normal or increased GFR	>90
2	Kidney damage with mild decrease in GFR	60-89
3a	Moderate decrease in GFR (early)	45-59
3b	Moderate decrease in GFR (late)	30-44
4	Severe decrease in GFR	15-29
5	End Stage Renal Disease requiring renal replacement therapy	<15 or dialysis

1.3. Cardiovascular mortality and morbidity in CKD

Cardiovascular (CV) disease in adult patients with CKD is highly prevalent with a 10-30 fold increase in mortality in patients on dialysis when compared with age matched

individuals from the general population.^{18,19} Adult patients with predialysis CKD also have very high rates of mortality secondary to CV disease that presents even before onset of ERF.^{17,20,21}

The risk of cardiac death is increased almost 700 times in young adults with ERF when compared with age-matched healthy peers.²² With modern management and advancements in dialysis therapy, increasing number of children now survive through childhood to early adulthood but as young adults continue to exhibit an increased risk of mortality when compared with age-matched healthy peers. Young adults with *childhood-onset* ERF, exhibit mortality rates about 30 fold higher than children with no renal disease and a cardiac cause for death in them accounts for 40-45% of all mortality.^{19,23} Recent data with extended follow-up of those with *childhood-onset* ERF from Northern Europe, now aged 30-50 years old shows a reduction in cardiovascular related deaths and an increase in infection related deaths.²⁴ Overall, though, moderate to severe renal disease results in a markedly reduced life expectancy for children on dialysis by 40-60 years and by 20-25 years for children who have received a kidney transplant when compared with age, gender and race matched individuals with no renal disease from the general population.^{19,22,25} Although cardiac disease accounts for a fraction of mortality in the general childhood population, for children with ESKD on dialysis CV disease *during childhood* accounts for 28-32% of mortality²⁵⁻²⁸, with little improvement in mortality, at 22%, secondary to cardiac causes following renal transplantation.^{25,26} During childhood the risk of death remains the highest in the youngest children on haemodialysis.^{11,29,30}

Table 2 extended from a review by Lillien *et al*³¹, outlines key studies that have provided medium to long term CV outcomes in children with chronic kidney disease.^{19,23,24,26,28-}

^{30,32} It is important to note that these studies provide minimal data regarding several risk factors of interest such as blood pressure levels or indices of calcium-phosphate metabolism during childhood.

Table 2: Key reports in children with chronic kidney disease that have provided medium to long term cardiovascular outcomes in children

Study	Setting	Number of pts (Number of deaths)	Duration of F/U	Cause of deaths secondary to cardiovascular disease only	Risk factors
McDonald et al¹⁹	ANZDATA National Registry	n=1634 with 26.7% deaths (436 deaths)	Median of 9.7 years	25% cardiac arrest; 14% myocardial ischaemia ; 11% pulmonary oedema and 22% other cardiovascular causes	Young age at time of commencement of RRT; treatment with dialysis.
	Era 1962-2002	SMR 30 45 % cardiovascular deaths (57% in haemodialysis patients versus 30% in renal transplant recipients)			
Parekh et al²⁶	US Renal Data Systems Registry	n=1380 deaths of which 22.5% deaths (311 deaths) were cardiovascular deaths (highest in haemodialysis patients 48.9% versus 23% in renal transplants)	Analysis of deaths in <30 year olds who commenced RRT aged <20 years between 1990-1996	52% cardiac arrest; 16% cardiomyopathy; 14% arrhythmia; 7% myocardial infarction	Aged 20-30 years; haemodialysis and Black patient ethnicity at highest risk of death
	Era 1990-1996				
Chavers et al²⁸	US Renal Data Systems Registry	n=1454 with 31.1% deaths cardiac deaths (452 deaths)	5-10 years	20% arrhythmia; 12% valvular heart disease; 10% cardiomyopathy; 6% cardiac arrest/death	Older adolescents, Black ethnicity and female at highest risk of death
	Era 1991-1996				

Study	Setting	Number of pts (Number of deaths)	Duration of F/U	Cause of deaths secondary to cardiovascular disease only	Risk factors
Groothoff et al²³	Dutch national registry report but with case note review LERIC cohort (<15 year born before 1979 and commenced RRT 1972-1992)	n=249 with 25.3% deaths (63 deaths) 41% cardiovascular deaths SMR 31	Average f/u 15.5 years	58% cerebro-vascular accidents; 15% CHF; 12% myocardial ischaemia; 8% cardiac arrest	Commencement of RRT at young age; long dialysis period and prolonged untreated hypertension had higher mortality secondary to CVA
Oh et al³²	Single center data Era 1970-1997	n=283 with 14.8% deaths (42 deaths) 50% cardiovascular or cerebrovascular deaths	More than 15 years	Not reported	49 patients lost to follow-up; No discussion regarding cause of death
Samuel et al³⁰	Canadian Renal Registry - national study Era: 1992-2007	n=843 with 12.7% deaths (n=107) in <18 year olds. 22% (n=23) were cardiovascular deaths	median f/u 6.8 (3.0, 10.6) years	4% hyperkalemia; 4% myocardial ischaemia/infarction; 4% cardiac failure; 1% fluid overload and 9% cardiac arrest, cause unknown)	No significant improvement in survival; no risk or advantageous factors found

Study	Setting	Number of pts (Number of deaths)	Duration of F/U	Cause of deaths secondary to cardiovascular disease only	Risk factors
Mitsnefes et al²⁹	US Renal Data Systems Registry Era 1990-2010	n=23401 with 12.7% deaths (n=2975) in <21 year olds. <5year olds at ESKD initiation n=705 deaths (20.4%). 36.9% (n=1098) were cardiovascular deaths	Analysis of deaths in those aged <21 years who commenced RRT between 1990-2010	Not reported	Age <5years at initiation of ESKD
Vogelzang et al²⁴	LERIC cohort (<15 year born before 1979 and commenced RRT 1972-1992) Extended follow up and report on mortality, and cause of death 2000-2010 and comparison with previous era 1972-1999	n=249 with 39% deaths (97 deaths) 62% cardiovascular related deaths Cardiovascular mortality rate (deaths per 100 patient years) reduced	Median f/u 25.5 (0.3-39) years CV mortality rate: 1972-89: 0.97 1990-99: 0.37 2000-10: 0.22	18% cerebro-vascular accidents; 31% cardiovascular; 4% myocardial ischaemia; 9% other (congestive heart failure, cardiac arrest, pericarditis, aorta dissection)	Highlighted a reduction in the rates of cardiovascular mortality when compared with previous era of follow up

Modified and extended from Lillien et al³¹

1.4. Causes of cardiovascular deaths in children with CKD

The cause of cardiovascular deaths in young adults with childhood onset CKD is often secondary to sudden cardiac death or 'cardiac arrest', and account for 6-52% of cases excluding cases secondary to hyperkalemia or cerebrovascular events.^{24,29-31} Fatal arrhythmia, cardiomyopathy or valvular disease are next most common, with a smaller proportion of patients with ischaemic heart disease.^{19,23-26,28,30} This large variation in the prevalence rate of 'cardiac arrest' in reported series probably reflects the difficulties in establishing the correct diagnosis particularly in younger children.^{29,31} In adult patients with ERF sudden cardiac death is also a major cause of death and is seen more often than in adults with no renal disease in whom coronary artery disease and heart failure are the commonest causes of cardiac death.^{33,34} Fatal arrhythmia presenting as sudden cardiac death and the risk factors for these in adult patients on dialysis are now increasingly being recognised.^{34,35} In children on dialysis it is likely that a number of these risk factors are prevalent and include abnormal hemodynamic and biochemical factors, rapid changes during dialysis in both hemodynamic and biochemical factors, autonomic dysfunction and ischaemic damage to the myocardium.

Following kidney transplantation although renal function improves it by no means becomes normal. Restoration of renal function by transplantation reduces though does not eliminate this increased CV risk and CV disease as a cause of mortality remains one of the commonest causes of death in this cohort.²⁵

1.5. Cardiovascular risk factors in CKD

Most experts categorise cardiovascular risk factors in patients (both adult and children) with CKD as either *traditional* or *non-traditional* (**Table 3**).^{18,36} Traditional risk factors are similar to those from the Framingham Heart Study that have been used to estimate the risk of developing symptomatic ischemic heart disease.^{37,38} As patients with CKD have several additional risk factors for CV disease, *non-traditional* risk factors have been proposed.

Table 3: ‘Traditional’ and ‘non-traditional’ risk factors for cardiovascular disease in adults with chronic kidney disease

Traditional	Non-traditional
Older age	Malnutrition
Male gender	Albuminuria
White race	Abnormal calcium-phosphate metabolism
Diabetes Mellitus	Anaemia
Hypertension	Electrolyte imbalance
Dyslipidaemia (↑LDL, ↓ HDL)	Fluid overload
Tobacco use	Chronic inflammation
Physical inactivity	Homocystinaemia
Left ventricular hypertrophy	CKD related dydlipidaemia [↑LDL, ↓ HDL, ↑TG, ↑Lp(a)] with altered
Family history of CV disease	structure and delayed metabolism of lipids
Obesity	Oxidative stress (e.g. reduced nitric oxide balance)
	Thrombogenic factors

LDL, low-density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; Lp(a) lipoprotein a. Modified from Sarnak and Mitsnefes *et al*^{36,73}

Sarnak *et al*¹⁸, defined a *non- traditional* risk factor as one that should ideally meet all the following four criteria:

- (i) biological plausibility as to why the factor should promote CV disease risk;
- (ii) demonstration that the risk factor increases with worsening renal function;
- (iii) demonstration of an association between the risk factors and CV disease in CKD in observational studies; and
- (iv) demonstration in placebo-controlled clinical trials that treatment of the risk factor decreases CV disease outcomes.

CV risk factors for adult CKD patients are shown in **Table 3**. Following transplantation several of these risk factors remain (e.g. hypertension, LVH, obesity, dyslipidaemia and abnormal calcium-phosphate metabolism), some new risk factors such as diabetes emerge and some CV risk factors such as hypertension and obesity get amplified. **Table 4** highlights published data regarding CV risk factors in children with predialysis and dialysis dependent CKD. It is important to highlight here that in children, the majority of non-traditional risk factors listed in **Table 3** satisfy the first two criteria with few risk factors satisfying criteria 3 and none criteria 4, as defined by Sarnak *et al*.¹⁸

Risk factors may be modifiable and thus could be a target of clinical management to improve clinical outcomes. The modifiable risk factors proposed in children include prevention of malnutrition and appropriate management of anemia, insufficient blood pressure control, dyslipidemia, disorders of calcium-phosphate metabolism such as increased phosphate, high intake of calcium salts or active vitamin D and high serum

levels of intact parathyroid hormone.³¹ Indeed it has been suggested that the observed reduction in mortality in children and young adults with ERF, when compared with historic data may be secondary to improvement in achievement of treatment targets and pathways.^{24,25}

Table 4: Cardiovascular risk factors in children with CKD for which there is published evidence

Risk factor	Predialysis CKD	Dialysis dependent CKD
Hypertension	Prevalence 48-60% Ref : European ³⁹⁻⁴¹ , USA: CKiD ⁴²⁻⁴⁴ , NAPRTCS ⁴⁵	Prevalence 50-75% Ref : European ^{46,47} , NAPRTCS ^{48,49}
Dyslipidemia	Prevalence 45% Ref : CKiD ⁵⁰	Prevalence >50% Ref : European ^{51,52}
Anaemia	Prevalence 30-93% Ref : European ^{53,54} , CKiD ⁵⁵ , Canada ⁵⁶	Prevalence 40-70% Ref : European ^{47,57} , USA ⁵⁸⁻⁶¹
Abnormal Ca-P metabolism	Prevalence 17-45% Ref: European ^{54,62} NAPRTCS ⁶³ , Canada ⁵⁶	Prevalence >50% Ref: NAPRTCS ⁶³ , European ⁴⁷
Hyperhomocystinaemia	Ref: Europe ⁶⁴⁻⁶⁶	Prevalence 87-92% Ref: USA ^{67,68}
CRP	Ref: European ^{54,66}	Prevalence 76% USA ⁶⁷⁻⁶⁹
Hypoalbuminaemia	Ref: European ⁵⁴	Prevalence 40-60% USA ^{70,71}
Physical inactivity	-	Prevalence >90 USA ⁷²

Adapted from Mitsnefes *et al*^{73,74}

A complex issue in this multi-factorial relationship between cardiovascular disease and CKD has been to tease out the individual role of particular CV risk factors in a typical adult patient with CKD. This is because of the *over-representation* of traditional risk factors in adult patients. A typical adult patient with ERF on dialysis has diabetes or atherosclerosis as the primary cause of ERF, and in whom heart disease including hypertension often antedates onset of renal disease.⁷⁵ Children with CKD on the other hand offer a unique opportunity to investigate CV risk factors in the absence of significant co-morbidities such as diabetes, atherosclerosis, smoking and ischemic heart disease.

There is an increasing prevalence of risk factors with worsening stages of CKD although these data are indicative of association only. Further, several key questions regarding the *role* of CV risk factors in paediatric CKD remain unanswered and include (i) lack of clear evidence for the level of GFR beyond which cardiovascular risk begins in children; (ii) if both *traditional* and *non-traditional* risk factors are *real* CV risk factors in children in the earlier stages of CKD; (iii) if primary disease causing CKD has different CV risk factor profiles; (iv) are there some mechanisms unique to children that dictate the relationship between these CV risk factors and CKD; (v) what is the cause of the very high rate of 'cardiac arrest' and sudden cardiac death observed in children, in particular the youngest children; and (vi) if control of some *key* risk factors such as blood pressure may provide *greater* overall CV risk reduction than control of other risk factors.⁷⁶

1.6. Pathophysiological mechanisms

Cardiac and arterial disease is thought to result from the cumulative effects of numerous risk factors. Pathophysiological mechanisms include endothelial dysfunction, atherosclerosis, arterial stiffening or arteriosclerosis, left ventricular hypertrophy (LVH) and myocardial fibrosis.¹⁸ It is likely that these evolve in parallel and worsen over time, with a likely significant adverse impact of increasing duration of moderate to severe CKD and worsening GFR.⁷³

1.6.1. Endothelial dysfunction:

The healthy endothelium acts to maintain blood fluidity, protect the arterial wall from invasion by circulating monocytes and regulates vascular tone.^{77,78} Nitric oxide (NO) derived from endothelial nitric oxide synthase (eNOS) in response to shear stress on the endothelium generated from blood flow regulates vascular tone and has a number of anti-atherogenic actions. eNOS function has thus received much attention both in its own right and as a marker of more generalised endothelial function.⁷⁷ It is usually assessed by using high resolution ultrasound to measure flow-mediated dilation (FMD) of the brachial artery in response to an increase in flow generated by a short period of reactive hyperaemia in the distal forearm. Impaired FMD in CKD⁷⁷⁻⁸⁰ is thought to be due in part to reduced clearance of endogenous inhibitors of eNOS: asymmetric dimethylarginines (ADMA) and its structural isomer symmetric dimethylarginine (SDMA)⁸¹ which compete with the substrate L-arginine from which NO is synthesised by eNOS. Increased plasma concentrations of ADMA and SDMA have been shown to be associated with increased risk of CV disease in adult patients with CKD.^{82,83} Other proposed mechanisms causing endothelial dysfunction include chronic inflammation,

raised homocysteine and dyslipidemia.⁸⁴⁻⁸⁶ Lack of normal endothelial repair has also been implicated as a possible cause for endothelial dysfunction although there is no paediatric data regarding this.⁸⁷

1.6.2. Structural changes in the artery:

This may be due to atherosclerosis or arteriosclerosis. Atherosclerosis is thought to be initiated as a “response to injury” with the expression of adhesion molecules on the surface of the endothelium, migration of monocytes into the intima of the artery and interaction with lipoproteins to generate lipid rich plaques which may eventually obstruct the lumen.⁸⁸ Arteriosclerosis refers to a calcification of the medial layer of larger elastic arteries.⁸⁹ However, it is now clear that atherosclerosis and arteriosclerosis often co-exist and that atherosclerotic lesions show varying degrees of calcification.⁹⁰ Calcification may, however, represent a distinct process with degeneration of elastin and inflammation leading to switch of vascular smooth muscle cells to an “osteogenic” phenotype whereby smooth muscle cells express an array of genes that are usually involved in bone formation.^{91,92}

Calcification (both medial and of atherosclerotic lesions) is thought to be a major cause of arterial stiffening of large arteries, particular the aorta. This imposes a number of adverse haemodynamic effects such as increased systolic pressure and decreased coronary perfusion.

Atherosclerotic lesions seen in older adults with CKD, are often calcified, have a patchy distribution and often described as having increased medial thickness, whilst diffuse, non-occlusive medial calcification is often seen in the younger adults with ERF.⁸⁵

In addition to metabolic, mechanical, infectious and inflammatory injuries^{93,94}, disorders of calcium-phosphate metabolism may be key to the development of vascular calcification in CKD.³¹ These disorders of calcium-phosphate (Ca-P) metabolism include hyperphosphatemia, elevated calcium-phosphate product, increased calcium and active vitamin D intake and hyperparathyroidism. Hyperphosphatemia, increased active vitamin D and elevated levels of PTH fragments have all been shown to induce vascular smooth muscle cells transformation to osteoblasts.^{31,91,92,94,95}

1.6.3. Cardiac abnormalities in CKD:

Hypertrophy of the cardiac muscle is a physiological adaptation in response to an increased load imposed over some time on the myocardium.⁹⁶ Although this hypertrophied myocardium may be partially compensatory, it eventually may lead to heart failure.

Cardiac remodeling of the left ventricle (LV) causing LV hypertrophy (LVH) is seen in two predominant patterns, concentric remodeling and hypertrophy and eccentric hypertrophy. Concentric patterns of altered LV geometry are classically associated with systemic arterial hypertension and arteriosclerosis, and eccentric hypertrophy as a result of volume loading conditions anaemia, fluid overload and arterio-venous fistulae all of which are seen in CKD.¹⁸ The pathophysiology of this was first described by

Grossman *et al.*⁹⁷ According to their hypothesis, pressure overload of the LV leads to increase in peak systolic wall stress and parallel replication of sarcomeres, wall thickening, and concentric hypertrophy. The LV wall thickening continues until the peak systolic stress returns to normal, a feedback inhibition loop. Increasing pressure overload of the LV will therefore lead to progression of concentric hypertrophy. In contrast, upon LV volume overload, increased end diastolic wall stress leads to replication of sarcomeres in series, fiber elongation, chamber enlargement and eccentric hypertrophy. This chamber enlargement also causes increase in LV peak systolic stress and leads to wall thickening, until peak systolic stress is normalized. This symmetric increase in wall thickness to maintain the ratio of wall thickness and LV transverse radius normal, causes eccentric hypertrophy.⁹⁷

Increased LV mass and LVH seen in CKD are a consequence of the elevated systolic BP and pulse pressure, increased peripheral resistance and arterial stiffness. Over time maladaptive LVH develops leading initially to diastolic and then systolic dysfunction, compromised perfusion of the myocardium (coronary and subendocardial) causing ischaemia with decreased capillary density⁹⁸, fibrosis⁹⁹ and increased tendency to arrhythmia.³⁵ Several other processes are thought to be involved in the cardiac remodeling process and include role of angiotensin II, aldosterone, and inflammation amongst others.^{100,101}

1.7. Spectrum of CV abnormalities in children with CKD

There are currently no data from any intervention study in children that describe the impact of modifying one or more risk factors on cardiovascular outcomes in children with CKD. All evidence is therefore based on observational cross-sectional patient cohorts.

1.7.1. Endothelial dysfunction:

This has been evaluated mainly using FMD. Unlike results in adult patients with CKD where impaired FMD predicts CV morbidity and mortality^{79,80}, in children impaired FMD has been described in dialysis dependent patients¹⁰² but this has not been observed consistently in predialysis paediatric patients.¹⁰³⁻¹⁰⁵

1.7.2. Large artery structural and functional changes:

Large vessel intimal disease can be detected by measuring carotid intimal media thickness (cIMT) and there are now several small cross-sectional studies in children investigating this^{74,106}, confirming in the majority, the onset of arterial disease from early CKD stages (**Table 5**).^{105,107-117} Some investigators have also described femoral artery intimal media thickness. Previous studies have been performed in children with early stages of CKD, those on dialysis and following renal transplantation and observed worse cIMT in patients on dialysis. Following transplantation abnormal cIMT has been shown to stabilize or regress.¹¹¹

In addition to structural changes in large arteries, measures of arterial stiffening such as pulse wave velocity (PWV), a measure of arterial stiffness measured along elastic arteries, is widely used in adults and shown to be a good predictor of cardiovascular events and mortality in the general as well as dialysis population.¹¹⁸⁻¹²⁰ PWV has been evaluated in only small childhood cohorts limited in those with ESKD.^{109,110} Other measure of arterial stiffening such as arterial compliance have also been evaluated in small populations of predialysis and dialysis dependent children.¹⁰⁸

Table 5: Large artery structural and functional changes reported in children with predialysis and dialysis dependent CKD

Large artery health	Study population characteristics	Results	Conclusions
Litwin <i>et al</i>, 2005 ¹⁰⁷	n=55 CKD stage2-4; 37 on dialysis and 34 post-Tx; 270 controls Parameters : cIMT; femoral IMT; WCSA and lumen CSA of carotid	All parameters increased in all renal groups; worst in dialysis patients	Increased Ca-P product; young age and dialysis
Mitsnefes <i>et al</i>, 2005 ¹⁰⁸	n=44 CKD stage2-4; 16 on dialysis and 35 controls Parameters : cIMT; distensibility and stiffness of carotid artery	Increased arterial stiffness and cIMT	Increased Ca-P product; and dialysis
Covic <i>et al</i>, 2006 ¹⁰⁹	n=14 on dialysis and 15 controls Parameters : PWV and aortic Alx	BP, age, height, weight, Ca and P levels accounted for 57% of the variability in Alx. Dialysis no impact on Alx or PWV	SBP level higher in dialysis patents

Large artery health	Study population characteristics	Results	Conclusions
Shroff et al, 2007 ¹¹⁰	n=85 on dialysis and controls Parameters : cIMT and PWV. Also assessed cardiac calcification	Increased cIMT had stiffer vessels and a greater prevalence of cardiac calcification	iPTH level; dosage of vitamin D and calcium intake from phosphate binders
Muscheites et al, 2008 ¹⁰⁵	26 children with CKD 2-4, ERF and Tx; 24 controls Parameters : cIMT z-score; brachial artery diameter and FMD. Also LVMI	60% impairment of FMD; increased cIMT and reduced FMD in most CKD	Increased BP Reduced endothelial function, which may have preceded the development of carotid arteriopathy
Litwin et al, 2008 ¹¹¹ (longitudinal cohort)	24 CKD stages 2-3; 32 ERF (19 had transplantation between two observations) Parameters : cIMT; WCSA and lumen CSA of carotid at baseline and 12 months later	Patients post-Tx showed no further worsening and some showed regression of increased cIMT	Cumulative dialysis duration ; degree of arterial damage prevalent at the time of grafting are the main determinants of persistent arteriopathy 1 year after transplantation
Ziolkowska et al, 2008 ¹¹²	32 children with CKD 2-4, 28 ERF and 43 controls Parameters : cIMT. Also bone mineral density	60% abnormal cIMT (both CKD and dialysis)	lower fetuin-A and alkaline phosphatase levels and higher lumbar spine BMD with higher cIMT
Civilibal et al, 2009 ¹¹³	38 children on dialysis; 17 controls Parameters : cIMT. Also LV function		Worse cIMT with increased BP; Worse LV function with anaemia and LVH

Large artery health	Study population characteristics	Results	Conclusions
Chavarria et al, 2012 ¹¹⁴	60 children on dialysis Parameters : cIMT	48% abnormal cIMT	Hypercalcemia and total time on RRT
Brady et al, 2012 ¹¹⁵	n=101 eGFR: 43 and 97 healthy controls Parameter: cIMT	median cIMT was 0.43 mm (interquartile range, 0.38–0.48);	dyslipidemia and hypertension were associated with 0.05 mm (95% CI, 0.01–0.08) and 0.04 mm (95% CI, 0.003–0.08) greater mean cIMT, respectively
Kotur-Stevuljević et al, 2013 ¹¹⁶	10 children with CKD 3-5, 20 HD, 22 transplant and 36 controls Parameter: cIMT	Average cIMT in mm CKD: 0.304 (0.238 to 0.389); HD: 0.418 (0.374–0.477); Transplant: 0.411 (0.354 to 0.466)	Disease duration, BP, urea, lipid, and oxidative status parameters accounted for more than 90% of the variability of cIMT-SDS
Garcia-Bello et al, 2014 ¹¹⁷	n=134; 39 children with CKD 3-5, 53 PD, 42 HD Parameter: cIMT and Einc	Average cIMT in mm 0.528±0.089 (all) 0.474±0.072 (CKD) 0.533±0.079 (PD) 0.571±0.092 (HD)	dialysis (HD>PD), Ca x P product predicted cIMT cIMT and Einc strongly associate with several biochemical parameters and reduced GSH but not inflammatory/oxidative stress markers.

1.7.3. Vascular calcification:

Following the first description of coronary artery calcification (CAC) in a retrospective autopsy series by Milliner *et al* in 1990¹²¹, CAC has been evaluated in dialysis dependent children or young adults with childhood onset ERF, in several studies over the past 10 years.⁷⁴ The prevalence rate of CAC observed in these studies varies between 10-20%^{34,110,123-127} in paediatric patients and prevalence rates between 47-92% for CAC in adults with childhood onset ERF.^{74,127} In the only longitudinal study assessing CAC in dialysis dependent children by Civilibal *et al*¹²⁷, 7 of 48 dialysis dependent children with CAC were observed to have an increase, and 1 patient with CAC at baseline had decrease, after 2-year repeat assessment using spiral computed tomography. No new patients developed new lesions of CAC after 2-years on dialysis. Ca-P product was identified as a risk factor for CAC.

1.7.4. Cardiac disease:

Increased LV mass and LVH are the most commonly reported cardiac abnormality in both adult and paediatric patients with CKD (**Table 6**).^{18,40,41,43,44,54,64,65,74,128-134} In children with CKD we have observed elevated systolic BP but not diastolic, mean BP or pulse pressure to be significantly associated with LVH.^{40,134}

Table 6: Studies in children with predialysis CKD reporting on increased LV mass and LVH

LVH	Number of patients (n) & eGFR in ml/min/1.73m ²	Prevalence and geometry of LVH	Significant risk factors identified
Johnstone <i>et al</i> , 1996 ¹²⁸	n=32; eGFR:NR	22%; concentric hypertrophy	-
Mitsnefes <i>et al</i> , 2003 ¹²⁹	n=25; eGFR: 39	24%; Geometry NR	Reduced exercise capacity
Poyrazoglu <i>et al</i> , 2004 ⁶⁴	n=10; eGFR: 17-48	50%; Geometry NR	Anaemia
Malikenas <i>et al</i> , 2005 ¹³⁰	n=56; eGFR: 34	36%; 23% concentric & 13% eccentric	Lower eGFR
Matteucci <i>et al</i> , 2006 ⁵⁴	n=156; eGFR: 49	33%; 12% concentric & 21% eccentric	ESCAPE study cohort Male gender anaemia, increased PTH and BMI
Mitsnefes <i>et al</i> , 2006 ¹³¹	n=31; eGFR: 49 at baseline & 43 2-years later	19% at baseline and 39% after 2-years	High LVMI at baseline, anaemia, increase in iPTH and increased SBP load at night
Weaver <i>et al</i> , 2008 ¹³²	n=45; eGFR: 46	17%; Geometry NR	Increased cardiac output
Rinat <i>et al</i> , 2009 ⁶⁵	n=57; eGFR: 15-60	16%; Geometry NR	Systolic BP
Mitsnefes <i>et al</i> , 2010 ⁴³	n=198; eGFR: 43	17%; 6% concentric & 11% eccentric	CKiD study cohort Confirmed and masked hypertension on ambulatory BP monitoring
Sinha <i>et al</i> , 2010 ⁴⁰	n=49; eGFR: 26	49%; all with eccentric hypertrophy	EVELINA study cohort-1 Systolic BP (clinic>ambulatory) and Ca-load in g/day for calcium containing phosphate binders

LVH	Number of patients (n) & eGFR in ml/min/1.73m ²	Prevalence and geometry of LVH	Significant risk factors identified
Matteucci <i>et al</i>, 2013⁴¹	n=84; eGFR: 49	LVH prevalence decreased from 38% to 25%	ESCAPE Follow up study cohort improvement in myocardial function was associated with reduction in BP, independently of LVMI reduction
Mencarelli <i>et al</i>, 2014¹³³	n=34; eGFR: 52	38%; concentric 84%	No correlation of LVH with BP but with diastolic dysfunction
Kupferman <i>et al</i>, 2014⁴⁴	n=436 followed up at 1, 3 and 5 years eGFR: 44	16% at baseline	CKiD Follow up study cohort systolic BP, female sex, anemia, and use of non-RAAS class of antihypertensive medications
Sinha <i>et al</i>, 2015¹³⁴	n=83; eGFR: 33	36%; all with eccentric hypertrophy	EVELINA study cohort-2 elemental calcium content (g/kg/day) estimated from prescribed calcium-based phosphate binder dose and BMI z-score

Diastolic dysfunction, a sign of impaired LV relaxation, is one of the earliest functional abnormalities of the left ventricle. This has been evaluated in children with CKD^{128,135} and more recently using tissue Doppler imaging.¹³⁶⁻¹⁴⁴ These studies suggest worsening diastolic dysfunction with increasing loss of kidney function with reduced e' velocities, increased E/e' ratios with or without associated finding of LVH and increased myocardial performance index.^{135-138,141,143-145} Overt systolic dysfunction is unusually seen in children with CKD, particularly those with predialysis stages of CKD. Subclinical

systolic dysfunction though has been demonstrated in the predialysis population using 'midwall shortening fraction' impairment instead of the 'endocardial shortening fraction' assessment.^{145,146} More recently, cardiac magnetic resonance^{147,148} or other advanced cardiac echocardiography techniques^{149,150} have been used to demonstrate early cardiac changes in children with CKD even before development of LVH or LV dysfunction.

1.8. Risk factor: focus on blood pressure

Despite a strong association between left ventricular mass, IMT and arterial stiffness with blood pressure in adults with CKD, the role of blood pressure in determining target organ damage in children with CKD remains controversial. In childhood CKD too, both LVH and arterial stiffness are thought to be determined less by blood pressure and more by other mechanisms primarily via disturbances in CKD-associated mineral bone disorders.

Studies of pulsatile arterial haemodynamics have shown that although mean arterial pressure (MAP) and diastolic blood pressure (DBP) remains nearly constant along the arterial tree, systolic blood pressure (SBP) varies at different points along the arterial tree.¹⁵¹ Due to the phenomenon of pressure wave reflection and amplification peripheral SBP (pSBP) measured at the brachial artery usually exceeds central aortic SBP (cSBP) at the aortic root.¹⁵² In adults, cSBP is thought to relate more closely to target organ damage than peripheral SBP (pSBP).^{153,154} Differences between cSBP and pSBP are

more marked in younger compared to older adults¹⁵⁵ and thus may be particularly important in children.

The relative importance of cSBP in children has not been evaluated previously. Previous studies in children studying carotid and aortic structural and functional parameters have included cohorts of non-dialysis CKD, dialysis dependent and those following kidney transplantation. Importantly, they have reported limited carotid parameters without measuring cSBP. Litwin *et al*, reported carotid geometry, IMT and diameter but did not determine carotid stiffness and cSBP.^{107,111} Similarly, Mitsnefes *et al*, reported carotid IMT, geometry and carotid stiffness but did not use central blood pressure.¹⁰⁸ In dialysis dependent children, Shroff *et al*, measured carotid IMT and PWV¹¹⁰; whilst Covic *et al*, measured carotid IMT, PWV and carotid Alx¹⁰⁹ but both did not determine more specific parameters of carotid mechanics and did not measure central BP.^{109,110}

1.9. Summary

Childhood CKD is a devastating illness requiring life-long medical input, often progressing to ESKD, requiring dialysis and renal transplantation. Despite an increasing number of children now surviving through childhood and early adulthood, cardiovascular disease remains one of the major causes of death in individuals with childhood-onset CKD as young adults and it is likely this relates to onset of pre-clinical cardiovascular disease developing during childhood.

There are several modifiable CV risk factors in CKD, of which key risk factors relate to elevated BP and markers of abnormal Ca-P metabolism. Unlike in pre-dialysis CKD, in children on dialysis, abnormal mineral metabolism (high phosphorus, calcium-phosphorus product, PTH and vitamin D metabolism) is thought to be one of the major determinants of cIMT and pulse wave velocity; whilst in children with predialysis CKD, elevated BP is highly prevalent. Commonly reported measures of end organ damage include measures of arterial stiffness and left ventricular hypertrophy, which in adults with CKD relate strongly to BP. Improved management of hypertension is a key aspect of management of adult CKD patients with recommendations for more stringent blood pressure targets in adults particularly those with proteinuric CKD.¹⁵⁶ There are no current guidelines regarding optimal level of blood pressure control for cardiovascular outcomes in children with CKD.¹⁵⁷ Available recommendations for BP levels in this cohort are extrapolated from the ESCAPE study – a randomised controlled trial to reduce the progression of CKD.¹⁵⁸

1.10. Purpose of this thesis

Arterial stiffening relates to the severity of CKD, being greatest in those with dialysis dependent CKD, and is thought to be driven, at least in part, by excess body weight, hypertension and metabolic changes associated with CKD but their contribution to arterial disease progression remains poorly understood. The relationship of blood pressure with arterial disease remains unclear in the paediatric literature.

Previous studies performed in children pre-dialysis, those on dialysis and following kidney transplantation have measured PWV of the carotid-femoral pathway (i.e. mainly the aorta) and/or measures of carotid mechanics have been examined but these studies have been limited by lack of concurrent measures of carotid blood pressure (required to determine functional elasticity of the carotid artery). Furthermore, whilst the potential impact of age and blood pressure have been adjusted for, when comparing differences between children with and without CKD, this comparison has not been performed between age and blood pressure matched groups.

The objectives of my thesis are to 1) to examine the use of an easy to perform, well tolerated technique to measure PWV in children. 2) to develop and validate a technique for estimation for central aortic systolic pressure in children. 3) to determine typical estimates of systolic blood pressure amplification and 4) to determine the association of arterial function and structure with severity of childhood CKD and to examine the relation of these measures to blood pressure. 5) to design a controlled trial to evaluate effects of aggressive versus standard blood pressure control on cardiovascular target organ damage.

Chapter 2 : Methods

2.1. Blood Pressure

Blood pressure changes continuously throughout the cardiac cycle due to the interaction of the pressure generated by the ventricle with the impedance of the arterial tree. Systolic blood pressure (SBP) is the highest or peak pressure and occurs early after the start of contraction of the left ventricle and the lowest pressure, diastolic blood pressure (DBP) relates to the period of relaxation of the left ventricle. As a result of practical reasons relating to measurement, blood pressure has conventionally been assessed at the brachial artery through measurement of SBP and DBP. Mean arterial pressure (MAP) represents the average over time of blood pressure during the cardiac cycle and is often estimated from the empirical formula:

$$\text{MAP} = \text{DBP} + \frac{(\text{SBP} - \text{DBP})}{3}$$

3

However, the relationship between MAP, SBP and DBP depends on the “form factor” or shape of the pressure waveform.

2.2. Peripheral blood pressure measurement

Peripheral systolic (pSBP) and diastolic blood pressure (pDBP) were measured 3 times in succession at the brachial artery by a trained observer after children had been seated for at least 5 minutes using a calibrated aneroid sphygmomanometer with an appropriate sized arm cuff according to British Hypertension Society (BHS) guidelines. The aneroid sphygmomanometer instruments were checked for accuracy annually.

Hypertension was defined as systolic and/or diastolic BP above the 95th percentile for age and height or if the patient was on anti-hypertensive therapy, using the Fourth Report Criteria (National High Blood Pressure Education Program Working Group).¹⁵⁹

2.3. Central blood pressure

It is now well recognized that discounting hydrostatic pressure differences, MAP and DBP within conduit arteries are fairly constant throughout the arterial tree (being lower peripherally by only 1-3 mmHg than centrally at the heart).^{151,160} By contrast, SBP varies at different points in the arterial tree, with pSBP in the upper limb higher than central or aortic SBP (cSBP) measured at the aortic root. This phenomenon of peripheral pressure wave amplification is thought to be due to pressure wave reflections at the periphery of the arterial tree [Figure 2].¹⁶¹⁻¹⁶³

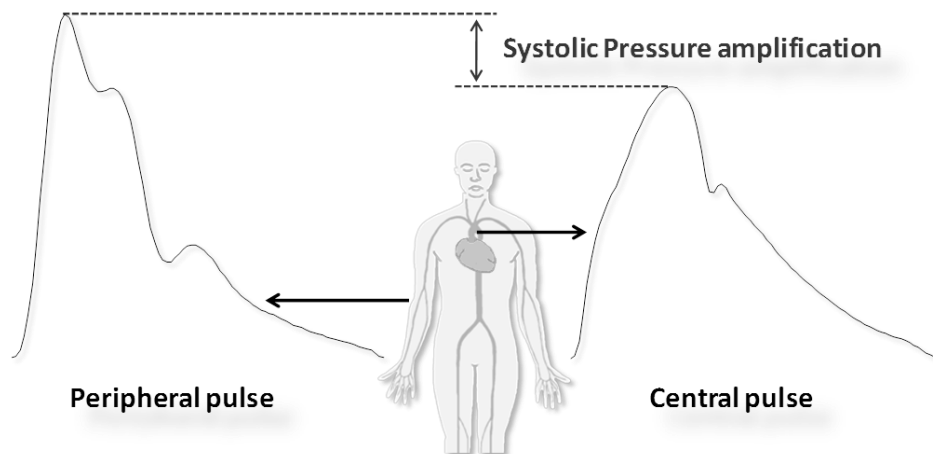


Figure 2: Schematic representation of the central pressure waveform, the peripheral pressure waveform and systolic pressure amplification as the pressure difference between the peripheral and central pressure values

Invasive catheter studies in adults have shown that the shape of the pressure wave in the ascending aorta is similar to the one recorded in the common carotid artery. Direct application of tonometry on the carotid artery, a superficial and easily accessible pulse point, has been widely used in adults to record central blood pressure.

2.3.1. Applanantion tonometry-derived estimate of cSBP ($cSBP_{Carotid}$) at the carotid artery

Direct application of tonometry at the carotid artery can be used to estimate non-invasively cSBP at the carotid artery ($cSBP_{Carotid}$) using the SphygmoCor system. This system uses a sensitive pressure transducer that lightly compresses the artery against underlying tissues (e.g. bone) [Figure 3].

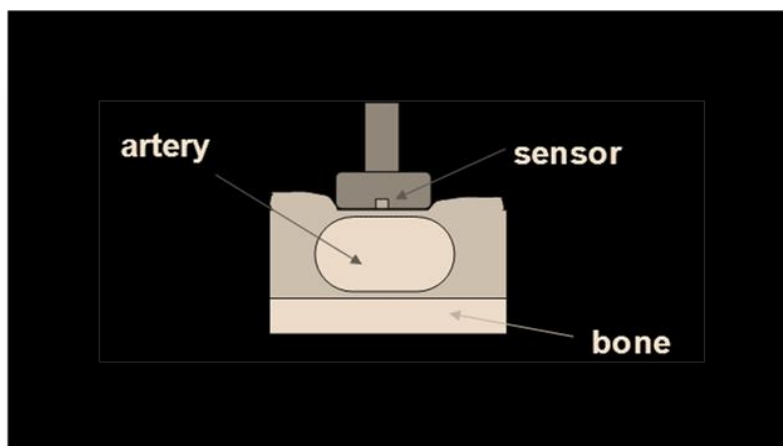


Figure 3: Schematic representation of the technique of applanantion tonometry

Following identification of the point of maximal arterial pulsation, pressing down lightly, the arterial wall is flattened against the underlying structure and the arterial pressure wave recorded. Carotid pressure waveforms are obtained from the right common carotid artery by applanation tonometry using a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX) and processed by the SphygmoCor device (Atcor Medical, Australia). This technique is possible to use in children but requires a significantly more experienced operator and a more co-operative older child. Overall, carotid tonometry is poorly tolerated in children [Figure 4].

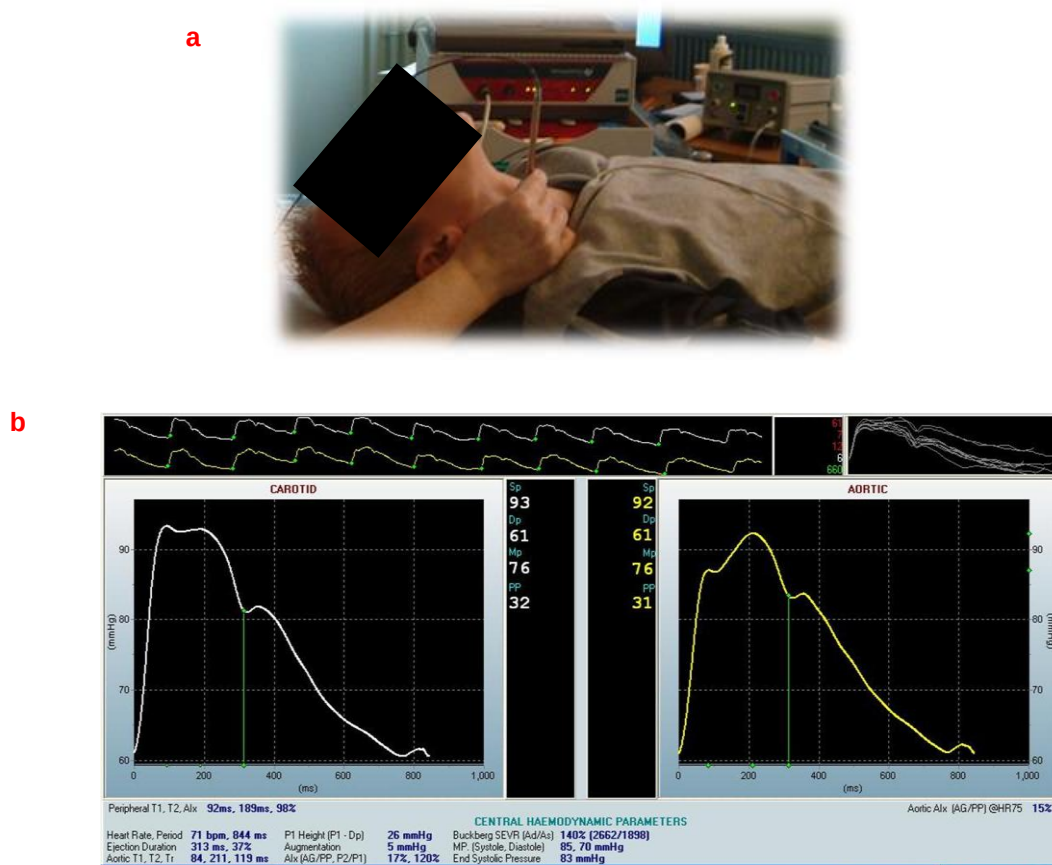


Figure 4: **a.** Carotid tonometry in an older child using the SphygmoCor device; and **b.** Acquisition of carotid pressure waveform obtained by the SphygmoCor system

2.3.2. Applanation tonometry-derived estimate of cSBP ($cSBP_{RT}$) at the radial artery

Central blood pressure can also be estimated non-invasively in adults “indirectly” using tonometry at the radial artery. This technique is also much easier to perform in children although still requires an experienced operator and co-operative patient young subject. Central blood pressure estimated using tonometric techniques has not been validated in children.

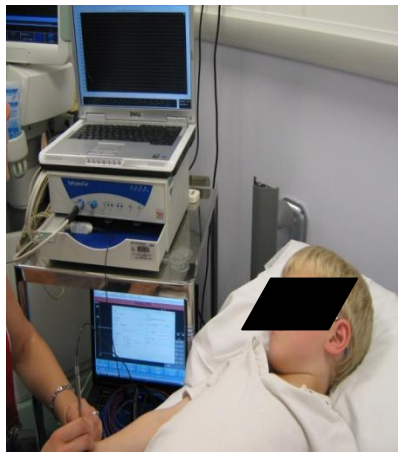


Figure 5: Radial tonometry in a 6-year old using the SphygmoCor system

Using this method radial waveforms meeting the inbuilt quality control criteria of the SphygmoCor device were ensemble averaged and converted to a corresponding aortic waveform using the inbuilt SphygmoCor generalised radial-to-aortic transfer function (derived in adults) from which central blood pressures were calculated (**Figure 6**).

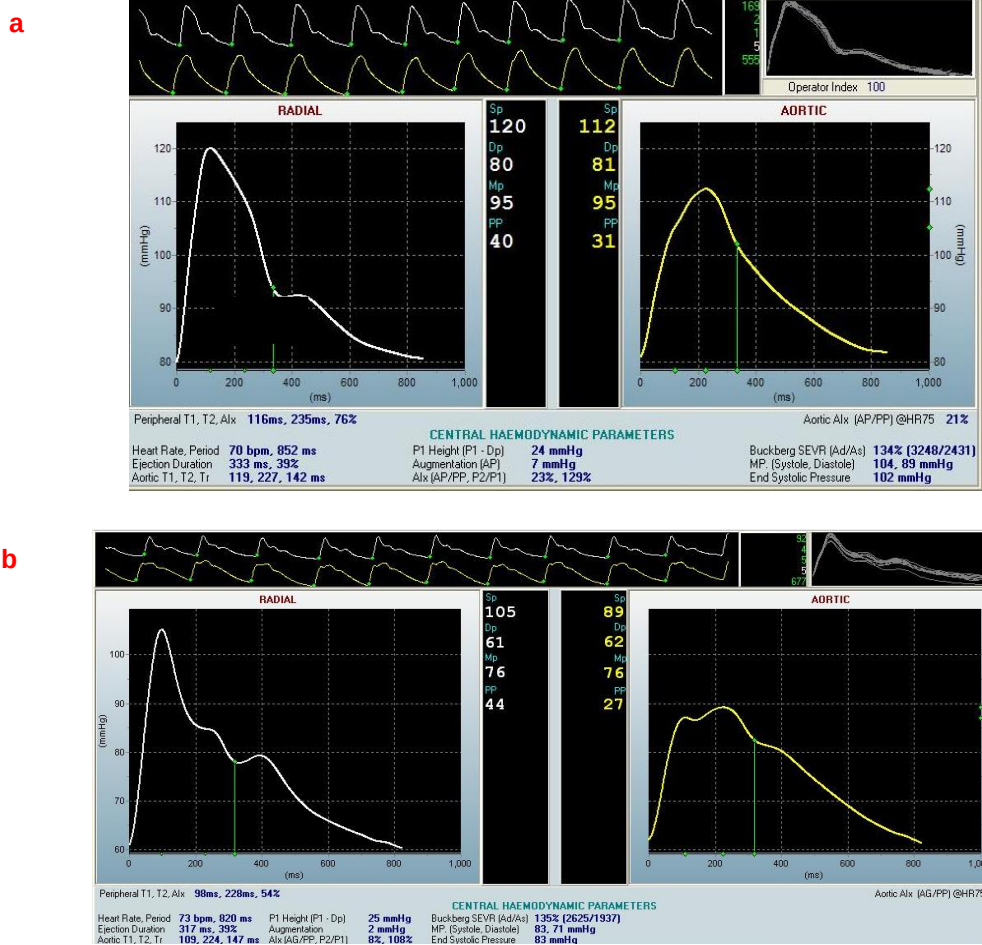


Figure 6: Acquisition of radial pressure waveforms obtained by the SphygmoCor system with radial to aortic transformation performed using the standard transfer function used in adults in a. 23 year old healthy woman; and b. 9 year old healthy girl

Radial waveforms were calibrated from peripheral brachial measures of pSBP and pDBP, from which peripheral MAP was calculated by integrating the radial waveform. Transformed radial waveforms (i.e. estimated aortic waveforms) were calibrated from these values of peripheral MAP and pDBP to give a radial tonometric estimate of cSBP (cSBP_{RT}) calibrated from non-invasive peripheral measures of SBP and DBP.

Estimates of cSBP in children using tonometric techniques have not been validated in children previously.

2.4. Central pressure waveform and augmentation index

2.4.1. Central pressure waveform

The aortic (central) pressure pulse is thought to be a result of the summation of forward and backward pressure waves. The forward pressure wave (FPW) is generated by the ejection of blood from the left ventricle. It then travels along the arterial tree until it is reflected at sites of impedance mismatch in the distal circulation. The reflected wave travels backward (backward pressure wave, BPW) to add up with the FPW. Thus, the central pulse pressure (cPP) could be reduced into two pressure component waveforms. The pressure to the first systolic shoulder which is assumed to be the consequence of the FPW, and the pressure from the systolic shoulder to the systolic peak, also called augmentation pressure (AP) which is thought to be due to the BPW (Figure 7).

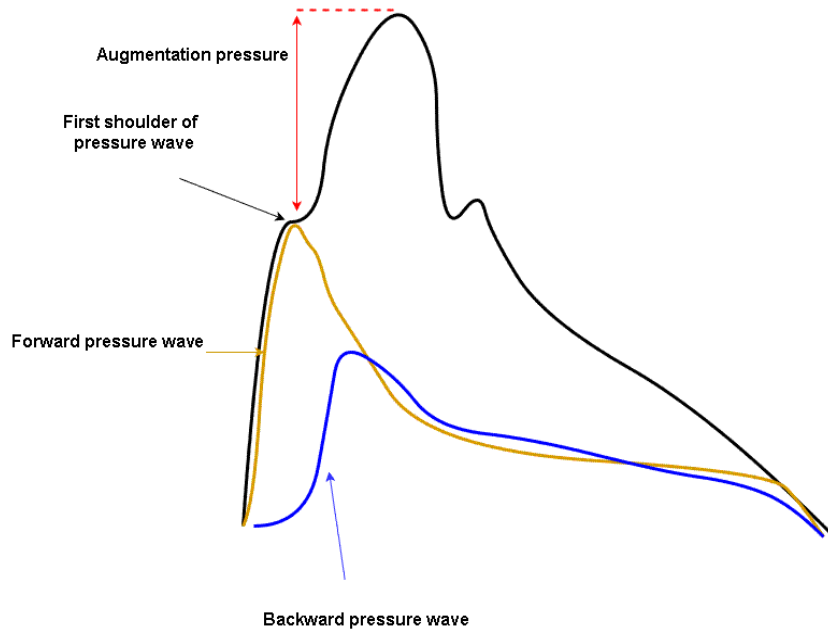


Figure 7: Schematic representation of augmentation pressure

In **Figure 8**, a schematic representation of the central pressure waveform, is shown with the components used for pulse wave analysis.

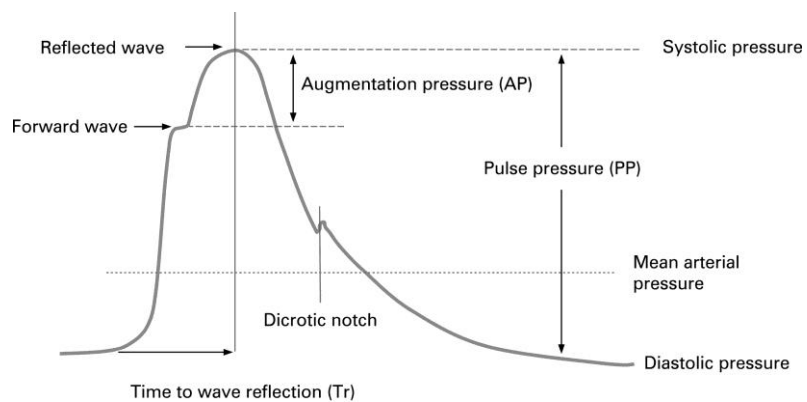


Figure 8: Schematic representation of the central pressure waveform and parameters used in pulse wave analysis

Using the SphygmoCor system, following tonometry at the radial artery and use of generalised transfer function, the transformed central pressure waveform in a healthy 5-year old child is shown (**Figure 9**).

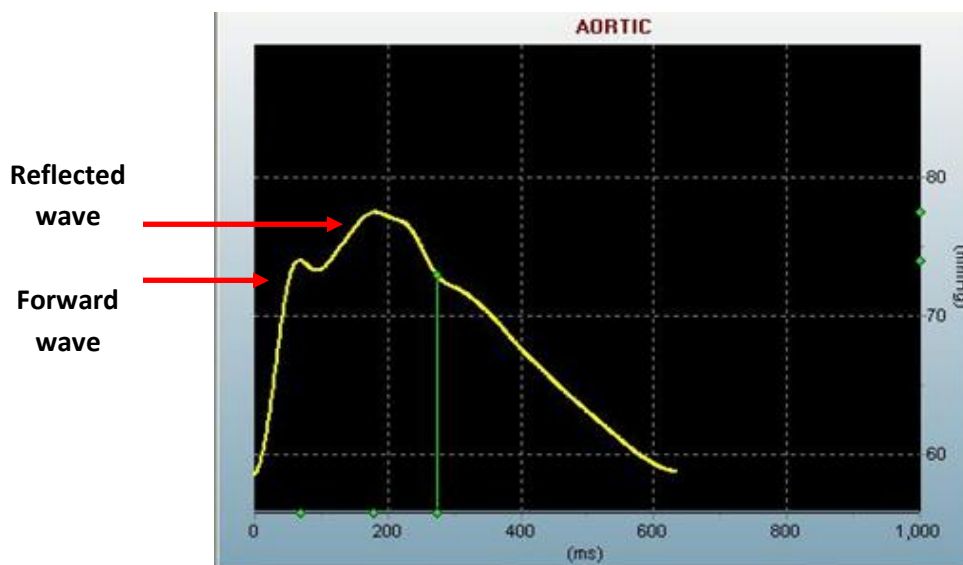


Figure 9: Synthesised aortic pressure waveform and components following pulse wave analysis. The radial pressure waveform was acquired using applanation tonometry with the SphygmoCor device in a healthy 5-year old.

2.4.2. Aortic augmentation index (AIx)

Augmentation index is defined as the ratio of augmenting pressure over pulse pressure, expressed as a percentage. Central pressure waveform analysis is used to calculate the aortic augmentation index (AIx).

Thus,

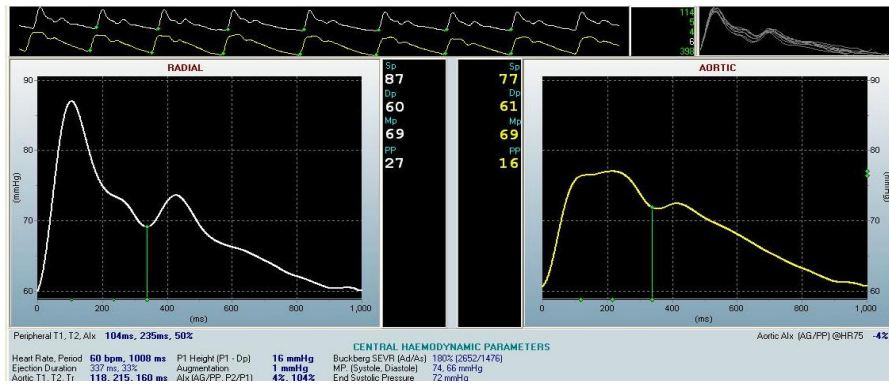
$$\text{Aortic augmentation index (AIx)} = 100 \times \frac{\text{AP}}{\text{cPP}}$$

where, AP is the augmented pressure, cPP is the central pulse pressure.

AIx provides an indication of the consequence of reflected waves on the aortic pressure waveform and it reflects the contribution of the reflected wave on systolic pressure and pulse pressure.

In young healthy adolescent subjects, aortic augmentation index is usually negative or near zero but has been found to be higher in children younger than 10 years old and thought to be significantly related to body height (**Figure 10**).^{164,165}

a



b



Figure 10: Acquisition of radial pressure waveform using applanation tonometry and computation of aortic Augmentation Index in a.13 year old healthy boy and b. 5 year old healthy boy

2.5. Large artery biomechanical properties: structural and functional

2.5.1. Pulse wave velocity

Pulse wave velocity (PWV) is the velocity of the blood pressure wave as it travels along the arterial tree and is determined by the elasticity, diameter and thickness of the arteries. For an artery that approximates an idealized thin walled elastic tube, PWV is given by the Moens Korteweg equation:

$$PWV = \sqrt{\frac{E_{inc} \cdot h}{2r\rho}}$$

Where E is Young's elastic modulus. PWV is thus proportional to the square root of the incremental elastic modulus (E_{inc}). The measurement of PWV is regarded as one of the most robust methods to measure arterial stiffness. It is usually determined by measuring the transit time (ΔT) of the pulse between a proximal and distal site:

$$PWV = \frac{\text{Distance between the two sites}}{\Delta T}$$

Although, PWV has been measured over different arterial segments including, brachial-ankle, brachial-femoral, the “aortic” PWV measured over the carotid-femoral pathway (PWVcf or PWVa) is considered to be the most informative measurement in adults. In children, only PWVcf has been described (**Figure 11**). PWVcf has been shown to be repeatable, reproducible and to be a significant predictor of mortality in adults with and without renal disease, including elderly, diabetic and hypertensive adults.^{120,166} It is considered the ‘gold-standard’ by most experts.

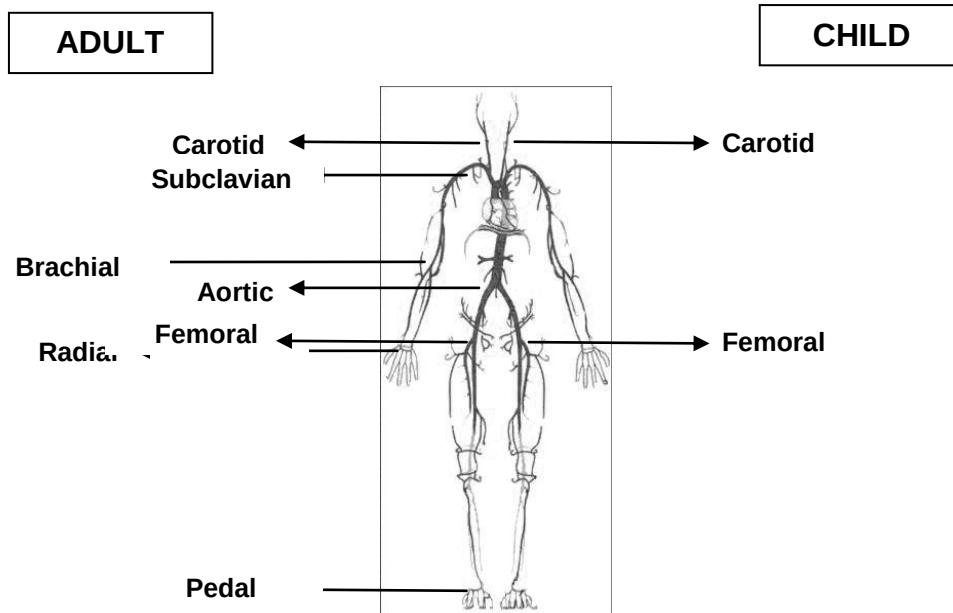


Figure 11: Schematic representation of examples of published pulse wave velocity measurement sites in adults and children

PWVcf is usually measured in adults by sequential recording at the carotid and femoral artery using electrocardiogram-referenced (ECG-referenced) applanation tonometry such as the SphygmoCor system (AtCor Medical, Australia).

The transit time, can be calculated by the time delay between the carotid and femoral sites (**Figure 12**).

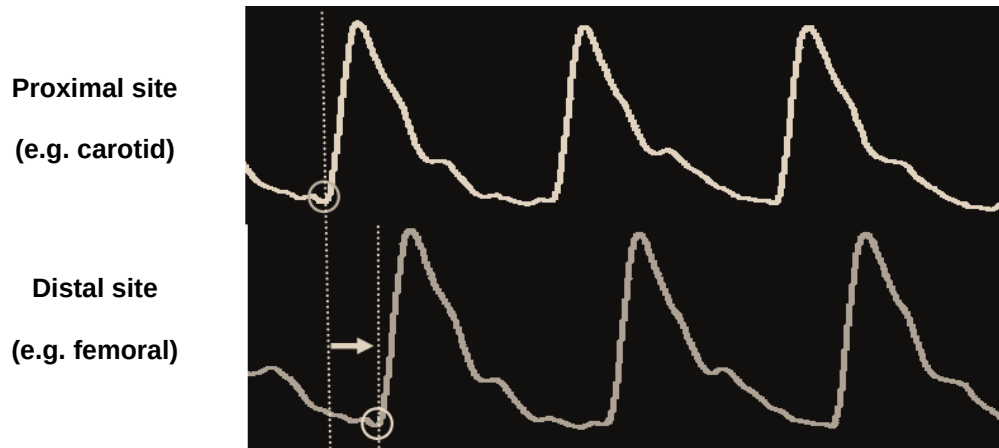


Figure 12: Schematic representation of the time difference ('delay') in pressure waves between a proximal and distal arterial pulse point when measured simultaneously.

2.5.1.1. PWV measured using the SphygmoCor system

In the Sphygmocor system, the R wave of the QRS complex of the ECG is the reference point and the *transit time* is calculated as the difference between 't1', the delay of the proximal pressure wave (e.g. carotid) and 't2', the delay of the distal pressure wave (e.g. femoral) with the R wave of the QRS complex of the ECG, both measurements being made sequentially (**Figure 13**).

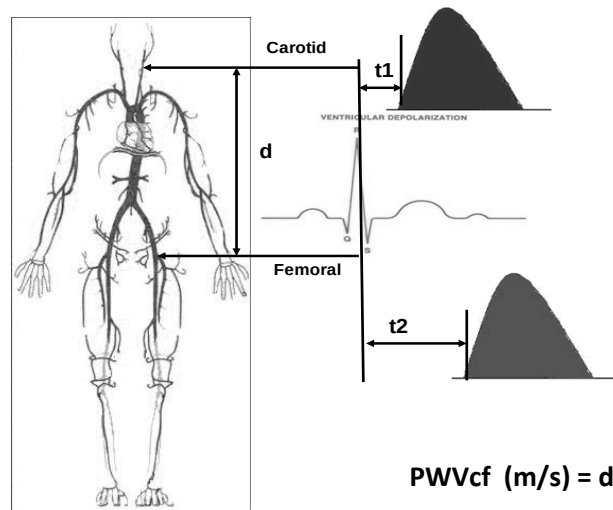


Figure 13: Schematic representation of the calculation of PWVcf, where t denotes $t_2 - t_1$, the time difference ('delay') in pressure waves between a proximal and distal arterial pulse point and 'd' the distance between the two pulse points. PWV is the ratio of t/d in m/s

Applanation tonometry using the Sphygmocor system remains most widely reported and can be performed in a co-operative child (**Figure 14**).

a



b



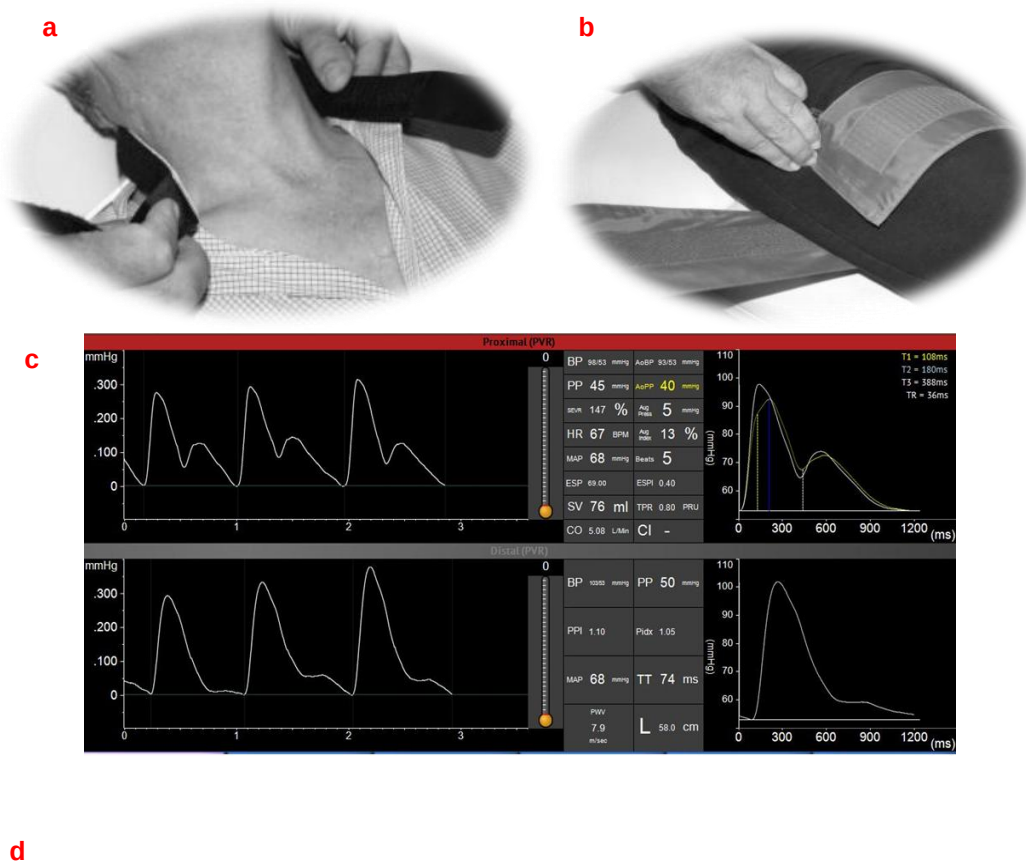
Figure 14: a. Using the Sphygmocor system to perform PWVcf in a older child; and **b.** Example of the output of the Sphygmocor system in a 14 year old healthy child with carotid artery pressure and femoral artery pressure waveforms shown in the upper and lower panels respectively (in white) together with the reference ECG (in yellow)

The Sphygmocor system though is expensive; with additional significant limitations to its use in children as it requires a trained operator, the carotid artery is difficult to applanate as the supporting tissues are elastic and access to the femoral artery in the inguinal area may be distressing, particularly in adolescents.

There are a number of instruments that allow PWVcf to be measured in adults using different techniques (applanation tonometry, ultrasound, pulse volume recording, plethysmography and Magnetic Resonance Imaging), using different algorithms – measuring PWV sequentially or simultaneously at two different pulse points. Measurements of PWV using differences technologies have limited agreement and this continues to be debated in the literature.¹⁶⁷

2.5.1.2. PWV measured using the Vicorder system

The Vicorder system (Skidmore Medical, UK) is a relatively recently introduced system that uses simultaneous volumetric pressure recordings from a sensor placed over the carotid artery and a thigh cuff placed over the femoral artery (which can be placed over light clothing, **Figure 15**). It is thought to be less operator dependent than a tonometric system and does not require the patient to undress.



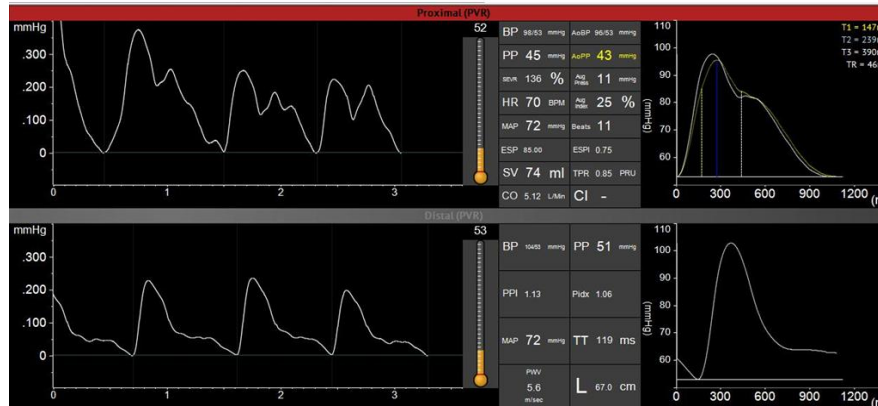


Figure 15: a. & b. Using the Vicorder system to perform simultaneous PWVcf in an older child; and **c. & d.** Example of output of the Vicorder system in a 12 year old healthy child with simultaneous recordings of the c. carotid and d. femoral pressure waveforms

The Vicorder system can also be used to measure brachial-femoral PWV (PWVbf), whereby the proximal sensor is a cuff is placed over the brachial artery. This may be more acceptable to young children than the neck cuff.

2.5.1.3. Measurement of distance

Path lengths are usually estimated from the distance between surface markings. For the SphygmoCor PWVcf path length was measured with a tape measure from the suprasternal notch to the femoral pulse at the point of applanation. For the Vicorder system, PWVcf was taken as the distance from the suprasternal notch to the top of the

thigh cuff. That for PWVbf path as the distance from the top of the arm cuff to the top of the thigh cuff.

2.5.1.4. Number and quality control of measurements of PWV

Data was considered acceptable from the Vicorder system when there were at least five sequential good quality waveforms were obtained from each cuff. For the Sphygmocor system, measurements were rejected if the standard deviation of the mean transit time exceeded 6% (automatically flagged by SphygmoCor). Three measurements were performed using each technique and average of 3 measurements calculated.

2.5.2. Carotid dimensions

2.5.2.1. Carotid intima medial thickness (cIMT) and diameter

The Mannheim Consensus provides recommendations for the measurement of IMT in adults only¹⁶⁸ although the underlying principles are the same for children. There are now some published normative data for carotid IMT (cIMT) in children and suggest significant positive correlation of body dimensions and SBP with cIMT with gender differences in particular between adolescents.^{169,170}

B-mode ultrasound is the most common method used to evaluate intima medial thickness (IMT). There are two main methods of measuring IMT using this technique. Most frequently used method involves the manual cursor placement technique, with IMT measurement at several points (usually three to six) on each side, and then averaging. The second technique is also based on manual cursor placement, but the investigator draws a line on the upper border of the intima and a second line on the lower border of the media. In this method, IMT is calculated digitally by a computer (**Figure 16**).

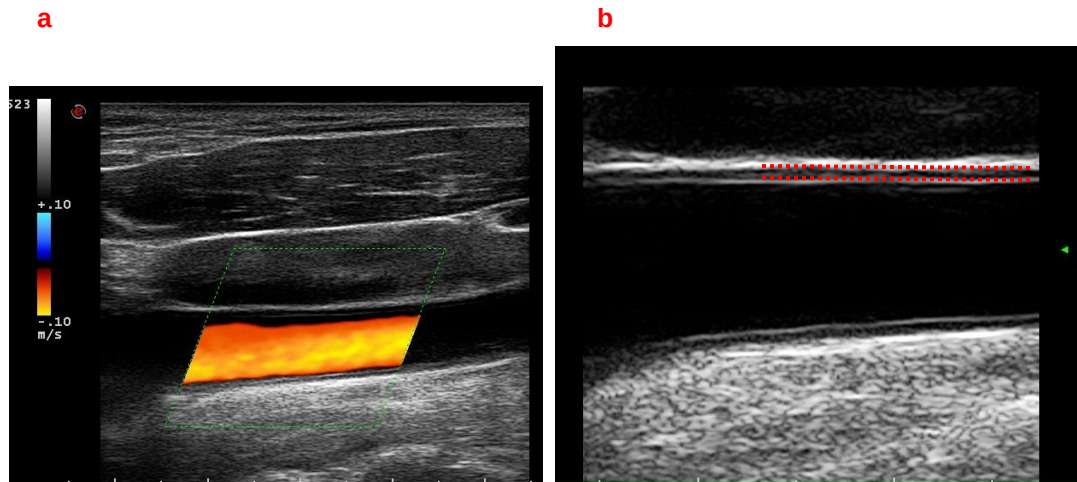


Figure 16: a. Acquisition of normal ultrasound scan of the carotid artery, with colour flow imaging; and b. an optimised image with walls seen more clearly and the upper border of intima and lower border of media on the posterior wall highlighted

In this study, we imaged the right common carotid artery (CCA) using the ART.LAB system.¹⁷¹⁻¹⁷³ A linear transducer (range 4 to 13MHz) was used to image a 4cm segment of artery approximately 1 to 5 cm proximal to the flow divider, with mean cIMT obtained from automated analysis of the posterior wall over this segment of the artery.^{174,175} This method is recognized as one of the most robust measures of IMT since the reproducibility of common carotid IMT measurements is higher than that of other segments.¹⁷⁶

After obtaining the desired longitudinal view of the CCA, the software highlights the diameter and IMT and, each radio frequency line is analyzed backward and forward in real time (**Figure 17**). Each interface is determined using a proprietary algorithm, taking advantage of the very high dynamic of the radiofrequency signal to define interfaces (blood intima and media-adventitia) and track them along the cardiac cycle.

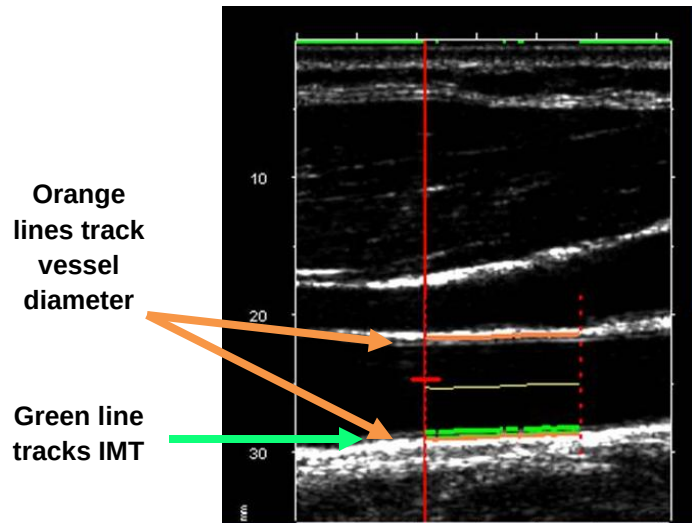


Figure 17: Acquisition of ‘Screen view’ for analyses of vessel diameter and IMT with the ART.LAB system

The ART.LAB system software extracts the raw radiofrequency data from image to calculate cIMT with greater precision. Measurements of the last 6 cardiac cycles are displayed on the screen with the mean diameter and IMT calculated (**Figure 18**). Three average values of cIMT were derived (each obtained over 6 cardiac cycles) and the averaged of 3 values calculated for each subject.

	DIAM	DIST	IMT
	[mm]	[um]	[um]
1	6.36		355
2	6.22		360
3	6.28		328
4	6.27		346
5	6.28		344
6	6.34		332
Mean	6.28		345
Stdv	0.05		18

Measurements for last six cardiac cycles displayed on the screen

Mean diameter and IMT calculated

Figure 18: Acquisition of 'Results view' for vessel diameter and IMT with the ART.LAB system

2.5.2.2. Carotid artery distension

Radiofrequency wall-tracking was used to obtain distension waveforms averaged over 6 cardiac cycles. Diastolic internal lumen diameter (D_d), systolic internal lumen diameter (D_s) and carotid distension ($\Delta D = D_s - D_d$) were derived from each distension waveforms (each obtained over 6 cardiac cycles) and averaged over 3 such waveforms (**Figure 19**).

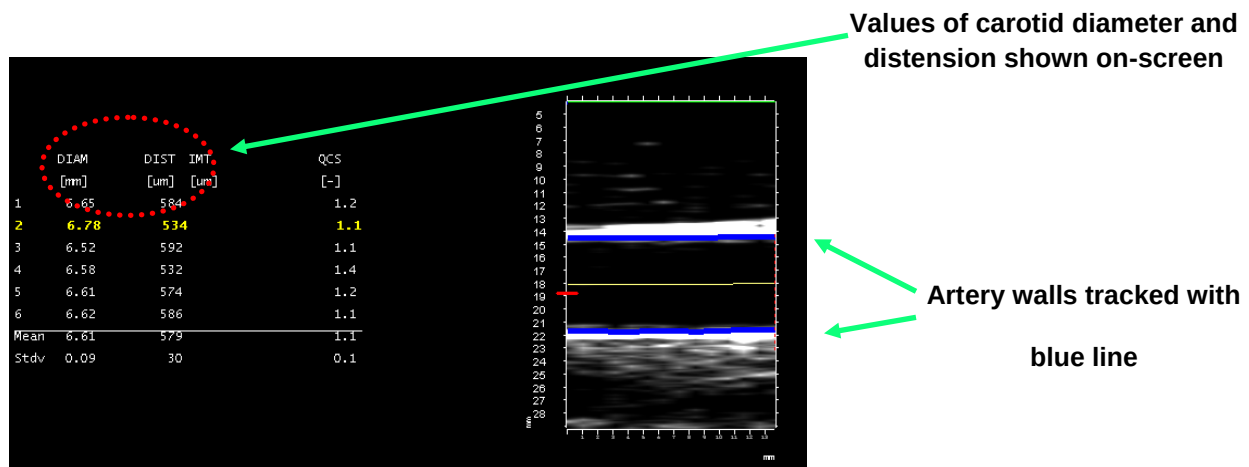


Figure 19: Acquisition of 'Results view' for artery distension and diameter with the ART.LAB system

The following measurements/indices of carotid artery geometry and elasticity were then derived from these measures of lumen diameter, distension and IMT¹⁷⁷

2.5.2.3. Other carotid artery structural measurements

a. Carotid wall thickness radius ratio (CWTR)

$$\text{CWTR} = \frac{2 \times \text{IMT}}{(D_d + \text{IMT})}$$

b. Wall cross-sectional area (WCSA)

$$\text{WCSA} = \frac{\pi [(D_d + \text{IMT})^2 - D_d^2]}{4}$$

2.5.2.4. Carotid artery functional measurements

a. Cross-sectional compliance coefficient (CC), the absolute change in lumen area during systole for a given pressure change.

$$CC = \frac{\Delta A}{PP}$$

where, ΔA is the change in cross-sectional area ($\Delta A = \pi \Delta D_d^2/4$) and PP the local pulse pressure.

b. Cross-sectional distensibility coefficient (DC), the relative change in lumen area during systole for a given pressure change.

$$DC = \frac{(\Delta A / A)}{PP}$$

where, A is lumen area in diastole ($A = \pi D_d^2/4$)

c. Circumferential wall stress (CWS), calculated using the Lamé equation^{178,179}

$$CWS = \frac{MAP \times D_m}{2 \times IMT_m}$$

where, D_m and IMT_m are the mean values of internal diameter and wall thickness during the cardiac cycle.

d. Young's incremental Elastic Modulus (E_{inc}), elasticity that is independent of the vessel geometry.

$$E_{inc} = \frac{[3(1 + A / WCSA)]}{DC}$$

2.6. Laboratory methods

Renal function was determined by estimating glomerular filtration rate (GFR) using the Schwartz formula¹³ with correction factor of 31 in our population with CKD.^{40,180,181} Stages of CKD were defined according to published definitions using KDOQI criteria.^{16,17}

All subjects including healthy volunteers (if agreed for blood tests) also had 'routine' biochemical investigations including haemoglobin, corrected calcium, phosphorus and intact parathyroid hormone (iPTH). All routine biochemical investigations were measured once and on the same day as the vascular assessments.

All subjects were evaluated at the time of a planned clinical review and underwent as part of routine clinical care clinical and study appropriate review of clinical history and physical examination, anthropometry including height, weight and body mass index, medication review and blood tests. In all subjects, blood and urine specimens were stored at -80°C in certified laboratories within 4-hours of collection.

Chapter 3 : Measurement of pulse wave velocity in children: comparison of volumetric and tonometric sensors, brachial-femoral and carotid-femoral pathways

3.1. Background

Pulse wave velocity (PWV) is a measure of arterial stiffness which when measured along large elastic arteries such as the aorta is highly predictive of cardiovascular (CV) risk in adults¹⁶⁶ particularly in patients with chronic kidney disease (CKD).¹²⁰ Children with CKD including those with Established Renal Failure (ERF) requiring renal replacement therapy have an increased risk of mortality secondary to cardiovascular disease both during childhood and as young-adults.^{19,23,27} These young adults with *childhood onset* ERF exhibit mortality rates about 30 fold higher than age matched peers with no renal disease.^{19,23} It is now widely accepted that the risk factors for these adverse cardiovascular events develop during childhood and that they are likely to evolve with progressive renal dysfunction.¹⁸²

In these children with moderate to severe CKD, arterial stiffness may contribute to cardiovascular morbidity/mortality either through remodeling of the left ventricle predisposing to sudden cardiac death⁷³ or through other mechanisms. Therefore, measurement of arterial stiffness in children with CKD may be particularly important. Reference values for PWV in children have recently been published¹⁸³, which may aid evaluation of PWV in paediatric clinical studies.

PWV is usually measured between the carotid artery and the femoral artery by sequential ECG-referenced arterial applanation tonometry (using a pressure sensitive transducer to lightly compress the artery) or other arterial pressure or flow velocity sensors. The SphygmoCor system (AtCor Medical, Australia) uses ECG-referenced applanation tonometry and is one of the most widely used systems in adults for measurement of carotid-femoral PWV (PWVcf). There are some limitations of this

system for use in children: tonometry requires a trained operator and access to the femoral artery in the inguinal area may be distressing particularly in adolescents.

The Vicorder PWV system (Skidmore Medical, UK) uses simultaneous volumetric pressure recordings from a sensor placed over the carotid artery and a thigh cuff placed over the femoral artery (which can be placed over light clothing) is less operator dependent than a tonometric system and does not require the patient to undress. The Vicorder system can also be used to measure brachial-femoral PWV (PWVbf), whereby the proximal sensor is a cuff is placed over the brachial artery. This may be more acceptable to young children than the neck cuff.

The relation of Vicorder brachial-femoral measurements to carotid-femoral measurements is unknown. Nor is it clear whether there is a close correlation between carotid-femoral measurements obtained by Vicorder and SphygmoCor systems. Good agreement between the devices has been reported in children and young adults^{184,185}, in older adults and in pregnant women.¹⁸⁶ However, others have questioned the suitability of the Vicorder device as an alternative to more established methods.¹⁸⁷

3.2. Objectives

The purpose of this study was to compare PWVcf and PWVbf obtained using the Vicorder system and to compare values of PWVcf obtained by the Vicorder and SphygmoCor in children with and without CKD.

3.3. Methods

3.3.1. Study population and protocol

The study population included a convenience sample of 156 children who were recruited from paediatric renal and hypertension outpatient clinics at the Evelina London Children's Hospital London, UK. No formal sample size estimate was performed. The local research ethics committee approved the study, and written informed consent was obtained from all parents or guardians. In older children, written assent was also obtained.

Seated blood pressure was measured in triplicate using a calibrated aneroid measurement by a single trained observer. All 156 patients had measurements of both PWVcf and PWVbf by Vicorder. Measurements were performed supine using appropriately sized cuffs as recommended by the manufacturer. Path lengths were determined using a measuring tape. That for PWVcf was taken as the distance from the suprasternal notch to the top of the thigh cuff. That for PWVbf path as the distance from the top of the arm cuff to the top of the thigh cuff. Three measurements were performed in each mode if the patient remained comfortable. Data was considered acceptable when there were at least five sequential good quality waveforms were obtained from each cuff.

Those patients who were able to tolerate the measurement also had measurements of PWVcf by SphygmoCor. SphygmoCor PWVcf path length was measured from the suprasternal notch to the femoral pulse at the point of applanation. Measurements were rejected if the standard deviation of the mean transit time exceeded 6% (automatically

flagged by SphygmoCor). As with Vicorder, measurements were done in triplicate whenever possible. All measurements were made by two experienced trained observers.

3.3.2. Statistics

Data analysis was performed using SPSS 19.0 (SPSS, Chicago, Illinois). Pearson's correlation was used to assess the correlation between measures obtained by the different methods. Agreement between methods was assessed using the Bland-Altman plots.¹⁸⁸ Differences between correlation coefficients were tested by using the Fisher r-to-z transformation. Within-subject standard deviation (SD) and coefficients of variation (SD as percentage of mean) were used to assess the repeatability of successive measures. Linear regression analysis was used to test the association of PWV with age and blood pressure. The regression equation between PWVcf and PWVbf Vicorder was used to adjust PWVbf (Vicorder) to obtain the best estimate of PWVcf (Vicorder), by multiplying PWVbf by 0.67, the reciprocal of the slope of the regression of PWVbf vs. PWVcf and adjusting for the small average difference, 0.5 m/s, between the two measures.

3.4. Results

3.4.1. Study population characteristics

One hundred and fifty six children (42% female) aged 3-18 years were recruited. Of these, 110 (71%) had chronic kidney disease (CKD), 27 (17%) had hypertension and 19

(12%) were normal healthy subjects. There were no significant differences in clinical characteristics between the entire cohort and the subset able to tolerate carotid and femoral tonometry. Children with CKD were of similar age, height, BMI and BP level to controls (data not shown) and therefore data for entire cohort are shown together (Table 7).

Table 7: Patient demographic data

Parameter	All Patients n=156		SphygmoCor cohort n=122		P
	Range	Mean±SD	Range	Mean±SD	
Age (years)	3.2 – 17.9	11.7±3.6	4.3-17.9	12.2±3.3	0.21
Height (cm)	89.5 – 189.2	146.9±22.4	102.8-189.2	150.5±19.8	0.16
Weight (kg)	12.8 – 116.1	48.0±22.9	16.6-111.8	50.3±22.0	0.41
BMI	13.1 – 41.7	20.9±5.6	13.6-41.7	21.1±5.4	0.78
Systolic blood pressure (mmHg)	71 – 147	107±16	71-147	108±17	0.64
Diastolic blood pressure (mmHg)	22 – 97	57±12	22-97	57-12	0.78

3.4.2. Comparison of PWV by Vicorder over carotid-femoral and brachial-femoral paths

The mean difference between Vicorder PWVcf and PWVbf was 1.81 ± 1.21 m/s. Vicorder PWVbf measurements were significantly higher than Vicorder PWVcf measurements ($P < 0.001$) but were closely correlated ($R = 0.75$, $P < 0.001$, **Figure 20a**). Limits of agreement (LoA) between Vicorder PWVcf and PWVbf were 0.62 to -4.25 m/s (**Figure 20b**) with a significant systematic difference between the two measurements, with a greater difference between devices as PWV increased. The observation of an increasing difference between devices as PWV increased was the rationale to perform a correction for this systematic difference which was made assuming a linear relationship between PWVcf and PWVbf by Vicorder. We obtained the best prediction of PWVbf, the mean difference between PWVcf (Vicorder) and corrected PWVbf (Vicorder) was 0.00 ± 0.77 m/s, with LoA -1.54 to 1.54 m/s (**Figure 20c**).

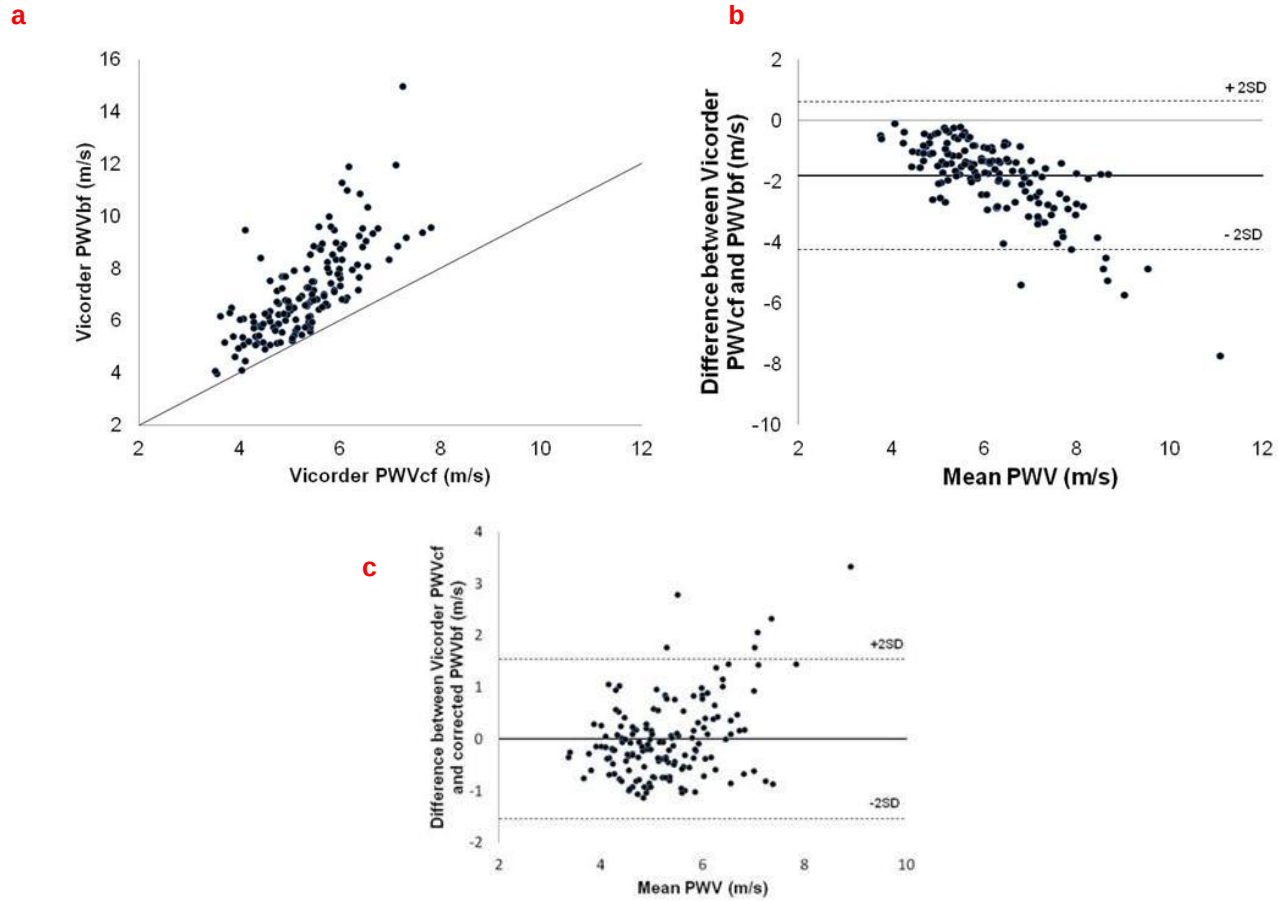


Figure 20. Comparison between pulse wave velocity (PWV) measurements by the Vicorder over different paths carotid-femoral (PWVcf) and brachio-femoral (PWVbf) **a.** Correlation between Vicorder PWVcf and PWVbf, **b.** Bland-Altman plot of agreement between Vicorder PWVcf and Vicorder PWVbf, **c.** Bland-Altman plot of agreement between Vicorder PWVcf and corrected PWVbf

3.4.3. Comparison of PWV by Vicorder and SphygmoCor

Of the 156 patients, 122 (78.2%) were able to tolerate carotid and femoral tonometry, with at least one valid result acquired. In comparison to the overall cohort, those with failed tonometry were significantly younger (9.84 ± 4.2 years vs 11.7 ± 3.6 , $p=0.008$), but with no difference between groups for BMI ($p=0.53$) and gender ($p=0.66$). Declining tonometry ($n=6$), failure to stay still ($n=6$), and failure to palpate one or more pulse and/or poor quality trace ($n=5$ each) were the commonest causes of failed tonometry.

PWVcf by Vicorder was significantly lower than that measured by SphygmoCor ($P<0.001$) and only moderately correlated with SphygmoCor PWVcf ($R=0.50$, $P<0.001$, **Figure 21a**). The mean difference between Vicorder PWVcf and SphygmoCor PWVcf was 0.31 ± 0.88 m/s. The LoA between Vicorder PWVcf and SphygmoCor PWVcf were -1.46 to 2.07 m/s. (**Figure 21b**).

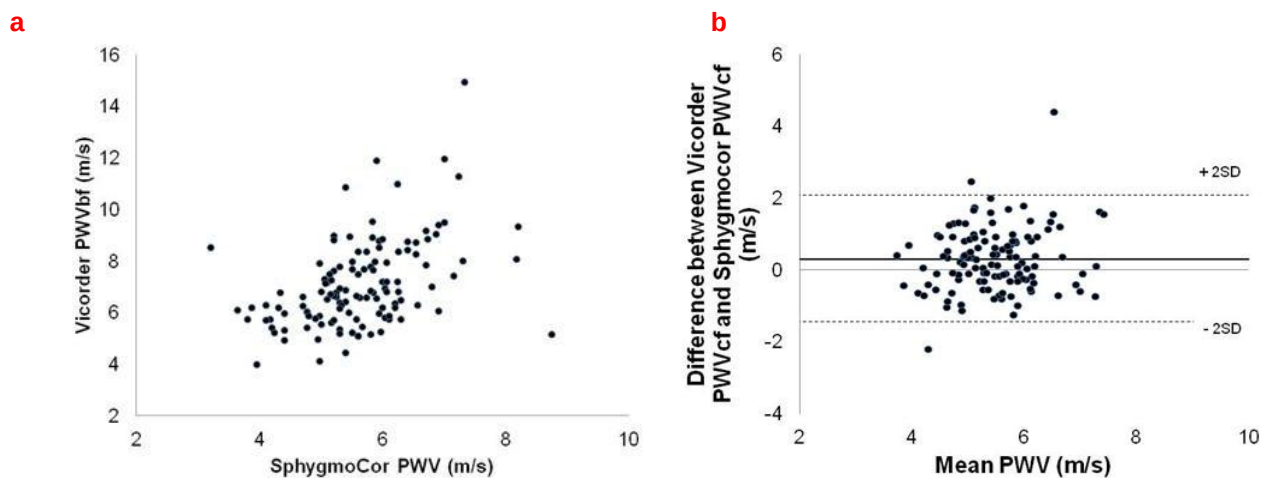


Figure 21: Comparison between pulse wave velocity (PWV) measurements by the Vicorder and SphygmoCor over the carotid-femoral path **a.** Correlation between SphygmoCor PWVcf and Vicorder PWVcf, **b.** Bland-Altman plot of agreement between SphygmoCor PWVcf and Vicorder PWVcf

The path lengths used for the two instruments were highly correlated ($R=0.84$, $P<0.0001$) and the correlation between Vicorder transit time and SphygmoCor transit time was similar to that between corresponding values of PWV ($R=0.52$ and $R=0.50$ for correlations between transit times and PWV respectively, both $P < 0.001$). Vicorder PWVbf was marginally less well correlated with SphygmoCor PWVcf ($R = 0.451$, $P < 0.001$), with a mean difference of $1.48\pm4.54\text{m/s}$. Using corrected values of PWVbf against SphygmoCor PWVcf, the mean difference was $-0.34\pm1.11\text{m/s}$ (**Figures 22a and 22b**) with LoA -2.56 to 1.88m/s .

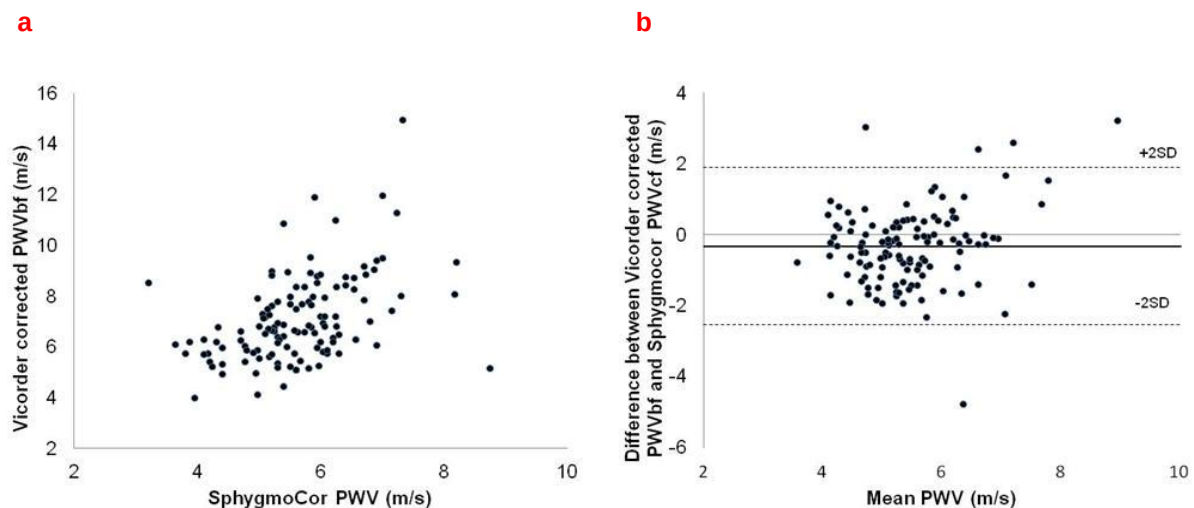


Figure 22: Comparison between pulse wave velocity (PWV) measurements by Vicorder over the brachio-femoral (PWVbf) and Sphygmocor over the carotid-femoral path (PWVcf) **a.** Correlation between SphygmoCor PWVcf and corrected Vicorder PWVbf, **b.** Bland-Altman plot of agreement between SphygmoCor PWVcf and corrected Vicorder PWVbf

We observed no significant difference between boys and girls for the difference between corrected PWVbf (Vicorder) and SphygmoCor PWVcf (-0.43 vs. -0.22 m/s; P=0.49). Similarly, there was no significant difference by age subgroups (<10, 10–15, >15 years) for the difference between corrected PWVbf (Vicorder) and SphygmoCor PWVcf (**Table 8**)

Table 8: Comparison of the differences between corrected PWVbf (Vicorder) and SphygmoCor PWVcf according to gender and age including n=122 subjects.

		Corrected PWVbf (Vicorder)	SphygmoC or PWVcf	Mean of corrected PWVbf (Vicorder) and SphygmoCor PWVcf	Difference between corrected PWVbf (Vicorder) and SphygmoCor PWVcf	P*
Subset	n	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Female	53	5.42±1.10	5.64±0.83	5.53±0.82	-0.22±1.05	0.41
Male	69	5.18±1.19	5.61±1.01	5.39±0.94	-0.43±1.15	
Aged < 10 years	34	4.95±0.73	5.10±1.10	5.03±0.79	-0.16±0.99	0.10
Aged 10-15 years	59	5.06±1.03	5.55±0.67	5.30±0.65	-0.49±1.15	
Aged > 15 years	29	6.12±1.41	6.38±0.69	6.25±0.95	-0.26±1.15	

* P value testing for the difference by gender and age subgroups for the difference between corrected PWVbf (Vicorder) and SphygmoCor PWVcf

3.4.4. Repeatability of Vicorder and SphygmoCor measurements

Within subject coefficients of variation were calculated for subjects with at least two measurements for each device with repeated measurements performed by the same researcher measured consecutively at the same study visit. Duplicate or triplicate PWVbf measurements were obtained for all patients. Six patients only had one PWVcf (Vicorder) recorded, as some measurements were discarded due to poor quality traces or intolerance of the neck cuff. Of the 122 patients included in the SphygmoCor subset, 101 had duplicate or triplicate measurements of acceptable quality. The time taken to perform 3 consecutive measurements was approximately 5 and 10 minutes for Vicorder and SphygmoCor respectively. Within subject variation for repeated measures were 5.9%, 7.8%, and 8.5% for PWVbf (Vicorder), PWVcf (Vicorder) and PWVcf (SphygmoCor) respectively.

3.4.5. Correlation of Vicorder and SphygmoCor measurements with age

Linear regression was used to determine the correlation between PWV and age for each device. Pearson's correlation coefficients were 0.50, 0.53 and 0.40 (each $P < 0.001$), for SphygmoCor, Vicorder PWVcf and Vicorder PWVbf (**Figure 23**). There was no significant difference in these correlation coefficients ($P = 0.36$).

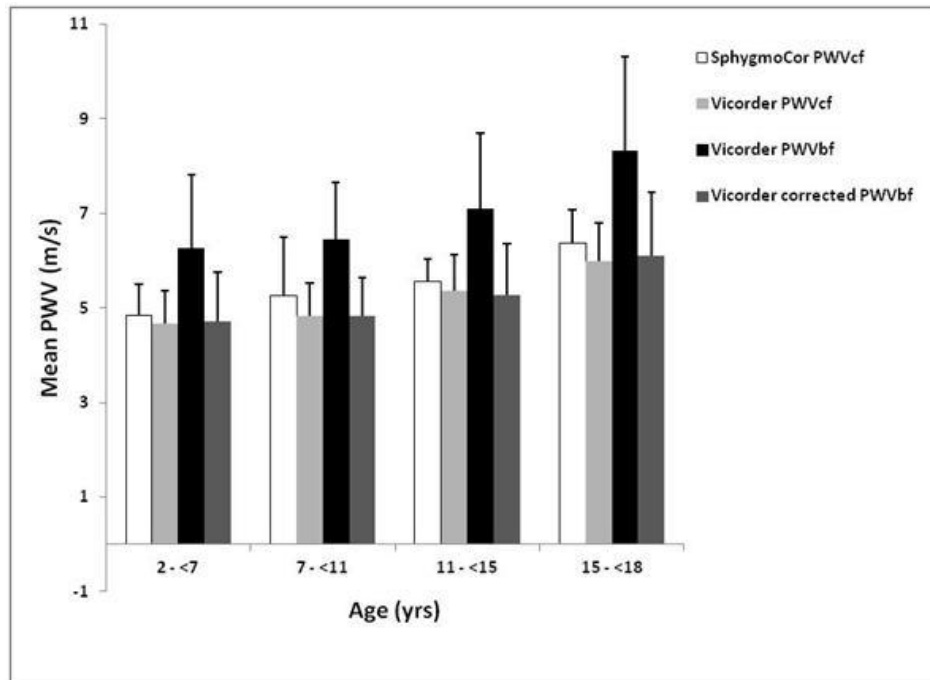


Figure 23: Variation of mean and SD of PWV according to age, as calculated by each device over different paths.

3.4.6. Discrimination of blood pressure with Vicorder and SphygmoCor

To investigate the discriminatory ability of the various PWV measurements performed by the two instruments we performed a sub-analysis in an age and gender matched cohort of two groups of children (n=20) at the extremes of the blood pressure distribution (and thus assumed to have stiff and compliant arteries for the high and low blood pressures respectively) to detect stiff and compliant aortae. Receiver operating characteristic curves show the sensitivity and specificity of the devices (**Figure 24**).

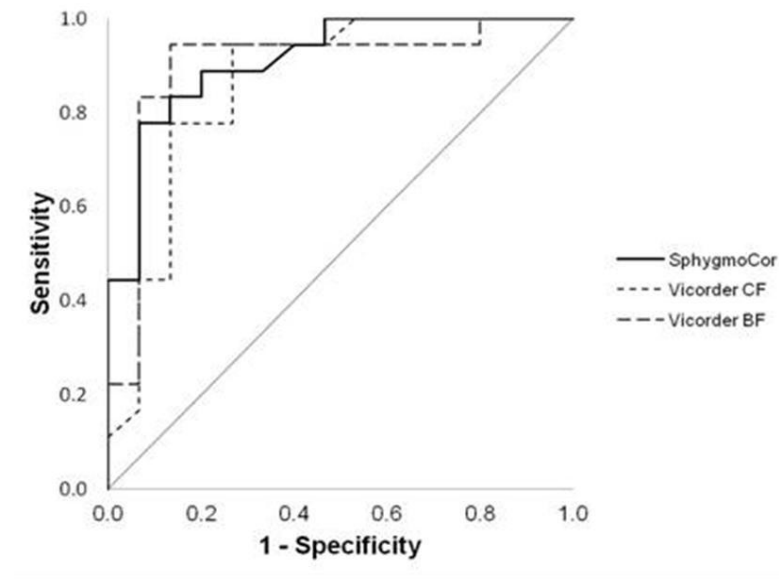


Figure 24: ROC curve showing area under the curve for each method of PWV measurement

The area under the curves was 0.913 (95% CI, 0.82-1.00) for PWVcf (SphygmoCor), 0.863 (95% CI, 0.73-1.00) for PWVcf (Vicorder) and 0.900 (95 CI, 0.78-1.00) for PWVbf (Vicorder). Therefore, there was no significant difference in the discriminatory ability of each method.

3.5. Discussion

Carotid-femoral measurements have until recently been used to estimate large artery PWV but such an approach can be difficult to use, especially in children. In the present study, carotid-femoral tonometry data could not be acquired in 22% of our children (34 out of 156). Usually this was due to practical problems in the younger children such as the need to sit still, difficulty in palpating pulses or obtaining traces of adequate quality.

In a few patients with arrhythmias, such as benign sinus tachycardia of childhood, the SphygmoCor was unable to compute the PWV accurately. Although we do not have any formal data regarding tolerance and comfort of subjects for either system, the Vicorder volumetric recoding system was used successfully (for both carotid-femoral and brachial-femoral measurements) in all children and measurements showed similar or better repeatability than those obtained using the SphygmoCor system. When comparing the repeatability and tolerability of Vicorder and SphygmoCor, it is important to note that our results could have been influenced by the order in which the instruments were used.

When comparing the agreement between Vicorder PWVcf and SphygmoCor PWVcf, there was only moderate correlation and LoA were relatively broad. LoA for carotid-femoral measurements for the two devices were -1.46 to 2.07m/s, slightly greater than the LoA of -1.0 to 1.7m/s obtained by Kracht *et al*¹⁸⁴ in a study on children and adolescents, the mean age (11.1 years) of which was similar to the children in our study (11.7 years). Our LoA were also slightly greater than those reported by Kis *et al* (-0.91 to 2.1, using the manufacturer's recommended calculation of path length) in a slightly older group (mean age 16.7 years).¹⁸⁵ Our LoA were, however, better than those reported by van Leeuwen *et al*¹⁸⁷ and thus sit within the range reported to date. Although the agreement between PWV obtained by the Vicorder and SphygmoCor methods has been described as "excellent", it is worth noting that since the mean PWV with SphygmoCor was 5.62m/s, a LOA of 2m/s represents an error of 36%. Thus values of PWV obtained by the two methods can hardly be regarded as interchangeable. Differences between methods could arise as a result of a) inaccuracies in estimating the

true path lengths, b) differences in the true path lengths, the point of pulse recording for the Vicorder system being distal to that for the SphygmoCor, c) differences in estimation of the timing of the arterial pulse waveforms at the two sites. The close correlation between the estimates of path length used for the two systems and the fact that the correlation between transit times was similar to that between values of PWV suggests that differences arise in large part through differences in timing algorithms. It is also noteworthy that in other studies which have used a variety of correction factors to adjust for inaccuracies in estimation of length, the impact on the SD of the mean difference is negligible which would again point to a difference in timing algorithm as the main source of variation between the methods. The SphygmoCor system (as in this study) is usually used with a well established intersecting tangent algorithm to identify the foot of the arterial pulse whereas the Vicorder uses a cross-correlation algorithm which may be influenced by differences in waveform morphology between the carotid and femoral sites.¹⁸⁹

To our knowledge, this is the first study to evaluate the measurement of brachial-femoral PWV. This is an even simpler technique requiring very little user training, which is well tolerated by children. Good quality waveforms can be acquired from most brachial arteries, with no venous artefacts as are often seen with a neck cuff. The brachial-femoral measurement measures the difference between pulse transit from the aorta to brachial artery and aorta to femoral rather than that between the aorta to carotid and aorta to femoral. The two measurements might, therefore, be expected to differ because of the longer and more muscular route from the aorta to the brachial artery compared to that to the carotid: the right innominate being common to both routes but

the former incorporating the subclavian and brachial arteries rather than just the common carotid. However, provided there is a close correlation between PWV in the carotid and subclavian/brachial arteries, PWV obtained over the two pathways might be expected to correlate well. This was indeed the case with a high correlation of $R=0.75$ between PWVbf and PWVcf. When adjusted for differences in estimation of path length, PWVbf by Vicorder agreed almost as well with SphygmoCor PWVcf as did PWVcf by Vicorder. We observed a systematic bias with greater difference between the PWVbf and PWVcf measurements by Vicorder with increasing PWV. The corrected PWVbf is likely to be generalisable to other paediatric populations but this needs to be tested prospectively because although the adjustment is likely to be driven by PWV it is possible that other factors such as age and/or height influence the relationship between the two methods of measurement. As PWV is predominantly driven by BP, our analyses to discriminate extremes of PWV measurement using different instruments in children with high and low BP must be interpreted with caution. Furthermore, we acknowledge limitations imposed by a relatively narrow range of PWV and BP. The correlation coefficient has limited value for assessing the agreement between two methods of measurement of the same quantity since the correlation coefficient depends on the range of values over which the correlation is assessed, being higher for a greater range.¹⁸⁸

In conclusion, the Vicorder technique is easy to use, is well tolerated by children and gives excellent repeatability when measurements are made by the same observer consecutively. Further data are needed to show inter and intra observer variability when measured over repeated visits. Values obtained over the brachial-femoral path are closely correlated with those from the carotid-femoral path. However, Vicorder

carotid-femoral values are only moderately correlated with those obtained from the SphygmoCor system with the difference likely to be due to differences in the timing algorithms used in the two systems. Although measurements are not interchangeable PWVbf appears as reproducible and as likely to discriminate between groups with differing arterial stiffness as other measures. Since it is the simplest measure to use we would recommend this is applied more widely in children and our findings tested in larger cohorts to ascertain its clinical utility.

Chapter 4 : Central aortic blood pressure from ultrasound wall-tracking of the carotid artery in children: comparison with invasive measurements and radial tonometry

4.1. Background

Systolic blood pressure (SBP) is amplified along conduit arteries such that peripheral SBP (pSBP) measured at the brachial or radial artery usually exceeds central aortic systolic pressure (cSBP) at the aortic root.¹⁵² By contrast, mean arterial pressure (MAP) and diastolic blood pressure (DBP) are almost identical at central and peripheral sites.¹²⁰ In adults, cSBP is thought to relate more closely to target organ damage than peripheral SBP (pSBP).^{153,154} Differences between cSBP and pSBP are more marked in younger compared to older adults¹⁵⁵ and may be particularly important in children.¹⁹⁰ However, non-invasive estimates of cSBP have not been validated in children and there is limited information on the magnitude of SBP amplification in children.^{191,192}

4.2. Objectives

The aim of the study was to i) compare estimates of central aortic systolic pressure obtained from radiofrequency ultrasound wall-tracking of the carotid artery^{171,172} with that measured directly using a pressure-tipped catheter placed in the aortic root at the time of arterial cannulation; ii) to compare the values of cSBP obtained from non-invasive radiofrequency ultrasound wall-tracking of the carotid artery with those obtained using applanation tonometry at the radial artery and a radial-to-aortic transfer function¹⁵²; iii) to determine typical SBP amplification in children with and without chronic kidney disease (CKD) and hypertension.

4.3. Methods

The study was performed at Evelina London Children's Hospital (ELCH), UK with the approval of the local Research Ethics Committee. Written Informed consent was obtained from parents and/or children for participation in the study.

4.3.1. Study 1: Invasive validation of carotid wall tracking-derived compared with measured aortic cSBP

Children aged 2-18 years (n=9) attending for diagnostic and/or interventional arteriography for the investigation/treatment of suspected renovascular disease or congenital heart disease were recruited from the Paediatric Nephrology and Cardiology departments at ELCH. Children with arrhythmias or clinical evidence of heart failure were excluded from the study.

4.3.1.1. Invasive measurement of central SBP (cSBP_{inv})

Central blood pressure was measured in children undergoing planned diagnostic and/or interventional arteriography for the investigation and/or treatment of suspected renovascular disease or congenital heart disease. The procedure was performed under general anaesthetic and access to the femoral artery established. A 4-FR catheter passed through the femoral arterial sheath over a guidewire and a high fidelity pressure-tipped wire placed at the proximal aortic root with radiographic guidance (**Figure 25**).

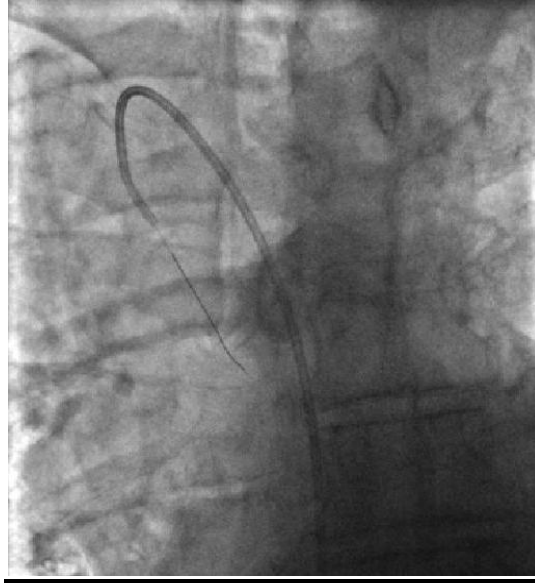


Figure 25: Catheter positioned at the proximal aortic root

ComboWire® XT 9500 series, pressure and flow wires (diameter 0.014", ComboWire®, Volcano Corporation, San Diego, CA, USA) were used. In addition to a pressure sensor mounted on the tip of the wire, this wire additionally also has a Doppler probe which enables the recording of the velocity of the blood flow (**Figure 26**). The aortic pressure waveforms were recorded digitally at 1 kHz on a computer through an analog-to-digital converter connected to the analog outputs of the ComboMap monitor.

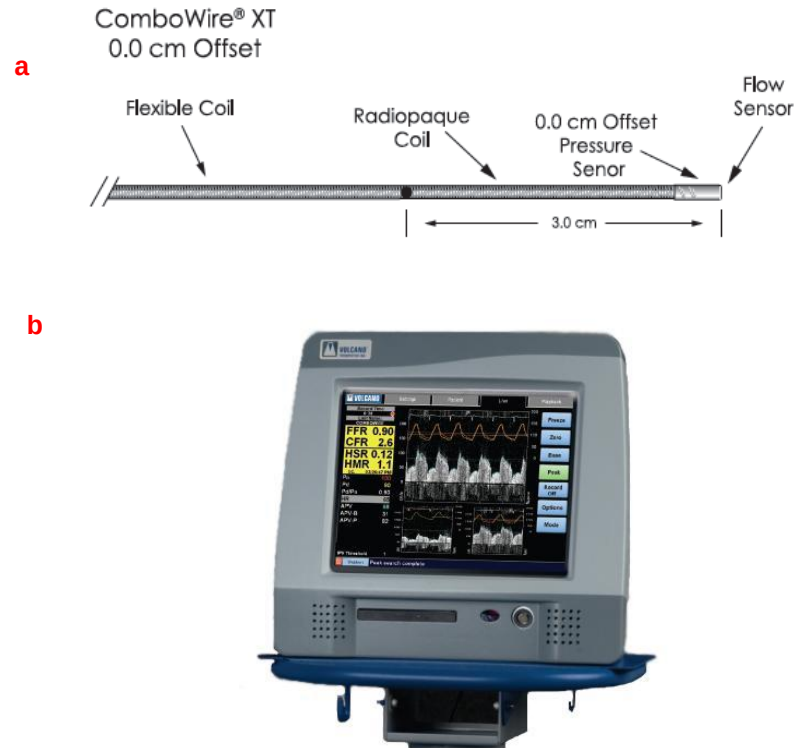


Figure 26: a. Schematic of the ComboWire® XT 9500 series, pressure and flow wire and b. The ComboMap monitor

Flow velocity and pressure waveforms were aligned according to the specifications provided by the manufacturer's engineers but for the purposes of my thesis only pressure waveform data was analysed.

4.3.1.2. Invasive validation of carotid wall tracking-derived aortic cSBP

Carotid distension waveforms were obtained by radiofrequency ultrasound wall tracking of the carotid artery, using the ART.LAB system (Esaote, Maastricht, Netherlands)

[Figure 27].^{120,152,153}



Figure 27: The ART.LAB system in use on a 6-year old child

The ART.LAB system provides very high resolution images, with a pixel size of $23\mu\text{m} \times 23\mu\text{m}$, and a very high frame rate of 600Hz and data is recorded in 14 lines perpendicular to the probe. The wall tracking system thus provides continuous beat to beat movement of the artery and allows measurement of the distention of the artery, its diameter and IMT (**Figure 28**).

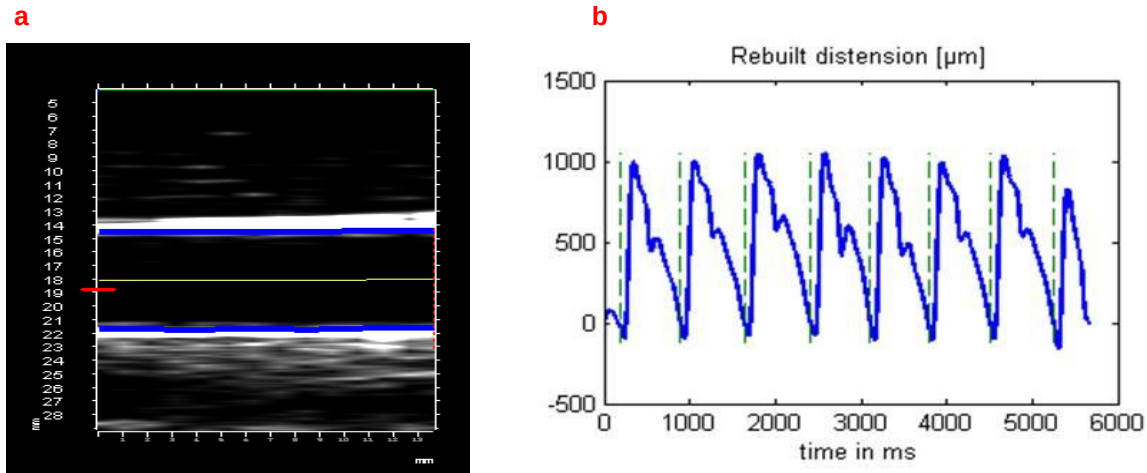


Figure 28: Acquisition of distension waveforms obtained by radiofrequency ultrasound wall-tracking of the carotid artery using the ART.LAB system

At the time of acquisition of the digital recording of the invasive aortic pressure waveform, carotid distension waveforms were obtained simultaneously over a 5 to 10 second period. Up to 5 repeat recordings of such 5-10 second periods of simultaneous carotid and aortic waveform recordings were obtained. Carotid distension and aortic waveforms were post-processed using custom in-house software developed in MATLAB (MathWorks, Cambridge, UK). Waveforms from each 5-10 second period of recording were ensemble averaged. Invasive values of cSBP, MAP and DBP (cSBP_{inv} , MAP_{inv} and DBP_{inv}) were obtained from the ensemble averaged aortic waveforms.

Distension waveforms were initially calibrated with mean and maximum distension values (**Figure 29**) and cSBP estimated ($\text{cSBP}_{\text{carotid}}$) from the ensemble averaged carotid distention waveforms that were calibrated from MAP_{inv} and DBP_{inv} as shown

previously by Kips et al (**Figure 30**).¹⁵³ Processing was performed automatically but an experienced observer first inspected all waveforms to exclude artefacts.

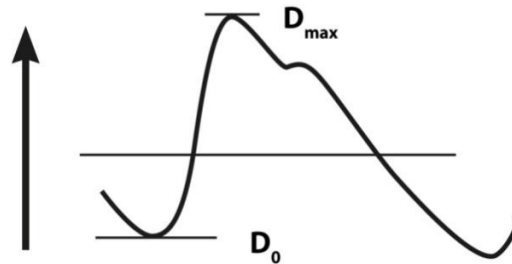


Figure 29: Calibration of the distension waveform with mean and maximum distension values

$$cSBP_{carotid} = DBP_{inv} + \frac{MAP_{inv} - DBP_{inv}}{D_{mean}} \times D_{max}$$

Figure 30: $cSBP_{carotid}$ estimated by the calibrated distention waveforms and the invasive values of the aortic pressure

4.3.2. Non-invasive comparison between radial tonometry-derived versus carotid wall tracking-derived estimates of cSBP

Children aged 2-18 years (n=84) were recruited from the hypertension and nephrology out-patient clinics of the ELCH and healthy control children were recruited from the local population. Children with arrhythmias or clinical evidence of heart failure were excluded from the study. Renal function was determined by eGFR using the Schwartz formula^{13,40} and CKD stage was defined according to published definitions.¹⁶

4.3.2.1. Peripheral blood pressure measurement

Peripheral systolic (pSBP) and diastolic blood pressure (pDBP) were measured 3 times in succession at the brachial artery by a trained observer after children had been seated for at least 5 minutes using a calibrated aneroid sphygmomanometer with an appropriate sized arm cuff according to British Hypertension Society guidelines. The aneroid sphygmomanometer instruments were checked for accuracy annually. Hypertension was defined as systolic and/or diastolic BP above the 95th percentile for age and height or if the patient was on anti-hypertensive therapy, using the Fourth Report Criteria (National High Blood Pressure Education Program Working Group).¹⁵⁴

4.3.2.2. Applanation tonometry-derived estimate of cSBP (cSBP_{RT}) at the radial artery

Radial pressure waveforms were obtained from the right wrist over a 10 second period by applanation tonometry using a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX) and processed by the SphygmoCor device (Atcor medical, Australia) [**Figure 5**].

Radial waveforms meeting the inbuilt quality control criteria of the SphygmoCor device were ensemble averaged and converted to a corresponding aortic waveform using the inbuilt SphygmoCor generalised radial-to-aortic transfer function (derived in adults) from which central blood pressures were calculated (**Figure 6**).

Radial waveforms were calibrated from peripheral brachial measures of pSBP and pDBP, from which peripheral MAP was calculated by integrating the radial waveform. Transformed radial waveforms (i.e. estimated aortic waveforms) were calibrated from these values of peripheral MAP and pDBP to give a radial tonometric estimate of cSBP (cSBP_{RT}) calibrated from non-invasive peripheral measures of SBP and DBP.

4.3.2.3. Radiofrequency ultrasound wall tracking-derived estimate of cSBP (cSBP_{CWT}) at the carotid artery

This was performed similar to the invasive study outlined previously. In contradistinction to the validation study, the carotid distension waveforms were calibrated using the same values of peripheral MAP and pDBP used to calibrate transformed radial waveforms to estimate cSBP (cSBP_{CWT}) [Figure 31]. ART.LAB waveform recordings were accepted if the SD of maximum distention was <50 µm.

$$cSBP_{CWT} = pDBP + \frac{pMAP - pDBP}{D_{mean}} \times D_{max}$$

Figure 31: Radiofrequency ultrasound wall tracking-derived estimate of cSBP (cSBP_{CWT}) estimated by the calibrated distention waveforms using the peripheral MAP and DBP pressure values

In all children, 3 sequential estimates of cSBP were attempted using radial tonometry and carotid wall tracking. The brachial BP measurements were performed initially followed by measurement of cSBP using ART.LAB and SphygmoCor devices (with

order varied but not strictly randomized). All cSBP estimates were obtained following calibration with peripheral BP components and thus, errors in the non-invasive peripheral measures of SBP and DBP contributed equally to both, radial tonometry and carotid wall tracking estimates of cSBP.

4.3.3. Statistics

Results are expressed as means $\pm$ SD. Agreement between methods was assessed by measuring the Pearson correlation coefficient, mean difference and SD of difference. Bland–Altman plots were used to examine systematic bias and random error. In the case of the comparison of carotid wall tracking-derived cSBP with invasive measurements, a modified Bland-Altman plot was used whereby the reference invasive measurement was substituted for the mean of the two methods on the abscissa of the Bland-Altman plot. All analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA). P-values <0.05 were considered statistically significant.

4.4. Results

4.4.1. Invasive validation of carotid wall tracking-derived compared to measured aortic cSBP

Of the 9 children studied, 4 were undergoing arteriography to rule out renovascular or aortic disease as a cause for hypertension, two had patent ductus arteriosus (PDA) and three had other complex congenital heart disease (CCHD). Demographics and blood pressure characteristics are shown in **Table 9**.

Table 9: Subject characteristics including n=9 subjects for the validation study and n=84 for the comparison study.

Characteristic	Study 1 (Validation Study)	Study 2 (Comparison Study)
Number of subjects	9	84
Age (years)	10.5 ± 5.0	13.2 ± 3.2
Male, n (%)	5 (44)	43 (51.2)
Caucasian, n (%)	6 (67)	69 (82)
Black, n (%)	2 (22)	5 (6)
Asian, n (%)	1 (11)	3 (4)
Other, n (%)	-	7 (8)
Height (cm)	135.5 ± 25.4	154.6 ± 20.3
Weight (kg)	35.3 ± 16.6	53.6 ± 22.4
BMI (kg/m ²)	18.0 ± 4.0	21.6 ± 6.1
Controls, n (%)	-	15 (18)
CKD Patients, n (%)	-	56 (67)
Hypertensive patients, n (%)	-	13 (15)
Antihypertensive drugs, n (%)	2 (22)	17 (8)
pSBP (mmHg)	96 ± 9.4	108 ± 15.1
pDBP (mmHg)	43 ± 11.0	59 ± 10.2
cSBP Catheter (mmHg)	90 ± 14.9	-
cSBP Carotid Ultrasound (mmHg)	94 ± 13.8	90 ± 11.9
cSBP Radial Tonometry (mmHg)	-	90 ± 11.7

Values are numbers or means ±SD. CKD, Chronic kidney disease; cSBP, central systolic blood pressure; pDBP, peripheral diastolic blood pressure; pSBP, peripheral systolic blood pressure

Values of $cSBP_{carotid}$ obtained from carotid distension-derived estimates of $cSBP$ (calibrated using MAP_{inv} and DBP_{inv}) were highly correlated with invasive measures, $cSBP_{inv}$ ($r=0.99$; $p<0.0001$, **Figure 32a**). Bland-Altman analysis demonstrated a small but significant systematic difference which did not vary significantly with $cSBP_{inv}$. The mean $cSBP_{inv}$ was 90 ± 14.9 mmHg and mean $cSBP_{carotid}$ was 94 ± 13.8 mmHg. The mean difference (i.e. aortic to carotid amplification), $cSBP_{carotid} - cSBP_{inv}$ was 3.9 ± 2.5 mmHg (**Figure 32b**).

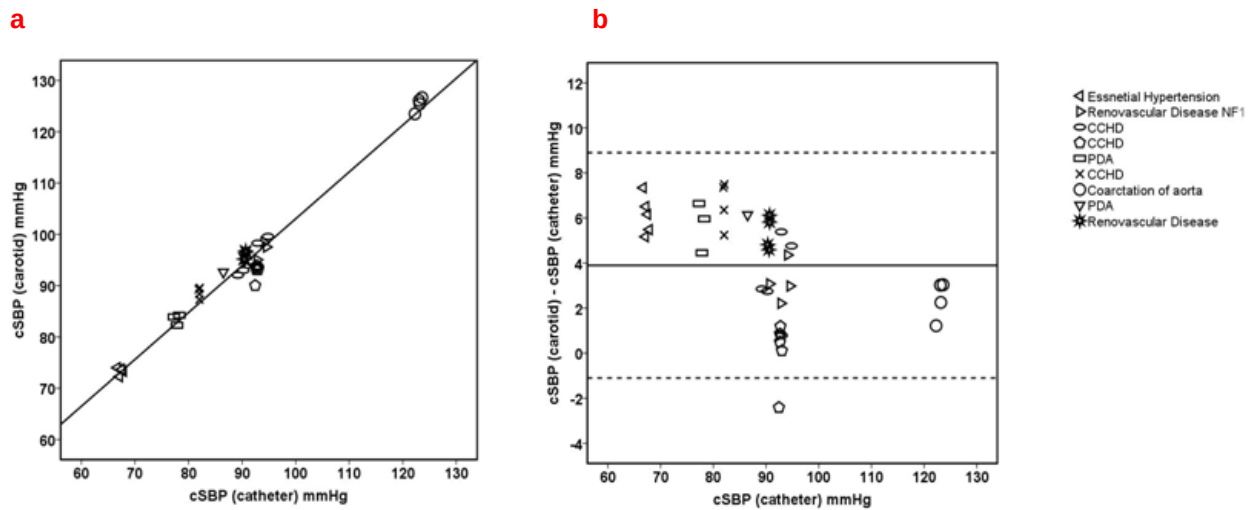


Figure 32: Invasive validation of carotid wall tracking-derived compared to measured aortic cSBP **a.** Correlation between central systolic blood pressure (cSBP) measured by invasive catheter and carotid ultrasound ($r=0.99$, $p<0.001$). **b.** The difference between cSBP measured by carotid ultrasound and catheter (mean $\pm$ SD, 3.9 ± 2.5 mmHg).

4.4.2. Non-invasive comparison of radial tonometry-derived versus carotid wall tracking-derived estimates of cSBP

Of the 84 children studied, 56 (31 boys) had CKD (stages 1, 2, 3 and 4 of CKD in 10, 21, 20, and 5 respectively) and 14 children with CKD were on antihypertensive medication. Thirteen children (6 boys) had hypertension with normal renal function of whom 3 were taking antihypertensive medication. Fifteen children (6 boys) were healthy control subjects. Characteristics of all subjects are shown in **Table 9**.

We obtained 3 sequential carotid wall tracking recordings of adequate quality in 71 children but radial tonometry recordings meeting SphmoCor quality control criterion were obtained in only 52 children, Estimates of cSBP obtained by radial tonometry and carotid wall tracking were closely correlated ($r=0.95$; $p<0.001$, **Figure 33**).

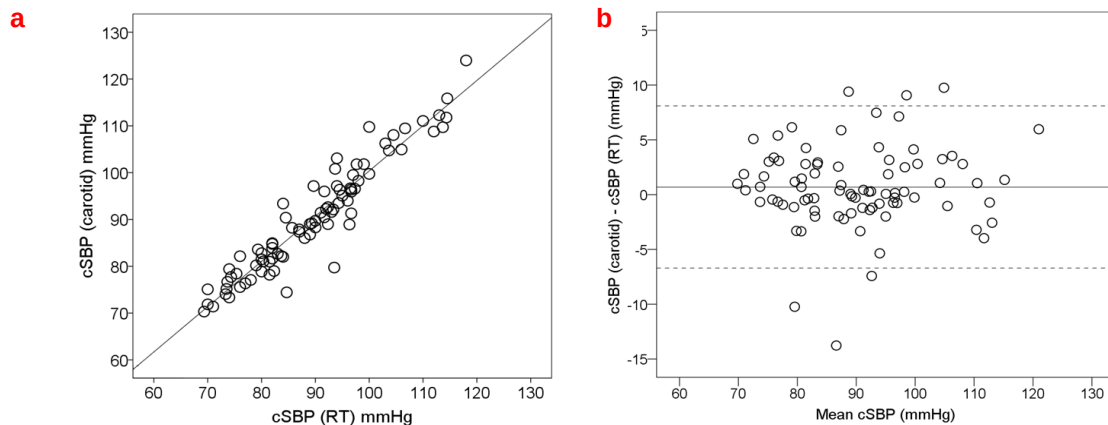


Figure 33: Non-invasive comparison of radial tonometry-derived versus carotid wall tracking-derived estimates of cSBP a. The correlation between cSBP measured by carotid ultrasound and radial tonometry ($r=0.95$, $p<0.001$); and b. The difference between cSBP measured by carotid ultrasound and radial tonometry (mean $\pm$ SD, 0.71 ± 3.7 mmHg). There is no significant difference in the mean cSBP obtained by both devices ($p=0.09$)

There was no significant difference between cSBP of the children on anti-hypertensive medication ($n=17$) and those without ($n=67$): 88 ± 11.8 mmHg vs. 90 ± 11.7 mmHg, respectively ($p = 0.43$). Bland-Altman analysis revealed no significant systematic error and no trend for error to vary with mean cSBP. The mean difference $cSBP_{\text{carotid}} - cSBP_{\text{RT}}$ was 0.71 ± 3.7 mmHg (95% CI = -1.53 to 1.01). Repeatability of measurements, for both methods, when using the same brachial systolic and diastolic BP for calibration was excellent: the coefficient of variation for repeated measurements was less than 2% for both carotid and radial measurements.

4.4.3. Peripheral to central amplification

Values of peripheral and central SBP as measured by both radial tonometry and carotid wall tracking are shown in **Figure 34**. The mean amplification (peripheral-central SBP) was 18.3 ± 6.6 and 17.7 ± 7.6 mmHg for radial tonometry and carotid wall tracking, respectively.

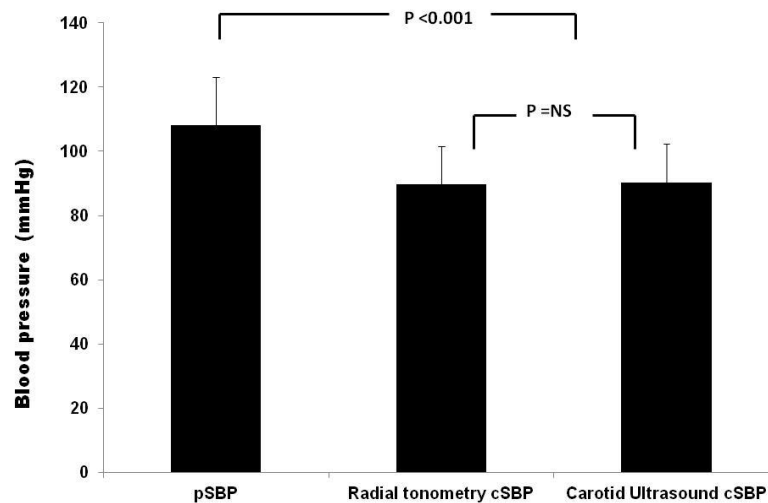


Figure 34: Peripheral-to-central amplification. Mean and SD peripheral systolic blood pressure (pSBP) and central systolic blood pressure (cSBP) measured by radial tonometry and carotid ultrasound. There is no significant difference in cSBP measured by tonometry and carotid ultrasound.

4.5. Discussion

To our knowledge this is the first study to examine the accuracy with which central aortic systolic pressure can be estimated non-invasively in children. The method that we elected to compare to invasive measures, carotid wall tracking, assumes carotid wall

distension to be proportional to local intra-arterial carotid pressure and for carotid pressure to approximate aortic root pressure.^{193,194} Theoretically, tonometric measurements obtained at the carotid artery would be expected to perform as well as carotid wall tracking.¹⁷² However, in preliminary studies we found high quality carotid tonometric recordings were more difficult to obtain than ultrasound wall-tracking in children. We compared agreement between cSBP estimated from carotid wall tracking with measured cSBP in a heterogenous group of children in whom central haemodynamics would be expected to vary widely. Despite this we observed good agreement between estimated and measured central aortic systolic pressures. This suggests that, in most children, assumptions relating to carotid wall distension being linearly related to local intra-arterial pressure and lack of pressure gradient at peak pressure from aorta to carotid are likely to be valid.

We observed a small difference between carotid-derived and measured cSBP with carotid SBP exceeding measured aortic cSBP. This may be due to some aortic-to-carotid amplification of SBP between the two measurement sites and could be corrected for by subtracting 3.9 mmHg from the carotid SBP with validation in a further study. It is important to note that we used invasive measures of mean and diastolic pressures to calibrate the aortic distension waveform to obtain a pressure waveform. This is the usual approach when assessing the accuracy of central systolic pressure determination because it removes the confounding effects of error in peripheral blood pressure determination.^{195,196} However, when the method is applied in practice, non-invasive measurements of peripheral pressures are required and any inaccuracy in these will influence the accuracy of the derived central aortic systolic pressure.¹⁹⁷

An alternative method (also requiring calibration from peripheral pressure measurements) for assessing central systolic pressure employs radial tonometry and a radial-to-aortic transfer function. We found that radial tonometry was more difficult to perform than carotid ultrasonography in children. The increased success of carotid wall tracking compared to tonometry (as assessed by the greater proportion of children in whom readings of adequate quality could be obtained) suggests this may be the preferred technique in children. However, when high quality recordings with an acceptable SD were used, and when the same peripheral blood pressure was used to calibrate both radial artery and carotid artery waveforms, we obtained excellent agreement between estimates of central aortic systolic pressure derived from the two techniques. Thus despite the radial-to-aortic transfer function being derived in adults, these results suggests that it holds to a close approximation in children. Whilst at first sight this might seem surprising, it is notable that when used to estimate cSBP in adults, the exact form of the transfer function does not appear critical and holds despite haemodynamic perturbations such as during pacing and vasodilator therapy which may to some extent mimic the circulatory state in children as compared to adults.^{195,197,198}

Amplification of SBP from the aorta to the upper limb in adults is usually in the order of 10 mmHg but varies with age being greater in younger compared to older adults.¹⁵⁵ Using an oscillometric device Elmenhorst *et al.*¹⁹¹ reported lower amplification in children and young adults aged 8-21 years, than we observed (by approximately 5 mmHg). However, when the oscillometric device was compared to the SphygmoCor, by Stoner *et al.*¹⁹⁹ cSBP was, on average, 4.5mmHg (95% confidence interval 4.0 to 5.2

mmHg) higher, by the oscillometric method. Thus, discrepancies in amplification may arise from the different measurement techniques.

Results of the present study show that in children, including those with and without hypertension and mild to advanced CKD, amplification is substantial with a mean amplification of ~ 20 mmHg and thus may be relatively more important than in adults. Although not all studies have shown a closer association of cardiovascular events with central compared to peripheral SBP in adults²⁰⁰, this may be because of limited sample size and/or inaccuracies in the measurement of peripheral BP, and target organ damage does appear more closely related to central rather than peripheral BP.¹⁵⁴

Results from the present study suggest that estimation of central aortic systolic pressure using either carotid wall tracking or radial tonometry will be helpful in determining whether central systolic pressure is equally or more important than peripheral blood pressure in children compared to adults. However, it should be noted that the absolute accuracy of estimation of central aortic systolic pressure will be dependent on that of the peripheral blood pressure and that a number of calibration issues remain. These include whether to calibrate from peripheral mean and diastolic pressures or from peripheral systolic and diastolic pressures and, if calibrating a radial waveform from a brachial systolic pressure, brachial to radial amplification.²⁰¹ Even if there are relatively large errors in determination of peripheral blood pressure, we have previously shown that radial tonometry provides a reasonable estimate of the difference between peripheral and central systolic pressure.¹⁹⁷ Non invasive estimates of cSBP in children obtained by either carotid wall tracking or radial tonometry should, therefore, be useful in determining: a) factors influencing amplification; b) whether measurement of central

aortic systolic pressure provides incremental value over pSBP when assessing target organ damage and c) interventions that have differential effects on central and peripheral systolic pressure.

4.5.1. Perspectives

This study provides data validating non-invasive measurement of central systolic blood pressure in children. Systolic pressure amplification in children is almost twice the levels of amplification described in adult cohorts and therefore likely to be more clinically relevant. Due to the small sample size of the invasive study results need to be interpreted with caution and further invasive validation is required. Further work should also investigate the factors determining amplification in children and whether measures of central blood pressure offer incremental value over peripheral blood pressure in the management of children with hypertension and those at risk of cardiovascular disease.

Chapter 5 : Decreased arterial elasticity in children with nondialysis chronic kidney disease relates to blood pressure and not to glomerular filtration rate

5.1. Background

In adults with chronic kidney disease (CKD), including those with onset of CKD in childhood, adverse cardiovascular outcomes are closely related to arterial stiffening.²⁰²⁻

²⁰⁴ Arterial stiffening relates to the severity of CKD, being greatest in those with dialysis dependent CKD, and is thought to be driven, at least in part, by metabolic changes associated with CKD.^{120,205,206} To what degree such change occurs early in childhood is unknown but could contribute to the greatly increased cardiovascular mortality and morbidity in young adults with childhood onset CKD.^{25,182}

Previous studies in children studying structural and functional properties of large arteries have included cohorts of nondialysis CKD, dialysis dependent and children after kidney transplantation. Pulse wave velocity (PWV) of the carotid-femoral pathway (i.e. mainly the aorta) and/or measures of carotid mechanics have been examined but the latter have been limited by lack of concurrent measures of carotid blood pressure (required to determine functional elasticity of the carotid artery).¹⁰⁷⁻¹¹¹ Furthermore, whilst the potential impact of age and blood pressure (BP) have been adjusted for, when comparing differences between children with and without CKD, this comparison has not been performed between age and blood pressure matched groups.

5.2. Objectives

The objectives of this study were to compare large artery mechanical properties including carotid-femoral PWV (PWVcf) and carotid mechanics derived from carotid blood pressures in children with non-dialysis CKD and healthy children in an analysis

incorporating a case-control design with appropriate matching for age and blood pressure and to examine the impact of blood pressure on these measures.

5.3. Methods

The study was performed at the Evelina London Children's Hospital (ELCH) UK with the approval of the Local Research Ethics Committee. Potential participants were attending CKD out-patient clinics and were identified following review of their health records at the authors tertiary paediatric nephrology centre serving the South East of England.¹² All participants included in this study report were enrolled sequentially and included if they had acceptable quality of blood pressure and vascular assessments. Written informed consent was obtained from parents and children if appropriate.

A total of 226 children including 188 children with CKD were recruited and 38 healthy children from the local population. Inclusion criteria were age 2-18 years, non-dialysis CKD irrespective of the presence or absence of hypertension. We excluded children with arrhythmias and those with clinical evidence of heart failure.

Hypertension was defined as systolic and/or diastolic BP above the 95th percentile for age and height or if the patient was on anti-hypertensive therapy using the Fourth Report Criteria.¹⁵⁹ Renal function was estimated using the modified Schwartz formula^{12,13,40}, and CKD stage was classified as described previously.¹⁶ Eighteen healthy children did not have any blood tests, and in them, the average eGFR for healthy children was imputed. Clinical markers of mineral bone disease including serum calcium, phosphate, calcium-phosphate product (Ca-P), intact parathyroid hormone

(iPTH) and (in a sub-sample of n=91) 25-hydroxy vitamin D3 concentrations were measured.

5.3.1. Peripheral blood pressure

Peripheral systolic (pSBP) and diastolic blood pressure (pDBP) were measured 3 times in succession at the brachial artery by a trained observer after children had been seated for at least 5 minutes using a calibrated aneroid sphygmomanometer with an appropriate sized arm cuff according to British Hypertension Society guidelines.

5.3.2. Carotid blood pressure, amplification and augmentation

Carotid systolic BP (cSBP) was obtained from radial artery tonometry using a transfer function and from carotid distension waveforms (with no transfer function). Radial pressure waveforms were obtained from the right wrist by applanation tonometry using a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX) and processed by the SphygmoCor device (Atcor Medical, Australia). Radial waveforms were calibrated from brachial measures of pSBP and pDBP, from which mean arterial pressure (MAP) was calculated by integrating the radial waveform. Transformed radial waveforms (i.e. estimated aortic waveforms) were calibrated from these values of MAP and DBP to give a radial tonometric estimate of cSBP ($cSBP_{RT}$) calibrated from non-invasive measures of pSBP and pDBP. Operators checked waveform quality and radial waveforms were only accepted if in-built quality control measures were achieved. Aortic augmentation index (Aix) was derived from the synthesised aortic waveform. cSBP was

also derived from carotid waveforms [obtained by echo-tracking as described below, (cSBP_{CWT})] calibrated from MAP and DBP. Amplification of systolic BP (pSBP-cSBP) was calculated using cSBP_{CWT} values.

5.3.3. Carotid dimensions and biomechanics

The right common carotid artery was imaged using the ART.LAB system.¹⁷¹⁻¹⁷³ A linear transducer (range 4 to 13MHz) was used to image a 4cm segment of artery approximately 1 to 5 cm proximal to the flow divider. Mean carotid intima medial thickness (IMT) was obtained from automated analysis of the posterior wall over this segment of the artery. Radiofrequency wall-tracking was used to obtain distension waveforms averaged over 6 cardiac cycles. Diastolic internal lumen diameter (D_d), systolic lumen diameter (D_s) and carotid distension ($\Delta D = D_s - D_d$) were derived from each distension waveforms (each obtained over 6 cardiac cycles) and averaged over 3 such waveforms. The following measurements/indices of geometry and elasticity as described by Laurent *et al*¹⁷⁷ were then derived from these measures of lumen diameter, distension and IMT:

Carotid wall thickness/radius ratio (CWTR)

$$CWTR = \frac{2 \times IMT}{(D_d + IMT)}$$

Wall cross-sectional area (WCSA)

$$WCSA = \frac{\pi [(D_d + IMT)^2 - D_d^2]}{4}$$

Cross-sectional compliance coefficient (CC), the absolute change in lumen area during systole for a given pressure change.

$$CC = \frac{\Delta A}{PP}$$

where, ΔA is the change in cross-sectional area ($\Delta A = \pi \Delta D_d^2/4$) and PP the local pulse pressure.

Cross-sectional distensibility coefficient (DC), the relative change in lumen area during systole for a given pressure change.

$$DC = \frac{(\Delta A / A)}{PP}$$

where, A is lumen area in diastole ($A = \pi D_d^2/4$)

Circumferential wall stress (CWS), calculated using the Lamé equation^{177,178}

$$CWS = \frac{MAP \times D_m}{2 \times IMT_m}$$

where, D_m and IMT_m are the mean values of internal diameter and wall thickness during the cardiac cycle.

Young's incremental Elastic Modulus (E_{inc}), elasticity that is independent of the vessel geometry.

$$E_{inc} = \frac{3(1 + A / WCSA)}{}$$

5.3.4. Carotid-femoral PWV (PWVcf)

PWVcf was measured in the supine position using the Vicorder volumetric system (Skidmore Medical, UK). Simultaneous arterial pulse waveforms were recorded using pulse volume recording measurements from standard vascular cuffs placed over the right carotid artery and the right femoral artery. All measurements were performed consecutively three times in succession and the average of three measurements was taken. The waveforms acquired at two sites simultaneously gave a transit time (TT) and PWVcf calculated from the distance between the suprasternal notch and the top of the thigh cuff divided by TT ($PWVcf = \text{Distance}/TT$).

5.3.5. Statistics

Subject characteristics are expressed as mean $\pm$ standard deviation (SD) for continuous variables, with two-group comparisons via Student's unpaired *t*-test, and three or more groups via an analysis of variance (ANOVA) test or Chi-squared test for categorical values. Given the age-related change in BP throughout childhood, peripheral BP measurements were presented both as mmHg and as standard deviation scores (SDS) (the number of standard deviations above or below a population mean assigned a value of zero) using published reference values.¹⁵⁹ To assess the impact of blood pressure, those with CKD were subdivided into subjects with systolic and/or diastolic BP $\geq 75^{\text{th}}$ percentile and those with systolic and/or diastolic BP $< 75^{\text{th}}$ percentile. We used the 75th percentile for BP as a cut-off based on the results of the 'ESCAPE' study which suggested that, in children with CKD, achieving a target BP below the 75th percentile delays the progression of renal dysfunction.¹⁵⁸ The 75th percentile has thus become an

important threshold and recommended by the European Society of Hypertension in its last guideline.¹⁵⁷ Multiple regression analysis was used to examine the relationship between measures of arterial stiffness, BP, GFR and other confounders. Confounders were included if they were identified from previous published relevant literature or of thought to be of pathophysiological significance and included age, gender, BMI, heart rate, anti-hypertensive treatment (yes/no) and biochemical markers of metabolic bone disease. Because arterial stiffness may lead to a rise in pulse pressure (PP) and systolic BP, the primary analysis was performed using MAP. To examine which components of BP were most closely associated with the measures of stiffness, an additional analysis was performed in which the MAP, cSBP and carotid PP (cPP) were included in a regression analysis. To investigate differences associated with renal dysfunction we performed a case-control sub-analysis with 2:1, age (to within 1 year) and gender matching (2 CKD subjects for each control), with similar peripheral and carotid blood pressure levels. All analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA) and a P-value <0.05 was considered statistically significant.

5.4. Results

5.4.1. Patient characteristics

All children with CKD were non-dialysis dependent and none had previously received a kidney transplant. There were 49 (26.1%), 47 (25%), 56 (29.8%), 31 (16.5%) and 5 (2.7%) in CKD stages 1 to 5 respectively.

The primary cause of CKD was congenital anomalies of the kidney and urinary tract (hypo/dysplasia, obstructive uropathy and vesico-ureteric reflux $\pm$ reflux nephropathy] in 92 (48.9%), glomerular diseases in 43 (22.9%), renovascular disease in 13 (6.9%), metabolic renal disease in 14 (7.4%), tubulointerstitial disease in 2 (1.1%), cystic diseases (autosomal recessive polycystic kidney disease and nephronophthisis) in 7 (3.7%) and unknown aetiology in 17 (9.0%) of the cohort. Subject characteristics are described in **Table 10**.

Table 10: Characteristics of children according to presence of CKD (n=188) and BP $\geq 75^{\text{th}}$ percentile and n=38 controls

Measures	CKD			Controls	ANOVA	CKD BP $< 75^{\text{th}}$	CKD BP $\geq 75^{\text{th}}$ versus
	All (A)	BP $< 75^{\text{th}}$ (B)	BP $\geq 75^{\text{th}}$ (C)	D	B-D	versus Control	Control
Number (%)	188 (83.2%)	144 (63.7%)	44 (19.5%)	38 (16.8%)			
Age (years)	11.9 $\pm$ 3.7	11.8 $\pm$ 3.5	12.2 $\pm$ 4.2	11.5 $\pm$ 3.3	0.70	0.65	0.43
Male sex, n (%)	129(57.1%)	90(62.5%)	26 (59%)	13 (34.2%)	0.002	0.002	0.025
Ethnicity					0.64	0.46	0.28
Caucasian	172 (76.1%);	108(75%);	36 (81.8%);	28 (73.7%);			
Asian	24 (10.6%);	14 (9.7%);	6 (13.6%);	4 (10.5%);			
Black	20 (8.9%);	14 (9.7%);	2 (4.6%);	4 (10.5%);			
other	10 (4.4%)	8 (5.6%)	0	2 (5.3%)			
Height (m)	1.47 $\pm$ 0.22	1.47 $\pm$ 0.21	1.46 $\pm$ 0.23	1.49 $\pm$ 0.21	0.77	0.57	0.49
Weight (kg)	45.6 $\pm$ 19.9	44.4 $\pm$ 18.8	49.4 $\pm$ 23.0	49.2 $\pm$ 23.4	0.22	0.18	0.97
Height SDS*	-0.29 $\pm$ 1.31	-0.23 $\pm$ 1.36	-0.47 $\pm$ 1.16	0.44 $\pm$ 1.26	0.005	0.007	0.001
Weight SDS*	0.28 $\pm$ 1.35	0.21 $\pm$ 1.32	0.52 $\pm$ 1.46	0.85 $\pm$ 1.49	0.03	0.01	0.31
BMI[†] SDS*	0.58 $\pm$ 1.33	0.46 $\pm$ 1.33	0.97 $\pm$ 1.27	0.76 $\pm$ 1.41	0.07	0.23	0.48
BSA (m²)[‡]	1.35 $\pm$ 0.38	1.33 $\pm$ 0.36	1.39 $\pm$ 0.43	1.41 $\pm$ 0.42	0.44	0.28	0.90

Measures	CKD			Controls	ANOVA	CKD BP <75 th	CKD BP ≥75 th versus
	All (A)	BP <75 th (B)	BP ≥75 th (C)	D	B-D	versus Control	Control
eGFR (ml/min/1.73m ²) [§]	64±33.8	63.9±34.8	64.3±30.4	104.5±9.0	<0.001	<0.001	<0.001
Antihypertensive drugs, n (%)	63 (27.8%)	47 (33.5%)	16 (80%)	0	<0.001	<0.001	<0.001
Serum Calcium (mmol/L)	2.33±0.10	2.34±0.10	2.31±0.10	2.30±0.07	0.18	0.20	0.81
Serum phosphate (mmol/L)	1.38±0.20	1.38±0.20	1.35±0.18	1.38±0.18	0.73	0.95	0.66
Serum Ca-P product (mmol ² /L ²)	3.19±0.52	3.21±0.54	3.13±0.45	3.18±0.42	0.72	0.84	0.74
Serum iPTH (ng/L) ¶	50 (31, 76)	49 (32, 76)	52 (29, 72)	25 (21, 30)	0.10	0.03	0.13
25 (OH) vitamin D3 (microg/L) [†] #	53 (41, 67)	55 (43, 67)	48 (34, 70)	65 (49, 226)	<0.001	<0.001	0.01

*SDS, standard deviation score; [†]BMI, body mass index; [‡]BSA, body surface area; [§]eGFR, glomerular filtration rate; ^{||}iPTH, intact parathyroid hormone; [¶]median (interquartile range); [#]measured in subset of sample.

Those with CKD were comparable with control children for age and ethnicity. There were more boys as compared to girls with CKD, reflecting the usual gender distribution of CKD in children. Those with CKD were significantly shorter ($p=0.002$) but had comparable body mass index (BMI) SDS ($p=0.46$) and body surface area ($p=0.38$) when compared with controls. There were no significant differences in characteristics other than BP between those with BP $\geq 75^{\text{th}}$ percentile versus BP $< 75^{\text{th}}$ percentile CKD sub-groups (**Table 10**). A higher proportion of children with stages 3-5 of CKD were on anti-hypertensive treatment, a surrogate marker for prior history of hypertension [83% (25/30), 72% (13/18) and 80% (4/5)] compared to children in stages 1-2 of CKD [26% (10/39) and 31% (11/36)] respectively. The CKD sub-groups were also comparable for markers of mineral bone disease including serum calcium, phosphorus and Ca-P product and iPTH. Vitamin D3 concentrations were significantly lower in children with CKD when compared with controls.

5.4.2. Peripheral and carotid BP and pulse wave analyses

Children with CKD and BP $< 75^{\text{th}}$ percentile had similar peripheral and carotid BP to normotensive control children, whereas those with BP $\geq 75^{\text{th}}$ percentile had significantly higher peripheral and carotid systolic and diastolic BP but not pulse pressure (**Table 11**). Carotid Aix was also significantly higher in the children with CKD and BP $\geq 75^{\text{th}}$ percentile compared to normotensive controls but similar in children with and without CKD in whom BP was similar.

Table 11: Peripheral and carotid blood pressure including n=188 with CKD and 38 healthy controls

Measures	CKD			Controls	ANOVA	CKD BP ⁺ <75 th versus Control	CKD BP ≥75 th versus Control
	All (A)	BP <75 th (B)	BP ≥75 th (C)	D	B-D		
Peripheral (brachial) blood pressure and heart rate							
pSBP [*]	104±15	100±11	117±19	103±11	<0.001	0.11	<0.001
pDBP [†]	57±13	53±10	71±14	56±10	<0.001	0.11	<0.001
MAP [‡]	73±12	69±9	86±12	72±9	0.001	0.053	<0.001
Pulse pressure	46±15	47±12	45±23	47±12	0.90	0.91	0.75
SBP SDS [§]	-0.21±1.22	-0.61±0.87	1.06±1.33	-0.31±0.74	<0.001	0.057	<0.001
DBP SDS [§]	-0.41±1.18	-0.80±0.86	0.85±1.21	-0.56±0.86	<0.001	0.13	<0.001
Heart rate (bpm)	76±13	76±13	78±14	76±12	0.68	0.85	0.59

Measures	CKD			Controls	ANOVA	CKD BP ⁺ <75 th versus Control	CKD BP ≥75 th versus Control
	All (A)	BP <75 th (B)	BP ≥75 th (C)	D	B-D		
Carotid blood pressure, amplification and augmentation							
cSBP _{CWT} [†]	89±14	85±10	102±17	88±11	<0.001	0.13	<0.001
cSBP _{RT} [#]	89±13	85±8	104±14	87±8	<0.001	0.20	<0.001
cPP ^{**}	32±13	32±11	31±20	32±9	0.91	0.83	0.84
Amplification	16±6	15±4	19±9	15±4	0.01	0.52	0.03
Aix (%) [^]	5±13	4±13	9±13	0.4±11	0.04	0.12	0.01

⁺BP, blood pressure; ^{*}pSBP, peripheral systolic BP; [†]pDBP, peripheral diastolic BP; [‡]MAP, mean arterial pressure; [§]SDS, standard deviation score; ^{||}bpm, beats per minute; [†]cSBP_{CWT}, carotid SBP measured using carotid-wall tracking; [#]cSBP_{RT}, radial tonometric estimate of cSBP; ^{**}cPP, carotid pulse pressure; Amplification, pSBP-cSBP_{CWT} calculated as the difference between peripheral and carotid systolic BP; [^]Aix, augmentation index (%) performed using radial tonometry based pulse wave analysis.

5.4.3. Carotid dimensions and biomechanical properties

Carotid lumen and wall dimensions were similar in all groups. By contrast, circumferential wall stress and functional measures of arterial stiffness differed between children with CKD and BP $\geq 75^{\text{th}}$ percentile compared to normotensive controls but not between children with and without CKD in whom BP was similar. Thus circumferential wall stress and Young's elastic modulus were significantly greater and cross-sectional distensibility and compliance coefficient were significantly lower in children with CKD and BP $\geq 75^{\text{th}}$ percentile than in controls (**Table 12**, each $P < 0.05$). Thus, the anatomy of the carotid artery was maintained and functional elastic properties of the wall were impaired only in children with CKD in whom BP was greater than in those without CKD. There was no significant difference between subgroups by CKD stage for any carotid biomechanical property evaluated.

5.4.4. Carotid-femoral PWV

PWVcf was similar in all 3 groups: 5.34 ± 0.82 , 5.24 ± 0.83 and 5.50 ± 1.11 m/s in healthy normotensive control children, those with CKD and BP $< 75^{\text{th}}$ percentile and those with CKD and BP $\geq 75^{\text{th}}$ percentile respectively.

Table 12: Arterial biomechanical properties including n=188 with CKD and 38 healthy controls

Measures	CKD [†]			Controls	ANOVA	CKD BP ⁺ <75 th versus Control	CKD BP ≥75 th versus Control
	All (A)	BP <75 th (B)	BP ≥75 th (C)	D	B-D		
Carotid-femoral Aortic stiffness (n=226)							
Carotid-femoral PWV (m/s) [*]	5.3±0.9	5.2±0.8	5.5±1.1	5.3±0.8	0.23	0.48	0.48
	(n=188)	(n=144)	(n=44)	(n=38)			
Anatomical measures							
Internal diastolic diameter (m x 10 ⁻³)	5.43±0.53	5.42±0.48	5.50±0.67	5.47±0.55	0.78	0.59	0.90
	(n=90)	(n=70)	(n=20)	(n=30)			
Internal diastolic diameter (m x 10 ⁻³ / 1.73m ²)	2.36±0.57	2.38±0.53	2.28±0.71	2.44±0.66	0.64	0.62	0.41
	(n=90)	(n=70)	(n=20)	(n=30)			
Intima-media thickness (m x 10 ⁻⁶)	437±65	435±61	441±80	430±61	0.83	0.67	0.58
	(n=102)	(n=79)	(n=23)	(n=32)			
Wall cross-sectional area (m ² x 10 ⁻⁶)	8.1±1.6	8.0±1.6	8.2±1.6	8.1±1.8	0.87	0.88	0.75
	(n=90)	(n=70)	(n=20)	(n=28)			

Measures	CKD [†]			Controls	ANOVA	CKD BP ⁺ <75 th versus Control	CKD BP ≥75 th versus Control
	All (A)	BP <75 th (B)	BP ≥75 th (C)	D	B-D		
Thickness / radius ratio (h/r)	0.14±0.02 (n=90)	0.14±0.02 (n=70)	0.14±0.03 (n=20)	0.14±0.02 (n=30)	0.65	0.37	0.46
Functional elasticity measures							
Circumferential wall stress (kPa)	67.7±17.7 (n=89)	63.3±12.8 (n=70)	83.6±23.5 (n=19)	68.7±14.9 (n=30)	<0.001	0.07	0.009
Cross-sectional Distensibility (kPa ⁻¹ x 10 ⁻³)	104±38 (n=88)	107±39 (n=69)	92±31 (n=19)	114±33 (n=30)	0.13	0.39	0.03
CS Compliance coefficient [‡] (m ² kPa ⁻¹ x 10 ⁻⁶)	2.3±0.7 (n=88)	2.4±0.7 (n=69)	2.1±0.7 (n=19)	2.6±0.7 (n=30)	0.06	0.13	0.02
Young's Elastic Modulus (kPa x 10 ³)	0.132±0.059 (n=88)	0.127±0.055 (n=69)	0.151±0.068 (n=19)	0.109±0.04 9 (n=29)	0.04	0.14	0.02

⁺BP, blood pressure; [†]CKD, chronic kidney disease; [‡]CS, cross-section; *PWV, pulse wave velocity

5.4.5. Relation of indices of carotid stiffness to blood pressure, eGFR and other characteristics

In all children with CKD, indices of elasticity except circumferential wall stress were independently related to age. Compliance was additionally related to MAP and circumferential wall stress to BMI. However, none of the indices of elasticity were independently related to GFR (**Table 13**).

Table 13: Multiple linear regression analysis of correlates of functional elasticity with brachial mean arterial pressure in children with non-dialysis CKD (n=88)

Variable	Coefficient	95% CI	P value	Model adjusted R ²
Circumferential wall stress (kPa)				0.088
BMI (kg/m ²)	0.817	0.153, 1.482	0.017	
Cross-sectional Distensibility (kPa ⁻¹ x 10 ⁻³)				0.124
Age (years)	-4.722	-7.661, -1.784	0.002	
CS Compliance coefficient (m ² kPa ⁻¹ x 10 ⁻⁶)				0.112
MAP (mmHg)	0.016	0.000, 0.031	0.046	
Age (years)	-0.094	-0.154, -0.033	0.003	
Young's Elastic Modulus (kPa x 10 ³)				0.088
Age (years)	0.006	0.002, 0.011	0.009	

Confounders in all models included MAP (not included in circumferential wall stress), GFR in ml/min/1.73m², age, gender, BMI, heart rate, anti-hypertensive treatment (yes/no) and markers of mineral bone disease.

There were no significant differences in arterial measures between boys and girls (Table 14).

Table 14: Comparison of arterial measures by gender in healthy children (n=38) and those with chronic kidney disease (n=188)

Measures	Controls			CKD [†]		
	Male	Female	P value	Male	Female	P value
Age (years)	12.4± 3.2	11.1± 3.4	0.248	11.8± 3.8	12.2± 3.4	0.463
eGFR (ml/min/1.73m ²) [*]	101.6± 4.9	106.0± 10.2	0.154	61.5± 31.2	68.2± 37.4	0.187
MAP (mmHg) [†]	74± 10	70± 8	0.257	72± 12	73± 12	0.639
cSBP _{CWT} (mmHg) [‡]	92± 11	86± 10	0.154	88± 14	89± 13	0.800
Circumferential wall stress (kPa)	73.4±12.7	66.6±15.6	0.257	66.0±17.5	69.7±17.8	0.319
Cross-sectional Distensibility (kPa ⁻¹ x 10 ⁻³)	110±35	116±34	0.692	105±41	103±35	0.855
CS Compliance coefficient [§] (m ² kPa ⁻¹ x 10 ⁻⁶)	2.8±0.8	2.6±0.7	0.579	2.4±0.8	2.3±0.6	0.438
Young's Elastic Modulus (kPa x 10 ³)	0.120±0.030	0.104±0.056	0.438	0.133±0.067	0.130±0.048	0.807

[†]CKD, chronic kidney disease; ^{*}eGFR, glomerular filtration rate; [†]MAP, mean arterial pressure; [‡]cSBP_{CWT}, carotid SBP measured using carotid-wall tracking. [§]CS, cross-section.

The relative impact (sequential R^2 coefficient) of age, gender, CKD or control group and MAP on arterial measures is shown in **Table 15**.

Table 15: Multivariate analyses with sequential R^2 changes for arterial measures showing the relative influence of age and mean arterial pressure following adjustment for gender and CKD (n=88) or control group (n=38)

Arterial parameter	Variable	β	P	R^2	Adjusted R^2
Circumferential wall stress (kPa)	Age	1.717	<0.001	0.112	0.105
	Age	1.736	<0.001	0.116	0.101
	Sex	-2.193	0.459		
	Age	1.768	<0.001	0.119	0.096
	Sex	-1.791	0.555		
	CKD*/ control group	2.19	0.533		
Cross-sectional Distensibility (kPa- 1 x 10 ⁻³)	Age	-4.569	<0.001	0.167	0.16
	Age	-4.565	<0.001	0.167	0.153
	Sex	-0.467	0.941		
	Age	-4.474	<0.001	0.173	0.151
	Sex	0.691	0.914		
	CKD/ control group	6.372	0.391		
	Age	-4.86	<0.001	0.178	0.149
	Sex	0.889	0.89		
	CKD/ control group	6.345	0.394		
	MAP [†]	0.248	0.416		

Arterial parameter	Variable	β	P	R ²	Adjusted R ²
CS Compliance coefficient [^] (m ² kPa ⁻¹ x 10 ⁻⁶)	Age	-0.046	0.025	0.042	0.034
	Age	-0.046	0.024	0.046	0.029
	Sex	0.083	0.532		
	Age	-0.042	0.039	0.075	0.051
	Sex	0.137	0.31		
	CKD/ control group	0.296	0.059		
	Age	-0.069	0.002	0.135	0.105
	Sex	0.151	0.25		
	CKD/ control group	0.294	0.054		
	MAP	0.017	0.006		
Young's Elastic Modulus (kPa x 10 ³)	Age	0.007	<0.001	0.168	0.161
	Age	0.007	<0.001	0.172	0.157
	Sex	0.007	0.47		
	Age	0.007	<0.001	0.184	0.163
	Sex	0.005	0.65		
	CKD/ control group	-0.015	0.194		
	Age	0.008	<0.001	0.197	0.168
	Sex	0.004	0.689		
	CKD/ control group	-0.015	0.198		
	MAP	-0.001	0.188		

*CKD, chronic kidney disease; [†]MAP, mean arterial pressure; [^]CS, cross-sectional

All measures of elasticity were related to cPP (**Table 16**).

Table 16: Results of multivariable regression analyses of relevant functional elastic carotid artery parameters with brachial mean arterial pressure, carotid systolic BP and carotid pulse pressure in n=88 children with CKD. A separate model was constructed for each blood pressure component and adjusted with the same confounders throughout (as shown in Table 13). Coefficients for the different components of peripheral and carotid pressure are shown from each of the models (coefficients for confounders not shown)

Variable	Coefficient	SE	P	Model adjusted R ²
Circumferential wall stress (kPa)				
Mean arterial pressure (mmHg)	-	-	-	-
Carotid systolic BP (mmHg)	0.821	0.131	<0.001	0.379
Carotid pulse pressure (mmHg)	-0.359	0.248	0.152	0.097
Cross-sectional Distensibility (kPa⁻¹ x 10⁻³)				
Mean arterial pressure (mmHg)	0.518	0.441	0.245	0.096
Carotid systolic BP (mmHg)	-0.198	0.443	0.657	0.078
Carotid pulse pressure (mmHg)	-	-	-	-
CS Compliance coefficient (m² kPa⁻¹ x 10⁻⁶)				
Mean arterial pressure (mmHg)	0.016	0.008	0.046	0.112
Carotid systolic BP (mmHg)	0.001	0.009	0.885	0.010
Carotid pulse pressure (mmHg)	-	-	-	-
Young's Elastic Modulus (kPa x 10³)				
Mean arterial pressure (mmHg)	-0.001	0.001	0.084	0.078
Carotid systolic BP (mmHg)	0.0001	0.001	0.811	0.030
Carotid pulse pressure (mmHg)	0.005	0.001	<0.001	0.470

On multiple regression analysis to examine the relationship of PWVcf and eGFR following adjustment for confounders [including MAP, age, gender, BMI, heart rate and anti-hypertensive treatment (yes/no)], age alone maintained a significant positive relationship with PWVcf ($\beta=0.423$, $p=0.003$) but there was no significant relationship between eGFR and PWVcf ($\beta=0.113$, $p=0.33$); model $r^2=0.385$.

5.4.6. Case control analysis

In the case-control analysis there was a significant difference in GFR between the CKD and control group (54.3 ± 17.7 vs. 103.5 ± 8.5 ml/min/1.73m², $p<0.001$). Despite this marked difference in GFR, measures of arterial stiffness were similar in age and gender matched control children with similar blood pressure (**Table 17**).

Table 17: Case control analysis of age and gender matched CKD children (n=58) with healthy controls (n=29), with similar peripheral and carotid BP levels

Measures	Controls	CKD	P value
Number (%)	29	58	
Age (years)	11.8± 3.4	11.7± 3.4	0.944
Male sex, n (%)	13 (44.8%)	33 (56.9%)	0.287
eGFR (ml/min/1.73m ²)*	103.5± 8.5	54.3± 17.7	<0.001
Height SDS [†]	0.46±1.15	-0.08±1.29	0.084
BMI [‡] SDS [†]	0.56±1.44	0.71±0.99	0.622
pSBP [§]	103± 11	102± 11	0.957
pDBP	56± 10	55± 11	0.838
MAP [¶]	71± 8	71± 10	0.863
Pulse pressure	47± 13	47± 9	0.891
SBP SDS	-0.39±0.75	-0.36±0.91	0.88
Antihypertensive drugs, n (%)	0	18 (31%)	<0.001
Heart rate (bpm) [#]	76± 13	74± 13	0.61
cSBP _{CWT} ^{***}	88± 11	87± 11	0.803
Carotid-femoral PWV (m/s) [^]	5.4±0.9	5.5±0.9	0.695
Internal diastolic diameter (m x 10 ⁻³)	5.52±0.57	5.39±0.45	0.32
Internal diastolic diameter (m x 10 ⁻³ / 1.73m ²)	2.47±0.70	2.45±0.60	0.912

Measures	Controls	CKD	P value
Intima-media thickness ($\text{m} \times 10^{-6}$)	434±62	443±53	0.542
Wall cross-sectional area ($\text{m}^2 \times 10^{-6}$)	8.3±1.9	8.1±1.3	0.749
Thickness / radius ratio (h/r)	0.135±0.014	0.142±0.016	0.112
Circumferential wall stress (kPa)	68.1±13.4	63.9±11.1	0.193
Cross-sectional Distensibility ($\text{kPa}^{-1} \times 10^{-3}$)	113±35	105±36	0.405
CS Compliance coefficient ($\text{m}^2 \text{kPa}^{-1} \times 10^{-6}$)	2.7±0.8	2.4±0.6	0.092
Young's Elastic Modulus ($\text{kPa} \times 10^{-3}$)	0.109±0.053	0.124±0.041	0.233

*eGFR, glomerular filtration rate; [†]SDS, standard deviation score; [‡]BMI, body mass index; [§]pSBP, peripheral systolic BP; ^{||}pDBP, peripheral diastolic BP; [¶]MAP, mean

5.5. Discussion

To our knowledge, this is the first study to provide a comprehensive characterization of arterial biomechanics in children with CKD. Our main findings are novel and twofold, (i) when children with nondialysis CKD are compared with healthy children with normal renal function, at similar levels of peripheral and carotid blood pressure, anatomical and functional elastic properties of the large arteries such as lumen diameter, wall thickness, PWVcf and elastic modulus remain comparable; but (ii) when children with CKD and sub-optimal BP control ($\geq 75^{\text{th}}$ percentile) are compared with normotensive controls there are significant differences in functional elastic properties of the carotid artery. Furthermore, we found no independent relationship between any biomechanical property and GFR when age, gender and blood pressure (both peripheral and carotid)

were adjusted for, nor a difference between biomechanical properties in children with or without CKD using an age and gender case control analysis in children with similar BP. This suggests that blood pressure rather than renal disease *per se* is the main determinant of functional arterial elasticity in children with predialysis CKD.

These findings contrast with previous studies that have reported structural and functional changes including increased arterial wall thickness, increased arterial wall cross sectional area and stiffness¹⁰⁷⁻¹¹¹ in children with CKD compared to healthy controls. Children with predialysis stages of CKD in these studies had abnormal mechanical arterial measures that were associated with increased BP, dyslipidaemia and markers of mineral bone metabolism^{107,108,111}, with stronger associations in subjects on dialysis¹⁰⁷⁻¹¹¹. Metabolic derangements associated with mineral bone disease in CKD are well known to contribute to arterial calcification and could explain the changes seen in children with advanced CKD.²⁰⁷ Mitsnefes *et al* have reported that, in a group of children with CKD, including children on dialysis, carotid IMT and arterial stiffness are associated with disturbances of Ca-P metabolism and hyperparathyroidism.¹⁰⁸ In this study children with predialysis CKD had increased carotid IMT and measures of arterial stiffness compared to control children but this may have been explained by BP which was higher in children with pre-dialysis CKD compared to controls. Thus their results are consistent with those of the present study.

However, Briet *et al* compared biomechanical properties of the carotid artery to (similar to those measured in the present study) in adults with CKD with those in patients with hypertension (but without CKD) and healthy controls.²⁰⁶ Their main finding was that, compared to controls with similar levels of blood pressure, patients with CKD had an

outward remodelling of the carotid artery with enlargement of the lumen diameter predominating over carotid wall thickening and stiffening. This may be a response to increased wall stress, which together with carotid diameter was independently related to age, blood pressure *and* GFR. The present study, by contrast, reveals no evidence of any form of remodeling in children with pre-dialysis CKD who, compared to adults, usually have a shorter duration of both hypertension and CKD. We did observe higher wall stress in the CKD group with higher blood pressure compared to normotensive controls (explicable by the difference in blood pressure alone) but, even in this group, there was no evidence of remodelling. This may reflect a relatively short duration of hypertension in children with CKD. Were hypertension sustained, this persistent increase in circumferential wall stress might result in arterial remodelling as described by Briet *et al.*²⁰⁶

Other studies have demonstrated childhood blood pressure to predict increased arterial stiffness in adulthood.²⁰⁸⁻²¹⁰ In the Young Finn's study reduced carotid arterial compliance and increased Young's elastic modulus was predicted by increased systolic BP and skinfold thickness/ BMI during childhood.²¹⁰ Findings with respect to PWVcf have been more variable with an association of adult PWVcf with childhood BP reported in the Bogalusa²⁰⁸ but not in the ARYA study.²⁰⁹ It is possible that Young's elastic modulus is a more sensitive index of susceptibility to the influence of BP than is PWV (which is proportional to the square root of E) and this would be consistent with findings in the present study where we observed increased Young's elastic modulus in the carotid but not increased PWVcf in children with CKD and higher blood pressure compared to controls. It is also possible that, as a more muscular artery, the carotid

artery is more susceptible to BP induced remodeling than the aorta (the major contributor to PWVcf). We observed higher Alx and reduced elasticity but similar PWV in those with CKD and sub-optimal BP when compared with healthy controls and CKD with optimal BP levels. This increased stiffness though was not accompanied by an increase in pulse pressure. This could be due to differential remodelling of arteries of different structure (muscular carotid versus elastic aorta) and might also reflect the result of treatment with anti-hypertensive medication, with more patients on treatment for hypertension with worsening stages of CKD.

In healthy children, both BP and BMI track throughout childhood and subsequently as young adult.^{211,212} In children with CKD, BP control deteriorates with worsening renal function^{213,214} and both BP and BMI increase following transplantation from pre-transplantation levels.^{46,215-217} We would suggest, therefore, that persistently high BP and BMI in children with pre-dialysis CKD is likely to lead to increased functional stiffness of the carotid artery. Blood pressure reduction may be an effective means to protect against arterial stiffening and/or remodeling in children with CKD and hypertension that persists into adulthood. In the present study we found no association of arterial properties with metabolic derangements. However, phosphate was well controlled in the children in the present study. In children with more advanced CKD, particularly those on dialysis it is likely that metabolic derangements contribute to arterial stiffening and in this case blood pressure reduction may be of more limited value.

We did not perform any formal sample size estimates to investigate an association of eGFR with measures of arterial remodelling evaluated here. This was because there

was limited data regarding this in children with pre-dialysis CKD at the time of study commencement. Data from adult studies is arguably not applicable to a childhood cohort, since adults with CKD often have more severe and more long-standing hypertension and additional co-morbidities including diabetes, smoking and dyslipidaemia.²⁰⁶ Available data from such adult cohorts highlights the limited influence of GFR on arterial properties including Young's Elastic modulus (2%) and carotid arterial diameter (3%). An important limitation of our study was that we had relatively few patients in stages 4 and 5 (mean $\pm$ SD eGFR was 64 ± 33.8 ml/min/1.73m²) and therefore our results do not preclude an association of arterial remodeling with GFR in children with more severe CKD.

The main limitation of our study is its cross-sectional nature that limits conclusions regarding causality. Limitations of the analyses include defining hypertension as a categorical variable (yes/no), with lack of data regarding duration of hypertension and of severity of hypertension (indicated by need for 2 or more anti-hypertensive agents). Nearly a third of children with CKD were receiving anti-hypertensive therapy (usually as monotherapy in the form of angiotensin converting enzyme inhibitors, ACEi). It is possible that ACEi may impact on arterial properties, although sub-group analyses did not suggest a specific effect of treatment. We acknowledge though that it remains difficult to discount the inherent confounding in this cohort particularly from treatments causally associated with CKD. However our results are applicable to the children with CKD representative of those in the general population with CKD (in whom a similarly high proportion are on anti-hypertensive treatment).

5.5.1. Perspectives

This study provides data that changes in functional elastic properties of the carotid artery are some of the earliest identifiable biomechanical properties in children with non-dialysis CKD. We observed no changes in carotid lumen and wall dimensions, findings that are in contrast to previous studies. These changes appear to be related primarily to BP and not GFR or markers of mineral bone disease. Due to the smaller number of subjects with more advanced stages of CKD these findings need further testing in a larger cohort with advanced CKD. Blood pressure reduction may be an effective means to protect against arterial stiffening and needs to be evaluated using a controlled clinical trial.

**Chapter 6 : The Hypertension
Optimal Treatment in Children with
Chronic Kidney Disease study:
The HOT-KID study- protocol of a
randomised trial to compare
effects of aggressive versus
standard targets in blood pressure
on target organ damage in
children with CKD**

6.1. Background

Despite a strong association between left ventricular mass, IMT and arterial stiffness with blood pressure in adults with CKD^{120,218,219}, the role of blood pressure in determining target organ damage in children with CKD remains controversial. Although there are increasing data regarding IMT and arterial stiffness, in children with CKD the association of IMT with blood pressure remains unclear.^{105,107-117} In children with dialysis dependent CKD, both LVH and arterial stiffness are thought to be determined less by blood pressure and more by other mechanisms such as hyper-parathyroidism leading to myocardial hypertrophy and arterial stiffening through calcification.^{54,107-111,123}

In children with predialysis CKD, the relationship of blood pressure with LVH continues to remain unclear following initial reports from the two largest multi-centre study reports, with one from Europe⁵⁴ and the other from the United States of America⁴³ showing conflicting relationships. The larger observational dataset from the multi-centre North American 'CKiD study' reported a significant association of elevated BP with LVH.⁴³ In contrast, the multi-centre European trial, the 'ESCAPE' study was designed to evaluate the reno-protective efficacy of intensified blood pressure control in paediatric patients with predialysis CKD.¹⁵⁸ The authors reported a significant reduction in renal failure progression in patients who had intensified blood pressure control (<50th percentile) as opposed to conventional BP control (50th-95th percentile). The ESCAPE study was not designed to evaluate the effects of aggressive versus standard BP control on LV mass or on arterial structure and function¹⁵⁸, but the investigators published an analyses on the study cohort at baseline and reported no association between LVH and BP but a significant association with male gender, anaemia, increasing weight,

hyperparathyroidism and reduced GFR.⁵⁴ Further recent reports from these two cohorts now suggest a more significant role of BP.^{41,44} The CKiD longitudinal studies suggest systolic BP, female gender, anaemia and use of anti-hypertensive medications other than RAAS inhibitors of to be predictive of LVH in those with nondialysis CKD.⁴⁴ The ESCAPE investigators on longitudinal study in a limited subset of n=84 subjects, were not observed to have the same impact of improved BP control on LVH but a predominant impact on LV function independent of BP control.⁴¹

In our paediatric predialysis CKD population at the Evelina London Children's Hospital, we have observed a significant association between LVH and blood pressure (**Figure 35**).⁴⁰ Despite having clinic BP measurements consistently within the currently accepted normal range (below 90th percentile) there was a significant difference in BP between those with and without LVH (**Figure 36**). Thus, in our own study, we have found a strong correlation between systolic BP *within the normal range* and indexed LV mass.⁴⁰

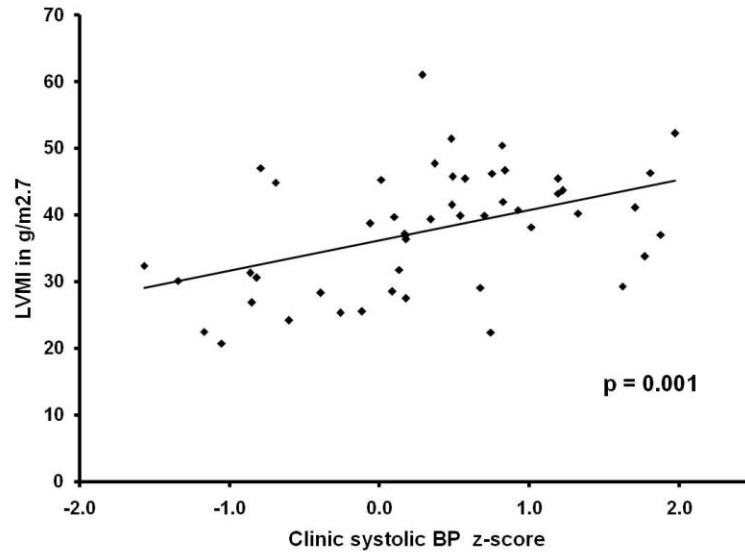


Figure 35: Relationship of clinic systolic BP z-score with indexed LV mass in all patients

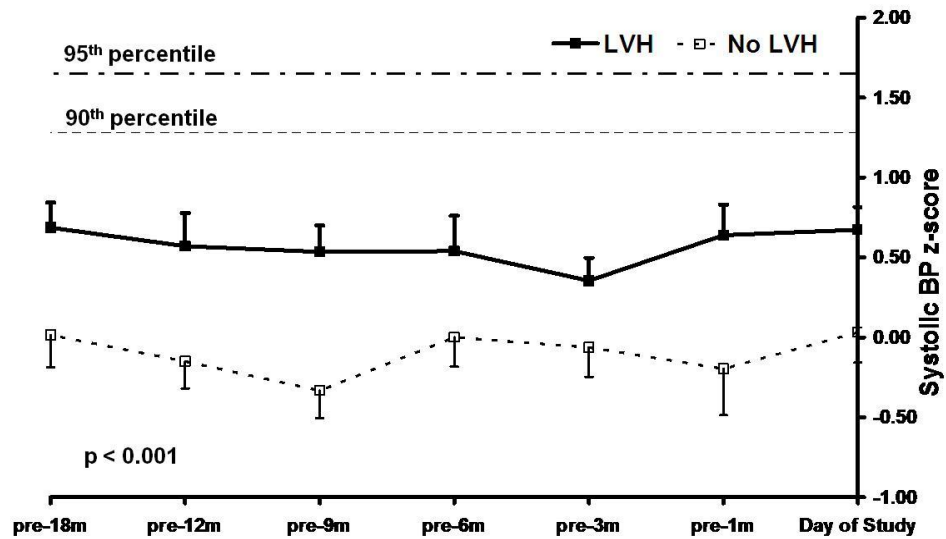


Figure 36: Clinic systolic BP z-scores in children with chronic kidney disease stage 3-5 patients with and without left ventricular hypertrophy (LVH). Interrupted line at the 90th and 95th percentile denotes the clinical definition of pre-hypertension and hypertension. Data shown as mean $\pm$ SEM

Our findings are at variance with the ESCAPE study both with regard to the finding of a positive association of LVH with BP and lack of an association with male gender, anaemia, inflammation, ponderosity or hyperparathyroidism.⁵⁴ The strong correlation of blood pressure with LVH in our own population may in part be because all blood pressure measurements were performed by the same observer using auscultation with mercury sphygmomanometer or calibrated aneroid instruments. When using 24-hour ambulatory BP monitoring (ABPM) we (and the CKiD investigators)⁴³ included all measurements as opposed to only a proportion of measurements (as in the ESCAPE study). We have previously shown that using a subset of the ABPM is likely to introduce significant clinical errors in the interpretation of ABPM.²²⁰ These differences may also be because of the difficulties in the measurement of blood pressure and the definition of “hypertension” during childhood as a categorical state with a value of systolic blood pressure above the 95th percentile for the population.^{221,222}

Overall, though our data suggest that even in those with blood pressure below the 90th percentile, blood pressure may still be responsible for at least a component of LVH. The association of LVH with systolic BP in the absence of overt hypertension, suggests that current targets for BP control should be re-evaluated in this population.⁴⁰ Extrapolation from data in adults with and without CKD suggests that the same may be true for IMT and arterial stiffness.

Commonly reported measures of end organ damage include measures of arterial stiffness and left ventricular hypertrophy, which in adults with CKD relate strongly to BP. Improved management of hypertension is a key aspect of management of adult CKD patients with recommendations for more stringent blood pressure targets in adults with

proteinuric CKD.¹⁵⁶ There are no current guidelines regarding optimal level of blood pressure control for cardiovascular outcomes in children with CKD.¹⁵⁷ Until recently, recommendations for children with CKD were to maintain BP below the 90th percentile for the child's age, gender and height.¹⁵⁹ Based on the findings of the ESCAPE study¹⁵⁸, the European Society of Hypertension now recommend maintaining BP below the 75th percentile primarily to retard progression of renal failure.¹⁵⁷ Our findings of a high prevalence of LVH in children with CKD and BP below the 90th percentile, together with the experience in adults, raises the important question of whether even this target for children with CKD is too high.

An additional consideration in children with CKD is the potential importance of central systolic blood pressure. Because of the limitations of cross-sectional association studies, optimal hypertension control can only be answered by a randomised interventional trial. We propose testing the benefit of maintaining blood pressure below the 40th percentile on cardiovascular target organ damage by a controlled trial: 'Hypertension Optimal Treatment in Children with Chronic Kidney Disease study: The HOT-KID study- a randomised trial to compare effects of aggressive versus standard targets in blood pressure on target organ damage in children with CKD'.

The HOT-KID study is designed to provide important definitive evidence of whether a lower than currently recommended level of clinic blood pressure ameliorates adverse cardiovascular target organ outcomes in children with CKD.

6.2. Methods/design

Study overview shown as a flow chart in **Figure 37**.

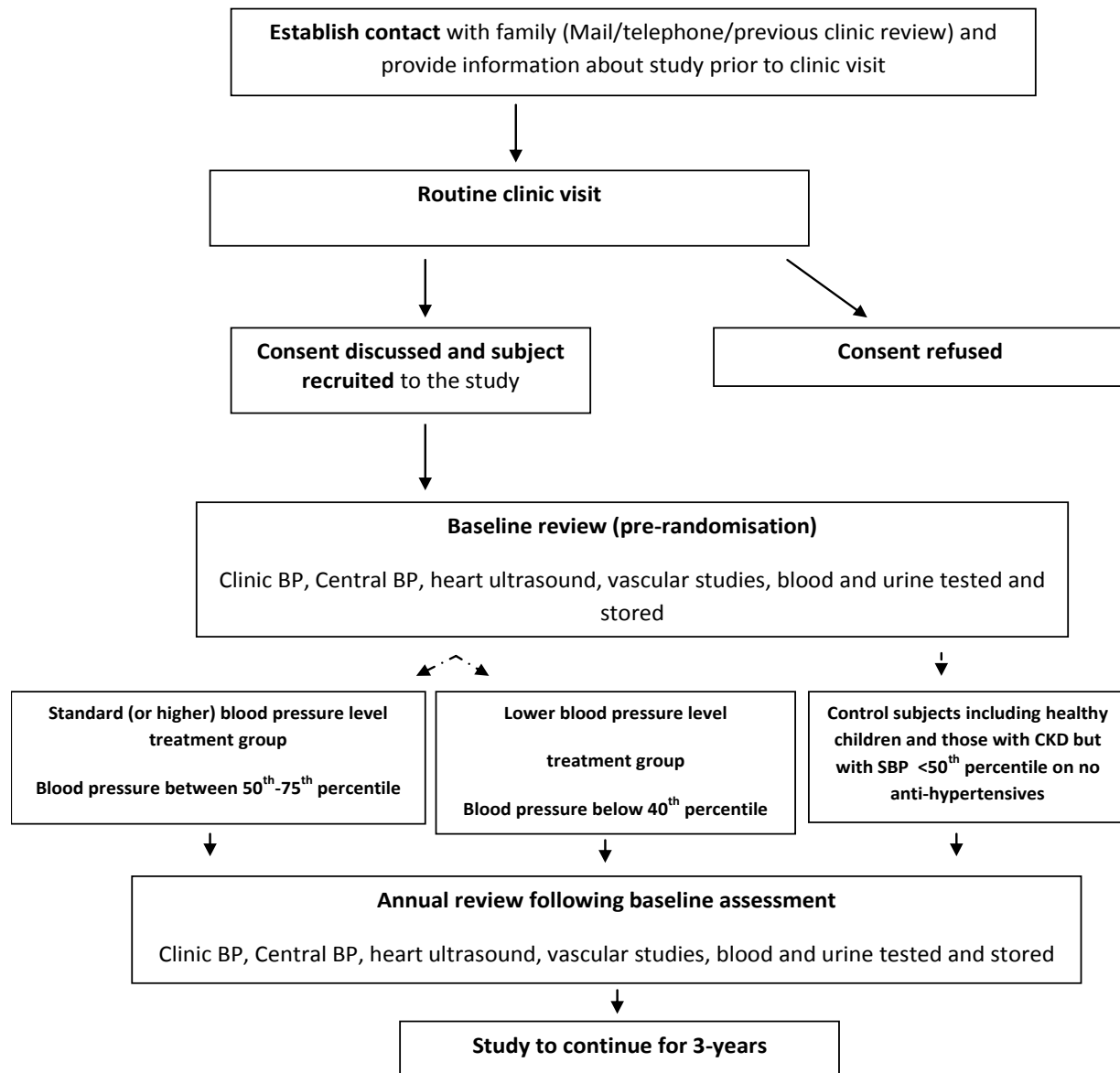


Figure 37: Trial overview shown as a flow chart.

6.2.1. Objective

Our primary objectives in this trial are to

1. a) Examine the relationship of LVM, IMT and arterial stiffness to blood pressure (following adjustment for confounders) in a cohort of children with and without CKD.
b) Compare these measures in children with and without CKD when adjusted for peripheral and central blood pressure.
2. Perform a randomised controlled trial to determine whether aggressive blood pressure reduction (below the 40th percentile) compared to standard care (below the 75th percentile) is effective in normalising left ventricular mass and arterial structure/function.

The secondary objectives are to examine the effects of aggressive versus standard blood pressure reduction on microalbuminuria and progression of renal failure and to determine if biomarkers of arterial injury/ventricular load are independently related to cardiovascular target organ damage.

6.2.2. Study design

The HOT-KID study is designed as a phase III randomised parallel group controlled non-blinded trial. Subjects randomised to the trial will be assigned to one of two arms: intervention arm where clinic SBP will be maintained <40th percentile and standard arm with SBP maintained between 50th-75th percentile for age and height. The trial subjects will additionally be compared with age-matched control subjects.

6.2.3. Recruitment

Children with CKD under the care of a paediatric nephrologist will be recruited from 14 centres including 11 of 13 tertiary paediatric nephrology centres in the UK thus representing the national childhood CKD population. Potential subjects meeting eligibility criteria will be identified from clinic lists and departmental databases following which their suitability for participation in the trial will be discussed with their lead clinician. Estimated GFR (eGFR) will be calculated using the Schwartz formula and CKD staged as per existing definitions.^{13,14,16} Study information sheets outlining the study will be mailed to the parents or guardians of potentially eligible children (and the child where age appropriate), 2 to 4 weeks prior to their next clinical appointment. At the time of this appointment, a member of the research team will discuss the study in further detail and confirm eligibility with regard to inclusion and exclusion criteria (as below). Informed consent will then be sought from the parents (or guardians) and subjects (informed consent or assent according to age) either at the time of this review or at the time of their next clinical review once the child and family have had an opportunity to consider and discuss the study information.

Subjects recruited in external centres will await a 'research study visit' for cardiac and vascular baseline investigations and subsequent randomisation. Thus, any intervention as part of the research study will commence only following baseline investigations. All cardiac and vascular investigations described in this study will be performed by core group of study investigators at the ELCH or at participating external centres. Children will be recruited over the first 12-months of the study and have at least 2-years of follow up.

6.2.4. Selection and withdrawal of subjects

6.2.4.1. Inclusion criteria

- Age 2 to 15 years
- Chronic kidney disease, stages 1-4 of CKD
- Children with or without antihypertensive/s medications (including any recent change/s in antihypertensive therapy).
- Children on anti-hypertensive medications must be able to tolerate either an Angiotensin Converting Enzyme inhibitor (ACEi) or Angiotensin Receptor Blocker (ARB) medication.
- Children with average clinic systolic BP <50th percentile and on no antihypertensive medication will be eligible as 'CONTROL-CKD' and
- Children with no renal disease, normal renal function and blood pressure will be eligible as 'CONTROL-HEALTHY'

6.2.4.2. Exclusion criteria

- Age <2 and >16 years
- Children who have/have had an arterio-venous fistulae
- Children who are on/have had chronic maintenance dialysis
- Children who have/have had a functioning kidney transplant
- Children with symptomatic BP or with past history of difficulty to control BP or

- Children in whom there is a clinical urgency to treat BP and inclusion in study may result in possible delay of treatment
- Children with arrhythmia or clinical heart failure
- Children with a known structural cardiac abnormality
- Children on treatment with angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) agents for treatment of proteinuria only or
- Children who are likely to be of clinical concern following up or down titration of BP levels as described later in section 6.2.6
- Children who are unable or intolerant to perform study related measurements e.g. height, echo or PWV
- Children who have/have had intolerance to ACEi and/or ARB drug/s or have any existing contraindications

6.2.4.3. Withdrawal of subjects

- Children will stop participating in the study if their eGFR $<15 \text{ ml/min/1.73m}^2$ or if they commence renal replacement therapy (dialysis or transplantation).
- Children will be withdrawn if they are unable to tolerate the performance of study related measurements e.g. height, echo or PWV or if they develop concerning adverse effects as a result of ACEi or ARB class of anti-hypertensive medications.
- If a child wishes to withdraw from the study this will be allowed. Identifiable data already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or

in relation to the participant. In principle we would aim to keep blood and urine specimen and data gathered up to the point that the consent is lost. However as patients are continually followed up by the clinical team we will be able to determine if the patient wants us to withdraw their data from the study. We will always follow the patient and their families' wishes.

6.2.5. Randomisation and allocation result dissemination

Study related investigations at 'baseline' will be performed before subjects are randomized. Subjects will be randomised to the two arms of the study: aggressive BP control (below the 40th percentile) or standard BP control (between 50th-75th percentile). Randomisation will be performed using an online platform at the the Institute of Psychiatry Clinical Trials Unit, Kings College London (IoP CTU KCL). The randomization will be stratified per centre to ensure matching of number of subjects to two trial arms. The results of randomization are not blinded and will be informed to their clinical teams.

6.2.6. Blood pressure targets and drug therapy

Following randomisation all subjects in whom systolic blood pressure is not to target will be reviewed at 2-4 weekly intervals. Up or down titration of blood pressure during the trial will be performed as outlined in **Figure 38**.

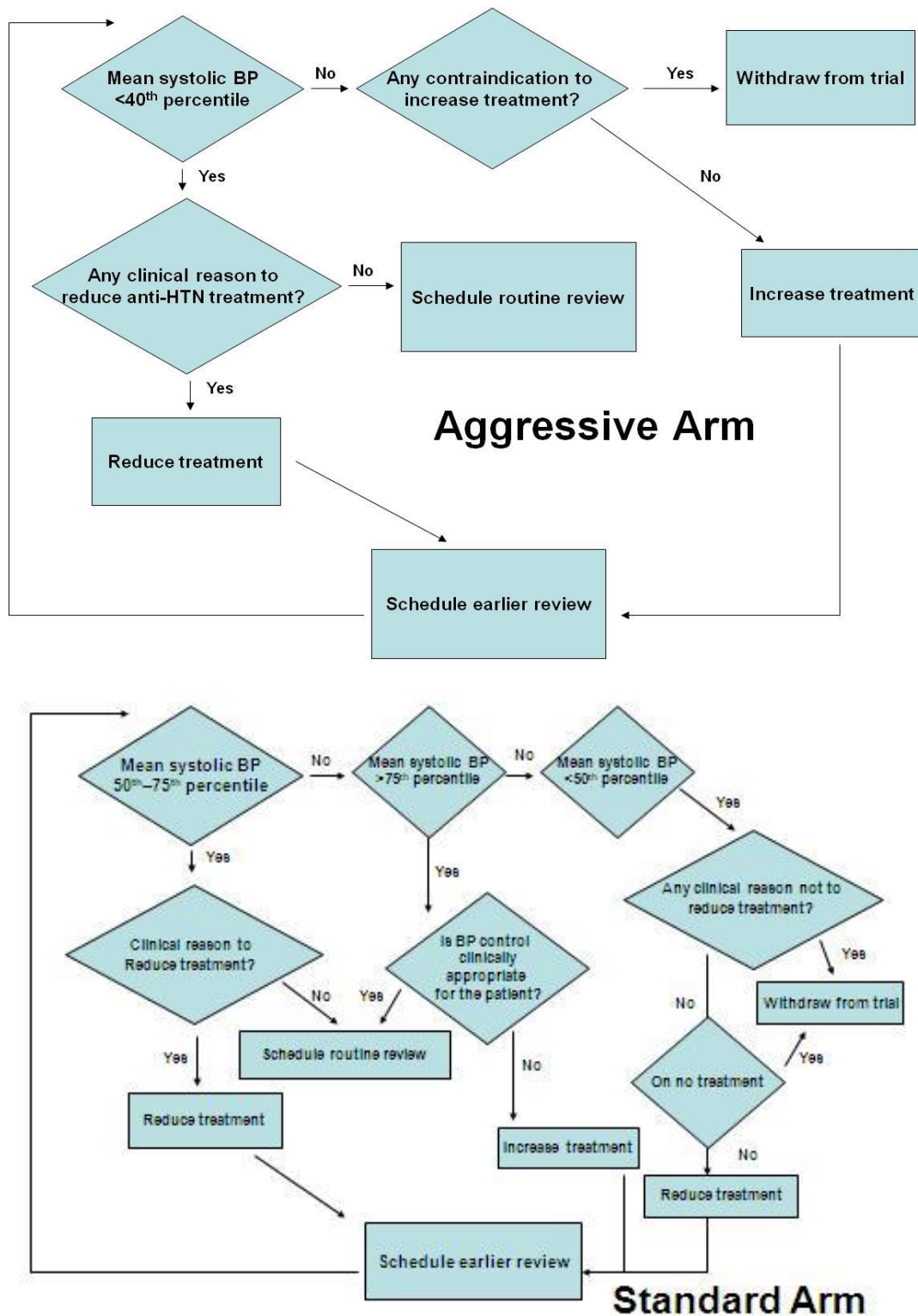


Figure 38: Schematic representation of up and down titration of blood pressure during the trial following randomisation – standard or intervention (aggressive) trial arm.

Once BP is to target, randomized subjects will be monitored at least 4-monthly at the time of routine hospital visits and have clinic BP maintained in the assigned target range over the duration of the study. The target BP will be titrated to the subject's age at annual intervals.

Patients already on anti-hypertensive medications at the time of study entry will continue on these or change as per clinical indication. ACEi or ARB's will be the recommended first line agents. The dose of the agent will be adjusted to achieve the target blood-pressure levels with initial follow up at 2-4 weekly intervals following commencement of medication. The following order of escalation will be used (1) calcium channel blocker (CCB) (2) beta-receptor blocker (3) others such as diuretic or an alpha channel blockers. The preferential use of long acting drugs with once daily dosing is recommended. This order of escalation will be used unless there are clear clinical indications for other agents. Amendments to the 'BP titration' procedures (**Figure 38**) will be made if necessary following review of data after 20 subjects.

6.2.7. Study assessments

Following initial entry into the study a detailed history will be taken and case records reviewed to determine the cause and duration of CKD, past and family history relevant to CKD. Ethnicity (self defined by parents) will be recorded.

6.2.7.1. Assessments at annual intervals

The following will be performed at baseline and subsequently at annual intervals for the duration of the trial in all subjects including those randomized and controls.

6.2.7.1.1. Clinical characteristics, examination and anthropometry

The clinical history will be updated together with details of current medications including antihypertensive treatment, phosphate binders and hydroxylated vitamin D. A clinical examination will be performed. Height (stadiometre), weight and body mass index (BMI) will be recorded.

6.2.7.1.2. Urinanalysis

Urine samples will be collected at baseline and at annual intervals. First morning voids on the three days prior to clinic visit will be required. The patient will keep urine specimens in a home refrigerator at 4°C before clinic visits. Specimens will be stored at -80°C. A mean value of three Ualbumin/Ucreatinine will be recorded.

6.2.7.1.3. Biochemistry

Blood biochemistry will be performed at baseline and at annual visits. In addition to serum urea, electrolytes, creatinine and cystatin C, the following biomarkers related to arterial injury, calcification and ventricular load will be determined:

- a) Arterial injury: lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and Apolipoprotein-B), homocysteine, high sensitivity CRP.
- b) Arterial calcification: plasma calcium, plasma phosphate and calcium-phosphate product ($\text{Ca} \times \text{PO}_4$), plasma intact parathyroid hormone (iPTH), fibroblast growth factor 23 (FGF23), 25(OH) vit D, 1,25 (OH) vit D, blood haemoglobin (Hb).
- c) Ventricular load and injury: N-terminal pro-B type natriuretic peptide (NT-proBNP), high-sensitive cardiac Troponin T (hs-cTnT).

6.2.7.1.4. Peripheral and central blood pressure

6.2.7.1.4.1. Clinic blood pressure

Peripheral BP will be taken as the mean of 3 measurements using appropriate sized cuff and a calibrated aneroid instrument according to British Hypertension Society guidelines. All observers will undergo appropriate training including the evaluation of their variation from independent experienced investigators. Normative criteria will be those defined in the Fourth report of the National High Blood Pressure Education Program Working Group in the United States ('Fourth report')¹⁵⁹ and BP will be expressed as z-scores.

6.2.7.1.4.2. Ambulatory BP monitoring (ABPM) over 24-hours

The performance of 24-hour ABPM is not mandatory but it is likely that for clinical reasons some children with CKD will have ABPM studies performed during the trial. Thus, in a subset of subjects both clinic and ABPM monitoring data will be available for analyses. ABPM studies will be performed in patients > 5 years and > 120 cm using Spacelabs 90217 oscillometric ABP devices (Spacelabs Inc, Redmond, Wash, USA). Measurements will be performed once every 30-minute throughout the period of monitoring. Daytime and nighttime periods during each ABP recording will be defined using information in the patient 'ABPM diary card'.²²⁰ An ABPM study will be judged to be of poor quality and excluded if (1) if there are more than 3-hours of interrupted recordings at any time during the 24-hour period; (2) if the duration of ABPM recording is inadequate and less than 20-hours in total, less than 12-hours continuously or does not include any nighttime measurements.⁴⁰ All mean ABPM parameters will be analysed as z-scores using the normative limits as per *Wuhl et al.*²²³ Nocturnal systolic and diastolic dipping status will be defined as a reduction in nighttime systolic (or diastolic BP) of greater than 10% of the daytime systolic (or diastolic) BP. All ABPM studies will be analysed centrally at the ELCH.

6.2.7.1.4.3. Central blood pressure

Central blood pressure will be estimated both from radial tonometry and modified BP measurement using a brachial cuff waveform-derived method with Centron cBP301 cSBP device.

The following research team members will be 'blinded' to the blood pressure percentile of the patient: (i) technologist/s performing the cardiac and vascular measurements and (ii) Research team member entering data to online database 'MedSciNet'.

6.2.7.1.5. Echocardiography

2D-guided M-mode echocardiography will be performed using images obtained in either parasternal long axis or short axis view of the left ventricle, as recommended by the American Society of Echocardiography.²²⁴ Echocardiogram studies will be performed by trained echocardiographer using a Philips EPIQ ultrasound system at ECLH and Philips CX50 at external centres (Philips Inc, Andover, Mass, USA). All studies will be stored digitally and analysed by a single investigator who will be blinded to the medical history. Left ventricular mass will be calculated using the Devereux equation.²²⁵ Left ventricular mass varies widely across the paediatric age range, therefore to allow standardisation it is usually expressed as left ventricular mass index (LVMI). We will use LVMI (LVM divided by height in meters raised to allometric power of 2.7 [$\text{g/m}^{2.7}$]) as a measure of LVH that accounts for body size.²²⁶ LVH will be defined as LVMI $\geq$ 95th percentile using age-specific reference intervals for normal children²²⁷, where appropriate, we will also calculate left ventricular mass for height z-scores.²²⁸ Relative wall thickness (RWT) will be measured to assess the left ventricular geometry. Patients with increased LVMI ($\geq$ 95th percentile) and elevated RWT ($\geq$ 0.41), have concentric LVH; with increased LVMI ($\geq$ 95th percentile) and normal RWT ($<$ 0.41) have eccentric LVH; and those with normal LVMI ($<$ 95th percentile) and elevated RWT ($\geq$ 0.41) have concentric remodelling. Shortening fraction will be calculated to estimate the LV systolic

function. Diastolic function will be assessed by pulsed Doppler interrogation of mitral valve inflow and tissue velocity imaging interrogation at the level of the mitral valve annulus both at the septum and left ventricular free wall.²²⁹ The mitral valve E/e' ratio will be used as a surrogate for filling pressures. Cross sectional images in both four chambers and multiple short axis views will be obtained to permit analysis of myocardial rotation and torsion using two dimensional strain ("speckle tracking") techniques.

6.2.7.1.6. Carotid intima-media thickness

High resolution ultrasound [Linear transducer for EPIQ and CX50 (15-6MHz)] will be used to obtain images of the common carotid artery. Mean common carotid intima-media thickness (cIMT) will be assessed using automated software (Medical Imaging Applications LLC, Iowa, USA) from digitized images obtained in diastole of the near and far walls of the both common carotid arteries 1-2 cm proximal to the flow divider.^{174,175}

6.2.7.1.7. Arterial stiffness

Arterial stiffness will be determined in all subjects by measuring carotid-femoral pulse wave velocity (PWVcf) and by measuring brachial-femoral PWV (PWVbf). All assessments will be performed by a core group of researchers from the central team from ELCH, by visiting each site. Carotid-femoral PWV will be determined using the SphygmoCor system (Atcor medical, Australia) in which ECG referenced sequential carotid and femoral applanation tonometry is performed. An alternative technique (Vicorder, Skidmore Medical) employs simultaneous measurement of pulse waveforms from pressure cuffs placed around the arm and thigh. This method is particularly well

tolerated in children. The clinical team will be blinded to results of echocardiogram and vascular studies.

6.2.7.2. Assessments at 4-monthly intervals

The following will be performed 4-monthly in all randomised subjects once their BP level is to target as per assignment to randomization arm.

6.2.7.2.1. Clinical characteristics, examination and anthropometry

Recent medical history will be updated and medication review performed. A clinical examination will be performed. Height (stadiometer), weight and BMI will be recorded.

6.2.7.2.2. Peripheral blood pressure – clinic blood pressure

Peripheral BP will be taken as the mean of 3 measurements using appropriate sized cuff and a calibrated aneroid instrument according to British Hypertension Society guidelines. Systolic BP level will be confirmed to be to study target for each subject but if not changes to medication made as appropriate and earlier review performed as per suggested schema in **Figure 38**.

6.2.7.3. All clinic visits

At all visits compliance with medications will be checked for and any reported missed medications/ periods of medications recorded. Any adverse events will be documented.

6.2.8. Trial schema and study visit schedule

All subjects will undergo comprehensive assessment at the baseline visit, following which they will be randomized and if clinic systolic BP is to target be seen at least 4-monthly for the duration of the trial. If clinic systolic BP is not to target they will be seen more frequently as suggested in **Figure 38**. All subjects will undergo study related assessments at annual intervals and these will be performed by a core-group of research investigators. The information captured at the baseline and subsequent annual visits is shown in **Table 18**.

Table 18: Trial schedule for study procedures and assessments

	Baseline	1 year	2 year
Informed consent	√		
Allocation of study number	√		
Randomisation (post-recruitment)	√		
Medical review and physical examination	√	√	√
Anthropometry	√	√	√
Medication review	√	√	√
Peripheral clinic BP measurement ⁺	√	√	√
Central BP evaluation	√	√	√
24-hour ABPM (optional) [†]	√	√	√

	Baseline	1 year	2 year
Arterial stiffness studies (cIMT[‡] and PWV[*])	√	√	√
Echocardiography	√	√	√
Urine sample	√	√	√
Blood sample	√	√	√

[‡]BP, blood pressure; [†]ABPM, ambulatory BP monitoring; [‡]cIMT, carotid intima-medial thickness; ^{*}PWV, pulse wave velocity

Data will be entered on a trial specific database using an online data entry platform 'MedSciNet'. This will be overseen by Mr Bola Coker, at the Biomedical Research Centre at King's Health Partners and Professor Janet Peacock, Trial Statistician. Mr Coker will also help with the safe running of study database and collation of results.

6.2.9. Subject retention

This visit schedule is in keeping with the frequency of routine follow-up of children with moderate to severe CKD. Subjects and their families will be updated with study progress at the time of any clinic attendances. Clinical teams will also be regularly updated with study progress and this will additionally help update the participants and therefore maintain subject retention to the trial. All subjects will be provided with contact details of their local research team to help maintain easy communication.

6.2.10. Sample size and power calculation

A total of 300 subjects will be recruited to the study to include 150 to randomise (75 in each trial arm) and 150 as control subjects.

Differences in outcome will be estimated using a random effects (mixed) model that includes the baseline value of the outcome. Sensitivity analyses will be performed to also include key prognostic variables including known factors other than blood pressure and those identified by univariate analyses. The outcome data are longitudinal and so a model that takes account of this data structure will be fitted. The random effects model is a 2-level multilevel model that allows for the repeated data within subjects, while allowing some measurements to be missing. (This is preferred to repeated measures analysis of variance that requires complete data on all subjects). For a particular outcome, eg LV mass, the baseline value for each subject will be included in the model to adjust for (random) baseline variability between subjects. The results of the analysis are in the form of mean difference in LV mass between the intervention and control groups, at the endpoint, with a 95% CI.

The original sample size calculations were based upon longitudinal measurements of LVMI at annual intervals in our cohort of children with CKD (n=47). The standard deviation (SD) of change in LVMI was $7.7 \text{ g/m}^{2.7}$ and this was obtained without the same standardized reading of LVM proposed in this study. To be conservative we assume a 20% improvement on our existing data giving an SD of $6\text{g/m}^{2.7}$ result from our own cohort. Thus with n=60 in each group we should be able to detect a difference in change in LVMI of $> 3.1 \text{ g/m}^{2.7}$ (=9% of baseline LVMI) over the 2 year duration with 90% power ($P<0.05$). This is one third of the difference ($9.4 \text{ g/m}^{2.7}$) between subjects in

our cohort that have blood pressure in the 50-75th and below 40th percentiles. Although we are assuming relatively rapid tracking of LV mass in relation to blood pressure, this is not unreasonable given the time frame of LV regression in adults. A recent meta-analysis of randomized studies shows a mean change in LVMI of 10.3% over duration of 6 months.²³⁰

Interim analysis will be performed at 1-year by the statistical team to check if the SD of LVMI is in keeping with initial projections. The choice of SD for the initial sample size was made using the best data available at the time of the study design. In accordance with good practice, we will monitor the SD during the study across the whole study group i.e. NOT by randomised group. In this way we can verify that the overall SD observed in the trial is in fact as expected. If the SD is found to be very different, either larger or smaller, we will consider whether the sample size calculations need to be revised accordingly. In this way we will be able to be as certain as is possible that the trial is powered as planned and ensure that the primary study endpoint is achieved We will inform the Research Ethics Committee of the results of the interim analysis.

With regard to PWV, the within subject SD for measurements separated by several months is 0.5 m/s so we should be able to detect a difference in change in PWV of 0.3 m/s. Although we do not have data relating PWV to level of blood pressure, this difference is small in absolute terms (being equivalent to only a few years of “vascular ageing” in adults) and therefore we feel it is likely that we will be able to detect a meaningful change in PWV.

6.2.11. Trial Outcome measures

The primary outcome measure for the controlled trial will be the differences in LV mass between aggressive and standard arms. The secondary outcome measures are the difference in carotid IMT and PWV between the aggressive and standard trial arms.

6.2.12. Statistical analysis

Differences in outcome will be estimated using a random effects (mixed) model that includes the baseline value of the outcome. Sensitivity analyses will be performed to also include key prognostic variables including known factors other than blood pressure and those identified by univariate analyses. An interim analysis at 1-year following commencement of study will allow detailed statistical analysis plan to be developed. Both SPSSv17 and SigmaStat v10 will be used for statistical analysis, in close consultation with statisticians at Kings College London.

6.2.13. Reporting of adverse events

Adverse events will be assessed at each study visit and data regarding these completed in case report forms. Related and unexpected Serious Adverse Events (SAE's) will be reported to the Research Ethics Committee (REC) for the duration of the trial.

6.2.14. Monitoring

6.2.14.1. Trial steering committee

This committee will comprise of Chief Investigator (Manish Sinha), at-least one of other co-applicants (John Simpson, Louise Watt and Professor Phil Chowienczyk), Research Nurse (TBA), Research Associate (Haotian Gu and Laura Milne), trial co-ordinator (Humra Chadwick) and trial statistician (Professor Janet Peacock). The committee will meet regularly to monitor recruitment and completion of study objectives with formal meetings annually to monitor data quality, safety and achievement of study objectives.

6.2.14.2. Independent data monitoring committee

An independent Data Monitoring Committee (DMC) will be established for this trial and will include members as per standard guidelines and include at least one member from each of the following designations: a statistician, an expert in renal disease and an expert in cardiovascular medicine. The DMC will aim to meet annually to review all collected data and may meet more frequently if required after analysis of the available data. The DMC will advise the Trial Steering Committee on the safety of continuing this clinical trial. The DMC will also review adverse event data annually or more frequently if needed.

6.2.15. Regulatory aspects

The HOT-KID study is sponsored by the Guy's & St Thomas' NHS Foundation Hospital's NHS Trust. The ISRCTN number for the trial is 25006406 and the UKCRN ID is 12925. This clinical trial will be conducted in accordance with the ethical principles as per the Declaration of Helsinki, and the principles of Good Clinical Practice and all applicable requirements as stated in the R&D approval.

6.2.16. Ethical committee approval

The Ethics for this study have been approved by the NRES Committee London – Westminster (REC reference 10/H0802/13). All protocol amendments will be submitted for approval to the REC before implementation and progress reports and copy of final study report will be provided to REC as per standard requirements.

Ethics approval letter for the HOT-KID trial as **Appendix A** and example of 'Parent Information Sheet' (29/12/2014 version 4.0) shown as **Appendix B**.

6.3. Discussion

6.3.1. Potential impact

Heart disease in this population is an important clinical problem. Our knowledge of its evolution remains rudimentary with a striking deficiency of studies examining 'cause and effect'. There is complete consensus by experts within the community that the optimal treatment target for blood pressure is of key importance. Whilst the multicentre, Effect of

Strict Blood Pressure Control and ACE Inhibition on the Progression of CRF in Pediatric Patients (ESCAPE) study¹⁵⁸, aimed to evaluate the renoprotective efficacy of intensified blood pressure control in paediatric patients with pre-dialysis CKD, has provided some data to support a target below the 75th centile (that now proposed as “standard treatment” in the present trial), this target is still high compared to that used in the adult population. The present study would provide definitive data to support a lower target.

This project offers a unique opportunity to determine whether a simple intervention that could readily be implemented in clinical practice is likely to reduce heart disease. It will answer a key translational research question and, if successful, will lay the foundations for a large scale multi-centre study which could provide a huge advance in reducing adult heart disease in adults with onset of chronic kidney disease (CKD) in childhood. This will be of relevance to not only in adults with onset of CKD in childhood but in the wider population of adult heart disease in which a degree of CKD is a ubiquitous component.

6.3.2. Dissemination

The study results will be published in accordance with the CONSORT statement and SPIRIT guidelines.^{231,232} Our findings will be submitted to major international paediatric nephrology and general paediatric meetings, and submitted for publication in a high impact factor journal, with open access. The results will also be communicated in plain English to study participants and their families.

6.4. Trial status

As of the 26th October 2015, 240 subjects (including 98 of 150 subjects randomized) had been recruited to the study. The study has been funded by a British Heart Foundation Project Grant (PG/11/90/28994).

Chapter 7 : Discussion

Children with chronic kidney disease (CKD) have several fold increased risk of cardiovascular (CV) death when compared with the general population both during childhood and subsequently as young adults. Through the life-span of their kidneys, markers of pre-clinical cardiovascular disease are highly prevalent, often observed to progress with worsening renal disease, and are likely to relate to subsequent CV morbidity and mortality.^{19,23,26,28-30,32}

Measures of end organ CV disease in childhood CKD that have been investigated to date include arterial stiffness and left ventricular hypertrophy (LVH) and these are often associated with several modifiable and non-modifiable CV risk factors.^{18,36,73} On balance, blood pressure (BP) remains one of the main modifiable CV risk factor in childhood CKD cohorts, particularly in those with pre-dialysis stages of CKD. Its optimal management is likely to improve the risk of future adverse CV outcomes. A substantial body of evidence over the past decade, including those from large observational studies suggests that uncontrolled BP is both under-recognised and under-treated and that the optimal level of BP to improve CV end-organ outcomes in this cohort is not specified.

Measures of large artery health in children with CKD have been less extensively evaluated than in adults. Structural measures such as carotid intima medial thickness have been reported but there are limited data regarding pulse wave velocity (PWV) and functional measures of arterial health.¹⁰⁸⁻¹¹⁰ Major hurdles include the need for trained skilled operators to perform studies on children who have poor tolerance of available instruments and methods widely used in adult subjects. An improved evaluation of arterial health in children with CKD may lead to a better understanding of CV disease and its evolution through childhood to young adulthood.

The measurement of PWV in children has been limited due to the lack of techniques that are easy to use and well tolerated across the childhood age-range. In chapter III of this thesis it is shown that using the Vicorder technique, PWV can be measured easily with minimal training and with excellent repeatability, and is well tolerated by most children. However, PWV measurements performed using different instruments over the same path are *not* interchangeable.

To understand the precise role of blood pressure on arterial health it is of course imperative to measure the BP in the artery whose stiffness is being evaluated. It is well known that systolic BP is amplified along conduit arteries, such that peripheral SBP (pSBP) measured at the brachial or radial artery usually exceeds central aortic systolic pressure (cSBP) at the aortic root, with little difference between mean arterial pressure and diastolic BP. Systolic amplification, the difference between pSBP and cSBP, is a result of pressure wave reflection and summation across the arterial tree. In chapter IV of this thesis the accuracy of central aortic systolic BP estimated in children using a non-invasive method was investigated. Systolic amplification was shown to be substantial with a mean amplification of ~20 mmHg, almost twice that seen in adults. Future work to understand the factors influencing systolic amplification, impact of interventions on peripheral and central systolic BP and if measurement of cSBP offers any improved value above pSBP when evaluating the end organ damage in children with CKD will be important.

In chapter V of this thesis we characterised large artery biomechanical properties. In this large cross-sectional study of pre-dialysis CKD, we demonstrate the key role of BP over other modifiable risk factors. When children with predialysis CKD are compared

with healthy children, we show that for the same peripheral and carotid BP anatomical and functional elastic properties of the large arteries are comparable but differences in functional arterial properties are significant when children with sub-optimal BP control ($\geq 75^{\text{th}}$ percentile) are compared with healthy children. Further, in age, gender and peripheral and central BP matched children with and without CKD, we show no differences in markers of arterial health. Thus data in this study suggests that although functional elastic properties of the carotid artery are some of the earliest identifiable biomechanical abnormalities in children with non-dialysis CKD they relate primarily to BP and not to level of renal function or markers of mineral bone disease. Important limitations to this cross-sectional study include lack of knowledge of duration of both hypertension and CKD and lack of power. Despite these limitations, this study suggests that effective BP control may be one of the most important ways of protecting children with CKD from arterial stiffening and this needs to be evaluated using a controlled clinical trial in this cohort.

In chapter VI of this thesis the protocol of such a controlled clinical trial has been presented. The trial is titled, 'Hypertension Optimal Treatment in Children with Chronic Kidney Disease study: The HOT-KID study- a randomised trial to compare effects of aggressive versus standard targets in blood pressure on target organ damage in children with CKD' and is designed to provide important definitive evidence whether a lower than currently recommended level of clinic blood pressure ameliorates adverse cardiovascular target organ outcomes in children with CKD.

In conclusion, systematic evaluation of arterial health in children remains the missing link to a more complete understanding of CV health in this cohort. Improved methods to

measure PWV and cSBP will help achieve this objective. Cross-sectional evaluation, in those with predialysis CKD suggests that BP is the key modifiable target in this cohort and of more significance than mineral bone disease. The ongoing controlled clinical trial, the HOT-KID study will determine whether a simple intervention that could readily be implemented in clinical practice is likely to reduce the unacceptably high incidence of cardiovascular disease observed in children with CKD.

APPENDIX A

Ethics approval letter for the HOT-KID trial



Health Research Authority

NRES Committee London - Westminster

(Formerly St Thomas' Ethics Committee)
Research Ethics Committee (REC) Centre, Charing Cross,
Room 12, 4th Floor West, Charing Cross Hospital
Fulham Palace Road,
London
W6 8RF

Tel: 020 3311 0100

Fax: 020 3311 7280

11 June 2012

Dr Manish D Sinha
Consultant Paediatric Nephrologist
Guy's & St Thomas's NHS Foundation,
Department of Paediatric Nephrology, Room 64, Sky level
Evelina Children's Hospital,
Lambeth Palace Road, London
SE1 7EH

Dear Dr Sinha

Study title: A randomised trial to compare effects of lower versus higher levels of blood pressure control on target organ damage in children with chronic kidney disease

REC reference: 10/H0802/13

Protocol number: N/A

Amendment number: Amendment 1 Date 21.05.2012

Amendment date: 21 May 2012

The above amendment was reviewed at the meeting of the Sub-Committee held on 06 June 2012.

Ethical opinion

No ethical issues raised.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Protocol	Version 1 The Hypertension optimal treatment in children with Chronic Kidney Disease	30 April 2012

	Study: The HOT -KID study	
Notice of Substantial Amendment (non-CTIMPs)	Amendment 1 Date 21.05.2012	21 May 2012
Covering Letter		21 May 2012

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

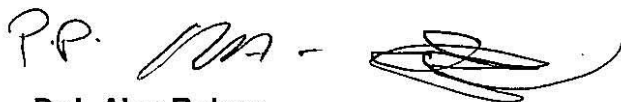
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

10/H0802/13:

Please quote this number on all correspondence

Yours sincerely



Dr L Alan Ruben
Chair

E-mail: Laura.Keegan@nhs.net

Enclosures:

List of names and professions of members who took part in the review

Copy to:

*Dr Shane Tibby, Guy's & St Thomas's NHS Foundation Hospitals
NHS Trust
Professor Philip Chowienczyk, King's College London*

NRES Committee London - Westminster

Attendance at Sub-Committee of the REC meeting on 06 June 2012

<i>Name</i>	<i>Profession</i>	<i>Capacity</i>
Dr Anthony Kaiser	Consultant Neonatologist	Expert
Dr L Alan Ruben	Retired General Practitioner	Expert
Miss Ros Stanbury	Ophthalmologist	Expert

APPENDIX B

Example of 'Parent information sheet' (29/12/2014 version 4.0)

(Form to be on hospital/institution headed paper)

PARENT INFORMATION SHEET

Version 4.0 29th December 2014

Study title

The Hypertension Optimal Treatment in Children with Chronic Kidney Disease study: The HOT-KID study- A randomised trial to compare effects of aggressive versus standard targets in blood pressure on target organ damage in children with CKD

A **randomised trial** is a scientific study used to test how effective a treatment is. It involves randomly allocating all the research participants or patients who have agreed to take part, to different treatments so that comparisons between the treatments can be fairly made.

Target organ damage – in this sense means possible strain on the heart and arteries caused by high blood pressure.

Chronic kidney disease (CKD) – chronic means lasting a long time. You have had this condition for a long period and continue to have it now.

Hypertension – this means high blood pressure and is a common complication of chronic kidney disease

We would like to invite your child to take part in a research study. Before you decide whether you want your child to take part in this study, it is important for you to understand why the

research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Do take time to decide whether or not you wish your child to take part.

What is the purpose of the study?

Hypertension is a frequent complication in children with chronic kidney disease and can lead to strain on the heart causing it to increase in size. It can also lead to strain on the health of the tubes carrying blood in the body called arteries and veins. Accurate detection and treatment of hypertension in chronic kidney disease is important for the health of the kidneys but perhaps also for the health of the heart and arteries. If your child has hypertension they may already be on medication to help control this.

We are comparing two levels of blood pressure to see which is better. The purpose of the study is to find out what difference this may make to the overall health of the heart and blood vessels. As part of the study if your child has chronic kidney disease and hypertension they may need to start taking medication or changing the dose of their current medication.

This study involves more accurate measurement of blood pressure along with assessment of the heart and arteries using non-invasive tests such as heart and artery ultrasound. We will also assess the health of the arteries by measuring 'blood vessel stiffness'. We will do this by using tests that do not involve any needles. We will also assess the health of your child's heart and kidney by performing tests on blood and urine specimens taken at the time of your routine clinic visits. Results from this study will help in improving our understanding of the role of blood pressure in chronic kidney disease and benefits of its improved control. Ultimately, this may lead to a better understanding of the high rate of heart disease seen in large number of young adults who developed chronic kidney disease during childhood.

The proposed duration of this study is 5 years.

Why has your child been chosen to participate in this study?

You have been invited to participate in the study because your child has got chronic kidney disease. To be eligible to participate in this study your child should be older than 2-years of age with an estimated kidney function >15% of normal. High blood pressure is a common complication of CKD and increases risks of developing heart problems. Your child is also eligible to participate in this study even if they have chronic kidney disease and no current problems with high blood pressure presently.

Your child may also have been invited to participate as they have normal blood pressure and no kidney disease but they are coming to the hospital for some other medical reason or their sibling has kidney disease or hypertension. Sometimes when doing new tests it is important to know what is 'normal' so that identifying 'abnormal' becomes easier. Your child may have been invited to take part in this study as we need to perform studies in 'normal' children who do not have chronic kidney problems.

We need your permission to perform these tests in either case.

Does your child have to take part?

Taking part in the study is voluntary. It is up to you and your child to decide whether or not they want to take part. If you do decide to allow your child to take part you will be given this information sheet to keep and be requested to sign a consent form. Even if you do consent for your child to take part you are still free to withdraw from the study at any time and without giving a reason. We would also want to reassure you that your decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives at any time.

What will happen to your child once you consent to take part?

We wish to compare the long term effects on the heart, arteries and kidneys of keeping the blood pressure at two different levels. Your child will be allocated into the 'control' or 'randomised' group. The control group will have children with CKD and normal blood pressure or healthy children. They will not be given any medication. The children in the randomised group will be randomly allocated using a special piece of computer software into one of two groups. One group will have their blood pressure maintained to that called 'standard care' i.e. What the current practice for blood pressure control is for patients with CKD. The second group will have their blood pressure maintained at a lower level than the first group.

In addition there are seven extra things we would like to do at the time of your child's routine clinic visit and once every 12 months after that for the next 5 years. These tests will not result in any extra hospital visits. None of these cause any pain or involve any needles.

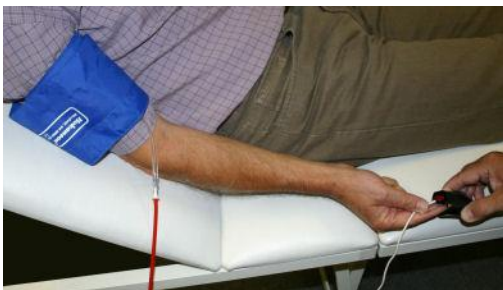
1. **A detailed ultrasound of the heart and arteries called an ECHO.** The ultrasound will be performed by experienced technicians who normally work with children. This will take about 20 minutes and will be performed while you are waiting to be seen by your regular doctors.



2. Around the time of the heart ultrasound we would perform a **blood vessel stiffness study (BVS)**. This test does not involve any needles and involves light pressure using a pencil-like device over one arm, the neck and the groin for 5-10 minutes each. This device also helps in measuring central blood pressure.



We will also perform **blood vessel stiffness study** using blood pressure cuffs on the arm, thigh and neck to measure the stiffness of the blood vessels. The two methods will take a total of 30-40 minutes. This test will be performed in the kidney clinic area by experienced technician or one of your doctors.





3. **Ultrasound of the carotid artery in the neck** This is a special ultrasound of an artery in the neck and will take 5 minutes.



4. **Central blood pressure** will be estimated from radial tonometry and modified BP measurement using Centron™ BP machine.
5. **In some instances if you agree your child may be given a blood pressure measurement monitor to use at home. It will measure blood pressure for 24 hours.** This test will only be performed if your child is over 5 years of age and over 4 feet tall. This will record blood pressure measurement twice every hour for 24-hours.
6. **Blood sample 5ml (one tea spoonful).** This blood will be collected at the same time that we take the usual blood tests in clinic. It therefore does not involve extra needles. We plan to analyse 2-3 ml of this blood specimen for tests that will measure additional risk factors that have an effect on the health of your child's heart. We will store any additional blood specimen for any tests that may become available in the future to detect high blood pressure and heart disease.
7. **Urine sample:** We also require first morning urine specimen (5-10mls each) to be collected on three consecutive days prior to a clinic visit once a year and a spot urine sample to be taken on the day of the clinic visit.

How will the study change my child's treatment?

This study is designed to test what level of blood pressure control is more beneficial for children with chronic kidney disease. If you agree to take part in the study your doctor will assign your child randomly to one of two levels of blood pressure control.

The two levels of blood pressure control are (i) maintaining blood pressure levels at currently recommended levels, children assigned to this study 'arm' or 'group' will be called the 'standard (or higher) blood pressure level treatment group' and (ii) maintaining blood pressure at lower levels than usual, if your child is assigned to this study group they will be included in the 'lower blood pressure level treatment group'.

We will recommend blood pressure medicines as per this research study to achieve the desired blood pressure target to your child's consultant but it is up to the consultant to decide whether this is appropriate for your child or to choose another medicine. Once assigned to a study group your child will remain in the same group throughout the duration of the study.

What will happen to your child after the initial investigations have been performed?

Following the initial set of investigations upon enrolment in the study your child will have all the investigations repeated at yearly intervals. The study duration is for 5-years. Participation in the research does not mean that your child will need to attend clinic appointments in the hospital more often than they have been previously doing.

What do I have to do?

As a participant you agree to encourage your child to complete the proposed tests if he/she is happy to participate. Your child should continue to take all prescribed medications as advised.

What is the drug that is being tested?

We are not testing any new drugs in this study but we are using standard blood pressure medicines in a different way.

What are the side effects of any treatment received when taking part?

More children in the low blood pressure group may need to take medicines or take their current medicines at higher doses. This may lead to more side effects. It is possible, if your child is in

the group with lower blood pressure targets, that they may have side effects such as generally feeling tired or headaches. Parents of children who have occasionally had these side effects have observed that their child has less energy than usual or complaints of feeling more tired and sleepy through the day. They may also complain of dull headaches. If this happens or you are concerned because of any new symptoms in your child please contact us so that we can give you advice. If your consultant believes that the lower blood pressure levels may be causing you to feel unwell we will stop the additional blood pressure medicine. If participation in the study shows that your child has high blood pressure then they will receive appropriate treatment. All changes to your child's treatment will be made after discussing the results of tests with you.

It may be that we are unable to collect the proposed volume of blood at a particular study. If this happens we will attempt to work with the volume of blood obtained or obtain additional samples at future clinic visits. If your child feels unwell during the blood test we will stop taking any blood at the time.

What are the possible benefits of taking part?

There are no direct benefits to your child. This study may eventually help us to identify what level of high blood pressure is optimal in children with chronic kidney disease. This may help to reduce heart disease arising from the strain that high blood pressure can put on the heart.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the disease/technique that is being studied. If this happens, your doctor will tell you about it and discuss with you whether you want your child to continue in the study. If you decide to withdraw your child from the study this decision will not affect the quality of your child's care. If you decide to continue in the study you will be asked to sign an updated consent form.

Sometimes, on receiving new information your research doctor might consider it to be in your best interests to withdraw your child from the study. She/he will explain the reasons and arrange for their care to continue.

What happens when the research study stops?

When the research study stops your child will continue to be seen and managed by their consultant as required. Any important results that are available before the completion of the research study will be known to your doctor.

What if something goes wrong?

If there is any harm to your child as a result of taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you or your child have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

This study is sponsored by the Guy's and St Thomas' NHS Foundation Trust. The sponsors will at all times maintain adequate insurance in relation to the study independently. The Trust has a duty of care to patients via NHS indemnity cover in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

Will my taking part in this study be kept confidential?

All information that is collected about your child during the course of the research will be kept strictly confidential. Any information about your child that leaves the hospital will have their name and address removed so that they cannot be recognised from it. We will inform your GP that your child is participating in the research study.

We have made sure that all the other parties participating in the study are aware of the laws and regulations that protect patient confidentiality. (These regulations include the Data Protection Act 1998 and similar legislations in other EU countries.) Under this protection, only authorized representatives connected with research are permitted access to names and other identifying characteristics. If we need somebody else to access your identification we will ask this permission from you in writing.

What will happen to the results of the research study?

The findings of the research study will be published in peer-reviewed journals and discussed at scientific meetings. It is likely that results of the research will be reported annually and at the end of the study. Copies of the published results could be obtained from your doctors. Any reports or publications will be anonymised for any patient details. This will ensure that the identity of your child is not revealed.

Who is organising and funding the research?

This research study has been generously funded by the British Heart Foundation. The nurses and doctors undertaking the research are not paid any extra money for undertaking this research.

Who has reviewed the study?

The ethics for this study has been reviewed and approved by the St Thomas' Hospital Research Ethics Committee now called the NRES Committee London - Westminster. They make sure that the research is fair and ethical in its treatment of your child as a research participant.

Contact for further Information

Please discuss any questions you may have with your trial doctor or members of the research team:

Your trial doctor is:

Name:

Contact phone number:

Your research/specialist nurse is:

Name:

Contact phone number:

You will be given a copy of the information sheet and a signed form to keep.

Thank you once again for taking the time to read this information sheet.

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