Findings from 4C-T Study demonstrate an increased cardiovascular burden in girls with end stage kidney disease and kidney transplantation

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Rizky I. Sugianto[1], Nima Memaran[1], Bernhard M.W. Schmidt[2], Anke Doyon[3], Daniela Thurn-Valsassina[1,4], Harika Alpay[5], Ali Anarat[6], Klaus Arbeiter[7], Karolis Azukaitis[8], Aysun K. Bayazit[6], Ipek K. Bulut[9], Salim Caliskan[10], Nur Canpolat[10], Ali Duzova[11], Jutta Gellerman[12], Jerome Harambat[13], Denise Homeyer[14], Mieczyslaw Litwin[15], Francesca Mencarelli[16], Lukasz Obrycki[15], Dusan Paripovic[17], Bruno Ranchin[18], Rukshana Shroff[19], Uwe Tegtbur[14], Jeannine von der Born[1], Ebru Yilmaz[20], Uwe Querfeld[12], Elke Wühl[3], Franz Schaefer[3,21] and Anette Melk[1,21]

[1]*Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany;* [2]*Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany;* [3]*Center for Pediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany;* [4]*Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria;* [5]*Department of Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey;* [6]*Department of Pediatrics, Faculty of Medicine, Cukurova Universitesi, Adana, Turkey;* [7]*Department of Pediatric Nephrology and Gastroenterology, Medical University of Vienna, Vienna, Austria;* [8]*Clinic of Pediatrics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania;* [9]*Department of Pediatrics, Faculty of Medicine, Ege University, Izmir, Turkey;* [10]*Department of Pediatric Nephrology, Faculty of Medicine, Istanbul University-Cerrahpaşa, Istanbul, Turkey;* [11]*Division of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey;* [12]*Charité Children's Hospital, Berlin, Germany;* [13]*Pediatrics Department, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France;* [14]*Institute of Sports Medicine, Hannover Medical School, Hannover, Germany;* [15]*Department of Nephrology, Kidney Transplantation and Arterial Hypertension, The Children's Memorial Health Institute, Warsaw, Poland;* [16]*Department of Pediatrics, S. Orsola-Malpighi Hospital, Bologna, Italy;* [17]*Department of Nephrology, Children's Hospital, University of Belgrade, Belgrade, Serbia;* [18]*Pediatric Nephrology Unit, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Université de Lyon, Lyon, France;* [19]*Department of Pediatric Nephrology, Great Ormond Street Hospital, London, UK; and* [20]*Department of Pediatric Nephrology, Sanliurfa Children's Hospital, Sanliurfa, Turkey*

Mortality in children with kidney failure is higher in girls than boys with cardiovascular complications representing the most common causes of death. Pulse wave velocity (PWV), a measure of vascular stiffness, predicts cardiovascular mortality in adults. Here, PWV in children with kidney failure undergoing kidney replacement therapy was investigated to determine sex differences and potential contributing factors. Two-hundred thirty-five children (80 girls; 34%) undergoing transplantation (150 pre-emptive, 85 with prior dialysis) having at least one PWV measurement pre- and/or post-transplantation from a prospective cohort were analyzed. Longitudinal analyses (median/maximum follow-up time of 6/9 years) were performed for PWV z-scores (PWVz) using linear mixed regression models and further stratified by the categories of time: pre-kidney replacement therapy and post-transplantation. PWVz significantly increased by 0.094 per year and was significantly higher in girls (PWVz +0.295) compared to boys, independent of the underlying kidney disease. During pre-kidney replacement therapy, an average estimated GFR decline of 4 ml/min/1.73 m^2 per year was associated with a PWVz increase of 0.16 in girls only. Higher diastolic blood pressure and low density lipoprotein were independently associated with higher PWVz during pre-kidney replacement therapy in both sexes. In girls post-transplantation, an estimated GFR decline of 4ml/min/1.73m^2 per year pre-kidney replacement therapy and a longer time (over 12 months) to transplantation were significantly associated with higher PWVz of 0.22 and of 0.57, respectively. PWVz increased further after transplantation and was positively associated with time on dialysis and diastolic blood pressure in both sexes. Thus, our findings demonstrate that girls with advanced chronic kidney disease are more susceptible to develop vascular stiffening compared to boys, this difference persist after transplantation and might contribute to higher mortality rates seen in girls with kidney failure.

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Correspondence: *Anette Melk, Children's Hospital, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. E-mail: Melk.Anette@mh-hannover.de*

[21]Equal contribution.

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Overall childhood mortality rates are declining.[1] In the general population, boys show higher mortality in most regions of the world,[2,3] largely because of more

accidents,[1] prematurity, respiratory distress during infancy,[2,4] and sepsis occurring postpuberty.[3] Inferior survival in girls is associated with poverty, marginalization, and a sociocultural preference for male offspring.[2] Mortality in children with end-stage kidney disease is >30 times higher than that in the general population.[5] Data from the United States Renal Data System on 14,024 children on kidney replacement therapy (KRT) suggest a higher mortality risk in girls (hazard ratio 1.36; 95% confidence interval 1.25–1.50) because of their higher risk of cardiovascular death.[6] Despite declining overall mortality rates in children with functioning grafts, the proportion of cardiovascular mortality remains unchanged and is ∼20% higher in girls.[7]

Cardiovascular events as the most common causes of death in children with end-stage kidney disease account for about one-third of deaths in children on dialysis and a quarter of those undergoing transplantation.[8] Data from the Australian and New Zealand Dialysis and Transplant Registry suggested an even higher mortality in pediatric kidney transplant recipients due to cardiovascular causes.[9] The post-transplant mortality due to cardiovascular causes is higher than that related to nonfunctioning grafts.[10]

Early measures of arterial stiffness such as increased aortic pulse wave velocity (PWV) are highly predictive for cardiovascular events and mortality[11] and associated with a faster decline in estimated glomerular filtration rate (eGFR) in adults with chronic kidney disease (CKD).[12] Aortic PWV can be measured noninvasively and reproducibly in children.[13,14] Higher PWV was demonstrated in children with CKD even after transplantation compared with their healthy peers.[15–18]

Findings in adults indicate that the global survival advantage of females is lost in end-stage kidney disease,[19] a phenomenon that is not sufficiently explained by disparities of access to transplantation due to higher levels of panel reactive antibodies in women[20] and pregnancy-induced incompatibility.[21] In the pediatric population, girls are less likely to undergo preemptive transplantation[6,22] and show poorer graft survival than boys, the latter being partly explained by receiving male donor organs.[23,24] Our own data indicated a higher susceptibility of girls for cyclosporin A–associated hypertension,[25] which could contribute to poorer graft survival and increased cardiovascular mortality.

Here we aimed to study the course of arterial stiffness in children with end-stage kidney disease who underwent transplantation either preemptively or after prior dialysis to uncover potential sex differences.

METHODS

Study design, setting, and participants

The 4C-T (Cardiovascular Comorbidity in Children with Chronic Kidney Disease –Transplantation) substudy is part of the 4C study,[26] a prospective observational study. Seven hundred four pediatric patients with CKD (age 6–17 years) with an eGFR below 60 ml/min per 1.73 m^2 not yet receiving KRT were enrolled between 2009 and 2011. Ethical aspects and details of the data acquisition were described previously.[26] The median follow-up time was 6 years, with a maximum of 9 years.

Data sources/measurements

PWV was assessed annually using the oscillometric Vicorder device (SMT medical), as described previously.[13,14] Every 6 months blood and urine samples, anthropometric parameters, casual blood pressure (BP), and medical history updates were obtained per standardized protocol. Laboratory measurements were performed centrally. eGFR was calculated using the Schwartz formula.[27]

Variables

Sex- and height-adjusted standardized scores (z scores) were calculated for PWV[13] as the primary end point.

The following parameters were considered as covariates: eGFR decline, body mass index, BP, lipids, hemoglobin, sodium, potassium, calcium, phosphorus, bicarbonate, parathyroid hormone, uric acid, and urea. Kidney diseases were categorized as congenital anomalies of the kidney and urinary tract (CAKUT) or non-CAKUT. Supplementary Table S1 provides a more granular classification of primary kidney diseases. Antihypertensive and immunosuppressive medications (including trough levels) were recorded. Systolic and diastolic BP (sex-, age-, and height-adjusted)[28] z scores as well as height and body mass index (sex- and age-adjusted)[29] z scores were calculated.

As ambulatory BP measurements were available only in a subgroup of patients, we provide data for the correlation between the BP and the ambulatory BP measurement in Supplementary Table S2.

Time variable

Time (in years) was assessed by the following variables: time since inclusion, time pre-KRT (time since inclusion but before KRT start), time post-transplantation (time since transplantation), time from eGFR $\leq$ 30 ml/min per 1.73 m^2 to transplantation, and time on dialysis (see Supplementary Figure S1 for more details).

Healthy control cohort

Longitudinal PWV measurements in 307 healthy children (girls, n = 145) from the REBIRTH active school study were used to assess possible sex differences in the physiological development of PWV. The study investigated cardiovascular parameters in healthy children during a school-based physical activity program[30] with 2 repetitive PWV measurements with an interval of 12.7 ± 3.3 months between 2017 and 2018.

Analysis steps

The analyses for PWV z scores (PWVz) were performed in 3 analysis steps: (i) all data comprising the whole observation time and then divided into 2 separate analyses according to transplantation: (ii) "pre-KRT" and (iii) "post-transplantation" (Figure 1).

Step 1: all data. We included patients with at least 1 visit during the observation period representing the complete observation time (n = 235, Figure 2). This includes data before KRT, on dialysis, and after transplantation. A spline regression was fitted to the data to visualize the course of PWVz and the sex difference on the development of PWVz. Linear mixed regression models (mixed models) for PWVz were performed as follows: (i) adjusted for time since inclusion and kidney disease category to understand the development of PWVz over time; (ii) adjusted for sex, time since inclusion, and kidney disease category to understand the development of PWVz over time depending on sex; and (iii) adjusted for the

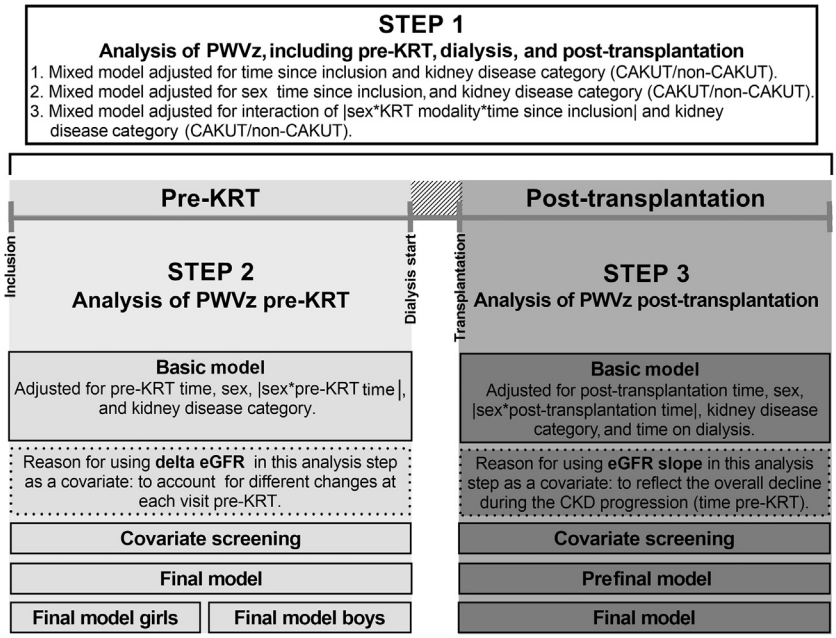


Figure 1 | Process flow of the analyses for pulse wave velocity *z* scores (PWVz). Step 1 describes the analyses for the complete observation period including pre-kidney replacement therapy (KRT), on dialysis, and post-transplantation. Step 2 describes the analyses flow for PWVz during pre-KRT. Step 3 describes the analyses flow for PWVz post-transplantation. CAKUT, congenital anomalies of kidney and urinary tract; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

interaction term |sex*KRT modality*time since inclusion| and kidney disease category to understand the development of PWVz stratified for each treatment modality depending on sex (Figure 1).

Step 2: pre-KRT data. All data before KRT start were included to study the development of PWVz during CKD progression. Patients with at least 1 visit pre-KRT were included ($n = 230$, Figure 2) in the mixed model for PWVz adjusted for pre-KRT time, sex, interaction term of |pre-KRT time*sex|, and kidney disease category to understand potential sex differences on the development of PWVz before KRT. This model was then set as the basic model for "pre-KRT" (Figure 1).

To further investigate the possible influencing factors on sex differences in the development of PWVz before KRT, we screened covariates that are assumed relevant for PWVz using the above predefined basic model. We included patients with at least 2 visits pre-KRT ($n = 158$) to assess the pre-KRT eGFR decline as one of potential covariates. Covariates showing significant associations with PWVz ($P < 0.05$) and/or eliminating the association ($P > 0.05$) between PWVz and the interaction term |pre-KRT time*sex| were included in the backward selection. Covariates that are highly correlated with each other (BP values and lipid levels) were grouped. If ≥2 covariates from the same group were eligible, the one with the better model fit (lower Akaike information criterion) was selected.

To assess CKD progression, eGFR decline was calculated. Delta eGFR (ΔeGFR) for each patient i at visit v was calculated as the

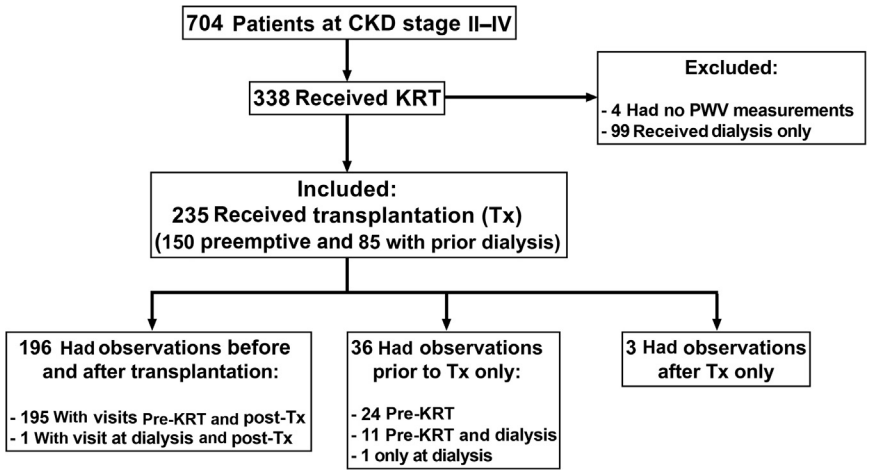


Figure 2 | Inclusion flowchart of the study population. CKD, chronic kidney disease; KRT, kidney replacement therapy; PWV, pulse wave velocity; Tx, transplantation.

Table 1 | Patients' characteristics at inclusion, at the last visit pre-KRT, and at 1 yr post-transplantation

Disease and transplant modality	Girls (n = 80) Frequency (%)	Boys (n = 155) Frequency (%)
Underlying disease		
CAKUT	33 (41)	98 (63)
Non-CAKUT	47 (59)	57 (37)
Transplantation		
Preemptive	48 (60)	102 (66)
With prior dialysis	32 (40)	53 (34)

At inclusion	Girls (n = 78) n	Girls (n = 78) Median (IQR)	Boys (n = 152) n	Boys (n = 152) Median (IQR)
Age (yr)	78	12.2 (9.24 to 14.3)	152	11.6 (8.98 to 14.2)
Height (cm)	78	139 (126 to 151)	152	138 (124 to 155)
Height z score	78	−1.45 (−2.09 to −0.32)	152	−1.16 (−2.03 to −0.19)
BMI (kg/m^2)	78	16.6 (15.3 to 18.4)	152	17.5 (15.7 to 19.9)
BMI z score	78	−0.62 (−1.08 to 0.18)	152	−0.08 (−0.79 to 0.84)
Systolic BP (mm Hg)[a]	78	110 (100 to 114)	152	114 (106 to 123)
Systolic BP z score[a]	78	0.4 (−0.08 to 1.29)	152	1.09 (0.38 to 1.74)
Diastolic BP (mm Hg)	78	65.5 (60.0 to 78.0)	152	69.0 (62.0 to 78.0)
Diastolic BP z score[a]	78	0.41 (−0.16 to 1.30)	152	0.72 (0.18 to 1.31)
eGFR (ml/min per 1.73 m^2)	78	21.5 (15.4 to 30.7)	152	19.3 (14.6 to 28.0)

At last visit pre-KRT	Girls (n = 78) n	Girls (n = 78) Median (IQR) or frequency (%)	Boys (n = 152) n	Boys (n = 152) Median (IQR) or frequency (%)
Age (yr)	78	13.5 (11.1 to 15.8)	152	13.7 (11.9 to 15.7)
Time since inclusion (yr)	78	1.2 (0 to 3.1)	152	1.9 (0 to 3.1)
Time from the last visit during CKD to KRT start (yr)	78	0.5 (0.3 to 0.8)	152	0.6 (0.2 to 0.8)
Height (cm)[a]	78	149 (135 to 157)	152	152 (137 to 165)
Height z score	78	−1.23 (−2.12 to −0.43)	152	−0.96 (−2.14 to −0.22)
BMI (kg/m^2)	78	18.0 (16.1 to 19.1)	152	18.1 (16.6 to 20.5)
BMI z score	78	−0.57 (−1.21 to 0.28)	152	−0.25 (−0.99 to 0.60)
Systolic BP (mm Hg)[a]	78	112 (105 to 119)	152	120 (109 to 128)
Systolic BP z score[a]	78	0.55 (−0.12 to 1.39)	152	1.09 (0.26 to 1.90)
Diastolic BP (mm Hg)	78	70.0 (63.0 to 80.0)	152	70.0 (65.0 to 80.0)
Diastolic BP z score	78	0.46 (−0.01 to 1.48)	152	0.81 (0.13 to 1.43)
eGFR (ml/min per 1.73 m^2)	76	13.9 (11.5 to 16.4)	148	13.3 (10.9 to 17.0)
Cholesterol (mg/dl)	77	185 (165 to 219)	150	169 (142 to 200)
HDL (mg/dl)[a]	77	54.0 (44.0 to 64.0)	150	43.9 (36.5 to 53.0)
LDL (mg/dl)	77	99.0 (81.0 to 123)	150	88.6 (69.5 to 122.3)
Hemoglobin (g/dl)[a]	77	10.6 (9.80 to 11.8)	145	11.2 (10.3 to 12.1)
Ferritin (μg/l)	72	99.8 (52.3 to 181)	134	101.5 (51.0 to 204)
Sodium (mmol/l)[a]	77	140 (136 to 142)	149	140 (137 to 142)
Potassium (mmol/l)	77	4.40 (4.00 to 4.80)	146	4.50 (4.10 to 4.98)
Calcium (mmol/l)[a]	77	2.30 (2.15 to 2.48)	147	2.38 (2.20 to 2.52)
Phosphorus (mmol/l)	77	1.61 (2.41 to 2.92)	150	1.67 (1.51 to 1.97)
Bicarbonate (mmol/l)	74	21.0 (19.0 to 23.0)	143	21.0 (19.0 to 23.0)
Parathyroid hormone (pmol/l)	75	16.3 (7.50 to 38.9)	138	24.9 (13.5 to 40.9)
Uric acid (mg/dl)[a]	77	6.12 (5.30 to 7.31)	150	7.17 (6.18 to 8.31)
Urea (mg/dl)[a]	76	60.7 (43.2 to 79.5)	148	73.0 (56.5 to 116)
Antihypertensive use		54 of 78 (69)		104 of 152 (68)
RAAS antagonists		37 (47)		54 (36)
CCB		29 (37)		57 (38)
β-Blockers		8 (10)		28 (18)
Peripheral α-blockers		5 (6)		8 (5)
Central α-blockers		0 (0)		1 (1)
Loop diuretics		6 (8)		13 (9)
Thiazide diuretics		4 (5)		2 (1)

Transplantation	Girls (n = 71) n	Girls (n = 71) Median (IQR) or frequency (%)	Boys (n = 127) n	Boys (n = 127) Median (IQR) or frequency (%)
Age at transplantation (yr)	71	15.2 (12.3 to 17.0)	128	14.7 (12.6 to 16.3)
Time from eGFR ≤ 30 ml/min per 1.73 m^2 to tx (yr)	71	2.3 (1.5 to 3.8)	128	2.5 (1.3 to 4.0)
Time since inclusion to tx (yr)	71	2.4 (1.4 to 4.4)	126	2.6 (1.4 to 4.1)
Time on dialysis (yr)	32	1.1 (0.7 to 2.1)	53	1.2 (0.9 to 1.6)
		42 (59)		82 (65)

(Continued on following page)

Table 1 | (Continued)

Transplantation	Girls (n = 71)		Boys (n = 127)	
	n	Median (IQR) or frequency (%)	*n*	Median (IQR) or frequency (%)
Transplanted >1 yr after eGFR ≤ 30 ml/min per 1.73 m^2 or after dialysis start				
Transplanted ≤1 yr after eGFR ≤ 30 ml/min per 1.73 m^2 or after dialysis start		29 (41)		45 (35)
At 1 yr post-transplantation	**Girls (n = 53)**		**Boys (n = 103)**	
	n	Median (IQR) or frequency (%)	*n*	Median (IQR) or frequency (%)
Age (yr)	53	15.7 (13.3 to 18.2)	103	15.3 (13.1 to 17.3)
Time since inclusion (yr)	53	3.1 (2.2 to 5.1)	103	3.2 (2.1 to 5.0)
Time post-transplantation (yr)	53	0.9 (0.7 to 1.1)	103	0.9 (0.8 to 1.2)
Height (cm)[a]	53	153 (143 to 160)	103	160 (147 to 168)
Height *z* score	53	−1.00 (−1.98 to −0.03)	103	−1.02 (−2.13 to −0.37)
BMI (kg/m^2)	53	20.3 (17.6 to 22.5)	103	21.1 (18.0 to 23.5)
BMI *z* score	53	−0.12 (−0.93 to 0.95)	103	0.37 (−0.50 to 1.11)
Systolic BP (mm Hg)[a]	53	115 (107 to 121)	103	120 (112 to 130)
Systolic BP *z* score	53	0.73 (−0.02 to 1.51)	103	1.04 (0.21 to 1.76)
Diastolic BP (mm Hg)	53	71 (63.0 to 77)	103	72 (65 to 79)
Diastolic BP *z* score	53	0.54 (−0.14 to 1.14)	103	0.71 (0.13 to 1.33)
eGFR (ml/min per 1.73 m^2)[a]	50	68.4 (50.3 to 82.3)	100	59.4 (46.7 to 74.0)
Tacrolimus trough level (μg/l)	41	5.00 (4.00 to 7.00)	82	6.00 (5.00 to 8.00)
Cyclosporin A trough level (μg/l)	11	100 (67.0 to 129)	16	104 (85.5 to 124)
Steroid dosage (mg per day)	46	5.00 (3.00 to 5.00)	79	5.00 (4.00 to 7.50)
Antihypertensive use		32 of 53 (60)		64 of 103 (62)
RAAS antagonists		9 (17)		16 (15)
CCB		25 (47)		47 (46)
β-Blockers		7 (13)		29 (28)
Peripheral α-blockers		0		4 (4)
Central α-blockers		1 (2)		1 (1)
Loop diuretics		1 (2)		6 (6)
Thiazide diuretics		0		4 (4)
Immunosuppresive use		53 of 53 (100)		103 of 103 (100)
Steroid		46 (87)		79 (77)
CNI		52 (98)		101 (98)
MMF		47 (87)		82 (80)
mTOR		6 (11)		13 (13)

BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; CCB, calcium channel blocker; CKD, chronic kidney disease; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; KRT, kidney replacement therapy; LDL, low-density lipoprotein; MMF, mycophenolate mofetil; mTOR, mechanistic target of rapamycin; RAAS, renin-angiotensin-aldosterone system.
[a]*P* value of < 0.05.
Data are *n* (%) unless otherwise noted.

difference between eGFR at visit v and the previous visit $v-1$ divided by the time T interval (in years) between both visits: $\Delta\text{eGFR} = [\text{eGFR}_{iv} - \text{eGFR}_{i(v-1)}] / [T_{iv} - T_{i(v-1)}]$ (Supplementary Figure S2). In the case of a missing eGFR value between 2 visits, ΔeGFR was interpolated.

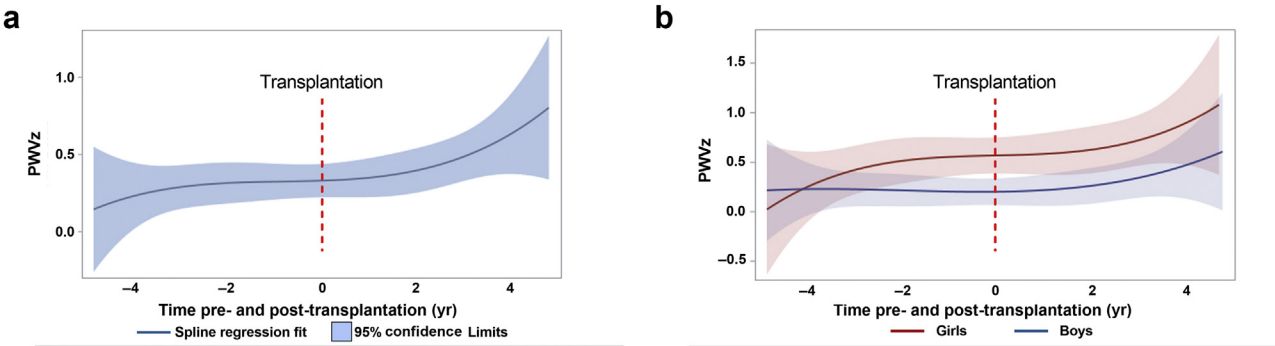
Step 3: post-transplantation data. Patients with at least 1 visit post-transplantation were included (n = 199, Figure 2). A mixed model for PWVz adjusted for post-transplantation time, sex, interaction term |post-transplantation time*sex|, kidney disease category, and time on dialysis was performed to understand the sex differences on the development of PWVz post-transplantation (Figure 1).

For further investigation, patients with visits at pre-KRT and post-transplantation were included to assess the eGFR slope pre-KRT (n = 195, Figure 2). Covariates were then screened using the above predefined basic model to identify the contributing factors. Covariates showing significant associations with PWVz ($P < 0.05$) and/or eliminating the association ($P > 0.05$) between PWVz and the variable "sex" were included in the backward selection. Similar to the analysis for pre-KRT, if ≥2 covariates were eligible but highly correlated with each other, the one with the lower Akaike information criterion was included.

We calculated individual eGFR slopes using the eGFR measurements pre-KRT to reflect the pace of the functional decline. The eGFR slope was computed as the function (regression coefficient, β) of the fixed effect of time pre-KRT (in years) for each patient i from linear regression of eGFR: $\text{eGFR}_i = \text{Intercept}_i + \beta_i(\text{time pre} - \text{KRT in years})$ (Supplementary Figure S2). eGFR slopes were defined as 0 in 20 cases undergoing dialysis and 17 who underwent preemptive transplantation. In all cases, final CKD stages were reached and KRT was initiated before a second measurement could be performed. Three children with only 1 eGFR measurement pre-KRT >12 months before the initiation of KRT were excluded. A sensitivity analysis for the final model including individual eGFR slopes computed from a single mixed model for eGFR pre-KRT was performed. To assess the time to transplantation, patients were grouped into shorter (≤12 months) or longer (>12 months) time to transplantation calculated as time to transplantation since eGFR dropped to ≤30 ml/min per 1.73 m^2 (preemptive) or since dialysis start (after prior dialysis).

Additional analyses for PWVz by kidney diseases

As the underlying kidney diseases differ between sexes, additional analyses had to be performed. Patients with at least 1 visit were



Mixed model for PWVz for the complete study observation (pre- and post-transplantation) (patients, n = 235; observations, n = 1368)

Effect	β	SE	P
Intercept	0.042	0.10	0.68
Time since inclusion (yr)	0.095	0.016	<0.0001
Non-CAKUT (ref.: CAKUT)	0.064	0.14	0.64

Mixed model for PWVz for the complete study observation (pre- and post-transplantation) (patients, n = 235; observations, n = 1368)

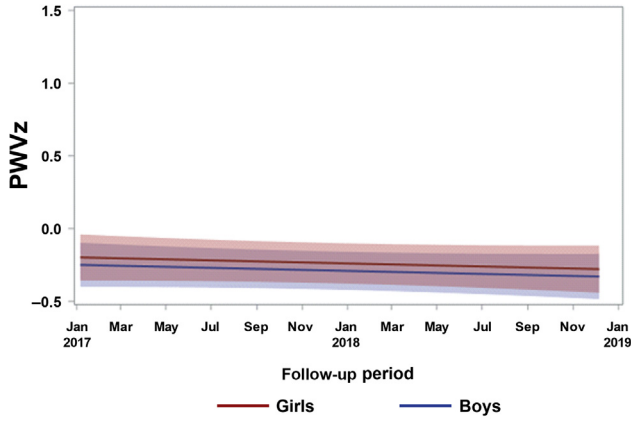
Effect	β	SE	P
Intercept	–0.032	0.11	0.77
Time since inclusion (yr)	0.094	0.016	<0.0001
Girls (ref.: boys)	0.295	0.15	0.045
Non-CAKUT (ref.: CAKUT)	0.006	0.14	0.97

Figure 3 | Analyses of pulse wave velocity *z* scores (PWVz) over the complete observation period. (**a**) Spline regression fit and 95% confidence interval (upper panel) and mixed model for PWVz adjusted for time since inclusion and kidney disease (the table in the lower panel). (**b**) Spline regression fit and 95% confidence interval differentiated by sex (upper panel), and mixed model for PWVz adjusted for time since inclusion, kidney disease, and sex (the table in the lower panel). β, regression coefficient; CAKUT, congenital anomalies of kidney and urinary tract; KRT, kidney replacement therapy; Ref., reference; SE, standard error.

included (n = 235). Two mixed models for PWVz were performed:

(i) a mixed model adjusted for the interaction term |time since inclusion*kidney disease category| to understand whether the PWVz development differs between patients with CAKUT and those without CAKUT. The corrected means and 95% confidence intervals of PWVz adjusted for the respective models were calculated for CAKUT and non-CAKUT groups; and

(ii) a mixed model adjusted for the interaction term |time since inclusion*sex and kidney disease category (girls–CAKUT, girls–non-CAKUT, boys–CAKUT, and boys–non-CAKUT)| to understand how sex influences the PWVz course in each kidney disease category.



Mixed model for PWVz in the healthy cohort (subjects, n = 307; observations, n = 614)

	β	SE	P
Intercept	–0.26	0.070	<0.001
Time since inclusion (yr)	–0.048	0.043	0.27
Girls (ref.: boys)	0.050	0.097	0.60

Figure 4 | Regression fit of pulse wave velocity *z* scores (PWVz) and 95% confidence interval in the cohort of healthy children differentiated by sex and adjusted for time since inclusion. The respective adjusted mixed model is given in the table below the graph. β, regression coefficient; Ref., reference; SE, standard error.

General statistical analysis

Data are given as median and interquartile range or absolute and relative frequencies. t tests were performed to test differences between sexes. Complete data analyses were performed, and covariates with missing >10% were not included in the covariate selection. Supplementary Figure S3 provides the number of observations over time. The pattern of missing data accounting for the variables included in the final models is provided in Supplementary Table S3A and B. Spline regression and mixed models were performed as described above. In the mixed models, patient ID and center were included as random effects to model the between-subject variation and time since inclusion as a repeated effect to model within-subject variation.[31] Statistical analysis was performed using SAS 9.4 (SAS Institute). This manuscript was written according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[32]

RESULTS

Patient characteristics

Of 704 children, 338 underwent KRT. Four patients without any PWV measurements and 99 patients only receiving dialysis without subsequent transplantation were excluded. Two hundred thirty-five patients (girls, n = 80) undergoing kidney transplantation (preemptive, n = 150; with prior dialysis, n = 85) were included. Of those, 196 had observations before and after transplantation, 36 only before transplantation, and 3 only after transplantation (Figure 2).

eGFR at inclusion and at the last visit pre-KRT, age at inclusion and at transplantation, time from eGFR ≤ 30 ml/min per 1.73 m^2 to transplantation, and time on dialysis did not differ between sexes. At the last visit pre-KRT, girls showed significantly lower height, systolic BP, hemoglobin, sodium, calcium, uric acid, urea, and higher high-density

Mixed model for PWVz with interaction term between KRT modality, sex, and time since inclusion (yr)
(patients, n = 235; observations, n = 1368)

Effect	β	SE	P
Intercept	0.076	0.10	0.46
Effect of time since inclusion in:			
Girls pre-KRT (yr)	0.17	0.057	0.004
Girls on dialysis (yr)	0.20	0.073	0.006
Girls post-Tx (yr)	0.13	0.024	<0.0001
Boys pre-KRT (yr)	0.022	0.038	0.56
Boys on dialysis (yr)	0.084	0.053	0.11
Boys post-Tx (yr)	0.075	0.019	<0.0001
Non-CAKUT (ref.: CAKUT)	0.015	0.14	0.92

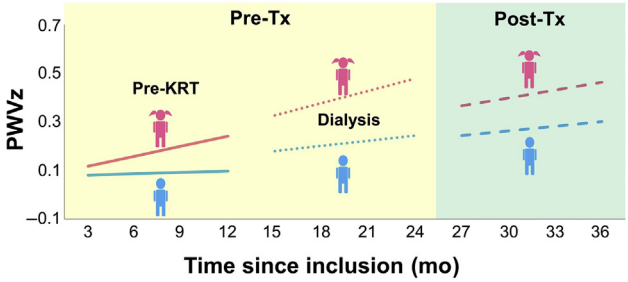


Figure 5 | Mixed model for pulse wave velocity *z* scores (PWVz) adjusted for the interaction term |time since inclusion*sex*kidney replacement therapy (KRT) modality (pre-KRT/dialysis/transplantation [Tx])|. The red shaded rows highlight the effect of time pre-KRT and dialysis in girls. The blue shaded rows highlight the effect of time pre-KRT and dialysis in boys (the table in the upper panel) and the different slopes of estimates according to the given linear mixed model differentiated by sex and KRT modality. The yellow area shows the time pre-Tx (including pre-KRT and dialysis), and the green area shows the time post-Tx. The pink lines show the PWVz slopes for girls and blue lines for boys. β, regression coefficient; CAKUT, congenital anomalies of kidney and urinary tract; Ref., reference; SE, standard error.

lipoprotein (HDL) than did boys. Table 1 summarizes patient characteristics at study inclusion, pre-KRT, at transplantation, and 1-year post-transplantation.

PWV and the effect of time and sex

Figure 3a shows the spline regression slope fitted for PWVz over the complete observation period. PWVz increased by 0.095 per year since inclusion ($P < 0.0001$), independent of kidney disease ($P = 0.64$). Figure 3b visualizes the sex-adjusted PWVz. The mixed model demonstrated that PWVz was 0.295 higher in girls ($P = 0.045$) than in boys.

We compared our study population with a cohort of healthy children with comparable height. Our cohort of healthy children demonstrated considerably lower PWVz (median −0.28; interquartile range −0.84 to 0.39) at study inclusion (Supplementary Table S4). PWVz in healthy children did not increase with time (PWVz −0.048/yr; $P = 0.27$) and did not differ between girls and boys (Figure 4).

As the spline regression of PWVz indicated an interaction between sex and time before transplantation, a mixed model

Table 2 | Mixed models for PWVz "pre-KRT": basic model adjusted for pre-KRT time, interaction term |pre-KRT time*sex|, sex, and kidney disease category; final model adjusted for the covariates included in the basic model, delta eGFR, interaction term |delta eGFR*sex|, diastolic BP *z* score, and LDL

	Basic model (AIC: 2221) 230 patients, 650 observations			Final model (AIC: 1340) 156 patients, 410 observations		
Variable	β	SE	P	β	SE	P
Intercept	0.14	0.14	0.33	−0.89	0.25	0.0005
Pre-KRT time	0.027	0.039	0.48	0.0003	0.048	0.99
Pre-KRT time*girls	**0.15**	**0.072**	**0.039**	**0.13**	**0.093**	**0.18**
Pre-KRT time*boys		Ref.			Ref.	
Girls (ref.: boys)	−0.018	0.22	0.94	0.028	0.28	0.92
Non-CAKUT (ref.: CAKUT)	0.042	0.20	0.83	−0.11	0.20	0.59
Delta eGFR		—		0.002	0.012	0.89
Delta eGFR/year*girls		—		**−0.040[a]**	**0.017**	**0.017**
Delta eGFR/year*boys		—			Ref.	
Diastolic BP *z* score		—		0.47	0.064	<0.0001
LDL		—		0.007	0.002	0.0001

AIC, Akaike information criterion; β, regression coefficient; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; eGFR, estimated glomerular filtration rate; KRT, kidney replacement therapy; LDL, low-density lipoprotein; PWVz, pulse wave velocity *z* score; Ref., reference; SE, standard error.
[a]A delta eGFR (a decline of eGFR) of −1 ml/min per 1.73 m^2 per year was associated with an increase of 0.04 PWVz in girls compared with boys.
Bold data indicate that the association between the |pre-KRT time*sex| and PWVz in the basic model is no longer significant after the inclusion of |delta eGFR/year*sex| in the final model.

for PWVz adjusted for the interaction term |time since inclusion*sex*KRT modality| was performed. Girls showed a PWVz increase of 0.17 pre-KRT ($P = 0.004$) and of 0.20 during dialysis ($P = 0.006$) per year since inclusion. These time effects during pre-KRT and dialysis were not present in boys (Figure 5, upper panel). The lower panel of Figure 5 illustrates the different slopes of PWVz for girls and boys, depending on KRT modality and highlights the greater progression of PWVz in girls pre-transplantation. This indicated the need of separating the analyses according to KRT, that is, "pre-KRT" and "post-transplantation." A separate analysis for dialysis was not possible because of the low number of observations (only 25 of 62 patients had ≥2 visits).

The effect of sex on PWV pre-KRT

We analyzed 230 patients. A higher PWVz increase of 0.15 per year was shown in girls than in boys ($P = 0.039$; Table 2, basic model). One hundred fifty-eight patients were included in the covariate screening for the final model (Supplementary Table S5).

The final model revealed that ΔeGFR was a strong predictor for PWVz in girls. An eGFR decline of −4 ml/min per 1.73 m^2 per year pre-KRT was associated with a higher PWVz of 0.16 in girls ($P = 0.017$) compared with boys. A higher diastolic BP *z* score and higher low-density lipoprotein (LDL) were associated with a higher PWVz in both sexes (Table 2,

Table 3 | Mixed models for PWVz "post-transplantation": basic model adjusted for post-transplantation time, sex, kidney disease category, and time on dialysis; prefinal model adjusted for covariates included in the basic model, PWVz at the last visit during pre-KRT, diastolic BP *z* score, and cholesterol; and final model adjusted for covariates included in the prefinal model, eGFR slope, interaction term |eGFR slope*sex|, and interaction term |time to transplantation*sex|

	Basic model (AIC: 1900) 199 patients, 613 observations			Prefinal model (AIC: 1620) 190 patients, 540 observations			Final model (AIC: 1606.4) 188 patients, 533 observations		
Variable	β	SE	P	β	SE	P	β	SE	P
Intercept	0.0001	0.14	0.99	−0.76	0.28	0.007	−0.85	0.31	0.006
Post-transplantation time	0.12	0.04	0.003	0.13	0.03	<0.0001	0.14	0.033	<0.0001
Post-transplantation time*girls	−0.008	0.06	0.89		—			—	
Post-transplantation time*boys		Ref.							
Girls (ref.: boys)	**0.44**	**0.19**	**0.024**	**0.38**	**0.14**	**0.010**	**−0.048**	**0.27**	**0.86**
Non-CAKUT (ref.: CAKUT)	−0.02	0.15	0.92	−0.011	0.14	0.94	0.032	0.14	0.82
Time on dialysis	0.25	0.09	0.006	0.19	0.09	0.032	0.19	0.085	0.024
PWVz at the last visit pre-KRT		—		0.22	0.04	<0.0001	0.20	0.040	<0.0001
Diastolic BP *z* score		—		0.36	0.06	<0.0001	0.36	0.055	<0.0001
Cholesterol		—		0.003	0.001	0.059	0.002	0.001	0.10
eGFR slope pre-KRT		—			—		0.012	0.014	0.42
eGFR slope pre-KRT*girls		—			—		**−0.054**[a]	**0.026**	**0.039**
eGFR slope pre-KRT*boys		—			—			Ref.	
Girls–longer time to transplantation[b]		—			—		**0.57**	**0.24**	**0.017**
Girls–shorter time to transplantation[b]		—			—			Ref.	
Boys–longer time to transplantation[b]		—			—		0.29	0.18	0.10
Boys–shorter time to transplantation[b]		—			—			Ref.	

AIC, Akaike information criterion; β, regression coefficient; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; eGFR, estimated glomerular filtration rate; KRT, kidney replacement therapy; PWVz, pulse wave velocity *z* score; Ref., reference; SE, standard error.
[a]An eGFR slope of −1 ml/min per 1.73 m^2 per year (a declining slope) at pre-KRT was associated with a higher PWVz of 0.054 in girls after transplantation as compared with boys.
[b]Transplanted >12 mo (longer time to transplantation) or ≤12 mo (shorter time to transplantation) after eGFR ≤ 30 ml/min per 1.73 m^2 or after dialysis start.
Bold data indicate that the association between sex and PWVz in the basic model is no longer significant after the inclusion of |eGFR slope pre-KRT*sex| and |time to transplantatation*sex| in the final model.

final model). Supplementary Figure S4 illustrates the sex difference on the effect estimate of the influencing factors on PWVz as a result from the respective model stratified by sex (Supplementary Table S6).

The effect of sex on PWV post-transplantation

We analyzed 199 patients. PWVz for girls was 0.44 higher than that for boys ($P = 0.02$). PWVz increased by 0.12 per year post-transplantation ($P = 0.003$) and by 0.25 per year on dialysis ($P = 0.006$) for both sexes. An interaction between time and sex was not detected (Table 3, basic model; Supplementary Table S7 shows the basic model separated by the transplantation type, i.e., preemptive and after prior dialysis).

We further analyzed 195 patients and screened for potential covariates using the basic model (Supplementary Table S8). PWVz increased by 0.13 per year post-transplantation ($P < 0.0001$) and by 0.19 per year on dialysis ($P = 0.03$). A 1-unit higher PWVz at the last pre-KRT visit was associated with a 0.22 increase in post-transplantation PWVz ($P < 0.0001$); furthermore, a 1-unit increase in diastolic BP *z* score was associated with a post-transplantation PWVz increase of 0.36 ($P < 0.0001$). Importantly, the association of female sex and higher PWVz persisted ($P = 0.01$; Table 3, prefinal model).

The previously observed effect of the eGFR decline on PWVz before KRT in girls was further elucidated by introducing 2 interaction terms: |sex*eGFR slope| and |sex*-time to transplantation| (Table 3, final model). Although the global sex effect disappeared ($P = 0.86$), the eGFR slope pre-KRT and a longer time to transplantation (>12 months after eGFR dropped to or below 30 ml/min per 1.73 m^2 or after dialysis start) revealed significant associations with higher PWVz in girls. An eGFR decline, for example, of −4 ml/min per 1.73 m^2 per year pre-KRT was associated with a PWVz increase of 0.22 after transplantation in girls ($P = 0.039$). A longer time to transplantation (>12 months) was associated with a higher PWVz of 0.57 in girls ($P = 0.017$; Table 3, final model). The associations of other contributing factors with PWVz persisted (Table 3, prefinal and final models). A sensitivity analysis for the PWVz post-transplantation model including individual eGFR slopes computed from the single mixed model is provided in Supplementary Table S9 showing similar findings. A description of PWVz and absolute PWVz at different time points is given in Supplementary Table S10.

Sex differences are independent of the underlying disease

As expected, the distribution of the underlying kidney disease differed between the sexes with a higher proportion of CAKUT in boys (63%) and non-CAKUT in girls (59%). We explored the potential effect of the difference in disease distribution. An additional model showed that PWVz did not differ between the categories of underlying diseases, as demonstrated by the corrected means showing no differences

between the categories of CAKUT (mean 0.39; 95% confidence interval 0.20–0.58) and non-CAKUT (mean 0.40; 95% confidence interval 0.19–0.60; Supplementary Figure S5A). We then explored the potential differences between the combinations of the sex and kidney disease category (girls–CAKUT, girls–non-CAKUT, boys–CAKUT, and boys–non-CAKUT). The mixed model adjusted for the interaction term |time since inclusion*category for sex and kidney disease| also showed that the development of PWVz did not differ between the 4 categories (Supplementary Figure S5B). This demonstrated that the higher PWVz in girls was independent of the kidney disease distribution.

DISCUSSION

Our study characterized the evolution of vascular stiffness in girls and boys with progressing CKD and subsequent transplantation. Girls with advanced CKD showed more pronounced arterial stiffening than did boys. This is in contrast to the physiological development as demonstrated in a cohort of healthy children. The faster progression of arterial stiffening in girls occurred before transplantation, reflecting a higher vulnerability of girls' vascular system toward the magnitude as well as the duration of the exposure to an impaired kidney function. Our key finding is that time acts differently on the cardiovascular burden between boys and girls during CKD. Importantly, this was independent of the underlying kidney disease.

A higher susceptibility of females with CKD to develop arterial stiffness in conjunction with kidney disease progression has not been described to date. Studies in adults so far have shown more severe arterial stiffness in women than in men[33] and an association between arterial stiffness and eGFR decline without sex differences.[34] A tendency toward faster decline in kidney function in girls than in boys has been demonstrated especially before puberty.[22] Although in the general population and in patients with CKD eGFR declines at a faster rate in males, a meta-analysis of a large number of postmenopausal women suggested a faster decline in women.[35–38] This could be explained by the absence of estrogens' nephroprotective effect.[39,40] In light of lower levels of sex hormones during puberty in children with CKD contributing to their well-known delayed puberty,[41] one could speculate that girls in our cohort are less protected by estrogen and kidney function decline should be similar between the sexes. However, this is not the case, suggesting sex hormones alone do not explain the differences seen.

Although the physiological development of PWV over time did not differ between girls and boys, we did see a significant difference in children with CKD. This difference occurred before transplantation and was associated with a longer time to transplantation, indicating that girls are particularly vulnerable during the final stages of CKD progression. Importantly, we can show that sex difference in PWV exists irrespective of the underlying kidney diseases, which are differently distributed between girls and boys.

Factors associated with bone and mineral metabolism (parathyroid hormone, vitamin D, and calcium, phosphorus, and their product itself)[42–44] are known contributors to the arterial stiffness increase during CKD progression. None of these factors explained the observed sex differences in PWV. In fact, serum calcium and parathyroid hormone were higher in boys. This, however, does not exclude the possibility that girls may develop a more pronounced PWV increase for a given calcium or parathyroid hormone level. Another important factor in mineral metabolism is fibroblast growth factor 23,[45] which was measured only at baseline in our cohort. Postmenopausal women without estrogen substitution show higher fibroblast growth factor 23 levels than do women with estrogen substitution or men.[46] It is conceivable that the fibroblast growth factor 23 pathway is more active in prepubertal girls or girls with an altered estrogen metabolism because of their uremic state. Similarly, osteoprotegerin, a cytokine that regulates bone resorption, is associated with cardiovascular events in patients with CKD and on hemodialysis.[47–49] In the Chronic Renal Insufficiency Cohort study, higher osteoprotegerin levels were associated with increased PWV and females had ∼10% higher osteoprotegerin levels than did male patients with CKD.[50]

Cholesterol and its subclasses LDL and HDL are known to influence PWV and predict cardiovascular risk.[51,52] In the general population, LDL is associated with an increased risk and HDL with a decreased risk. In children with CKD,[53,54] increased HDL promotes endothelial dysfunction and is associated with vascular damage, possibly because of a uremia-associated altered HDL functionality.[53] Our data showed higher HDL levels in girls, another factor that could explain the accelerated vascular damage in girls. This assumption is further supported by the performance of HDL in the model building process. HDL being in the causal pathway between the progression of CKD and PWV could explain why the introduction of HDL together with the interaction term "ΔeGFR/year*girls" did not reveal a significant result. LDL, however, showed an association with higher PWV in both sexes, but did not explain the sex differences. Our data highlight the importance of both cholesterol subclasses in pediatric patients with CKD and the need for early preventive strategies, especially because in adults with CKD and on dialysis, LDL lowering with atorvastatin and ezetimibe was successful in reducing atherosclerotic events.[55]

PWV increased further after transplantation. In previous studies, PWV tended to stabilize or slightly decrease during the first year after kidney transplantation[18,56–58] but longer observations in adults revealed an increase in PWV.[59] The increase in PWV post-transplantation with time likely reflects an ongoing damage even after transplantation in addition to the "preexisting" burden from the time pre-KRT. This implies the need of a better cardiovascular monitoring and cardiovascular disease prevention, especially before the onset of KRT. Our data also highlight the clinical importance of an even faster access for girls to transplantation, especially in

light of studies showing a slower access to preemptive transplantation.[6,22] A longer time on dialysis was associated with increased PWV, which is in line with our previous finding showing the advantage of preemptive transplantation compared with dialysis.[18] The observed association between higher BP and higher PWV in both sexes is in accordance with previous findings in the general pediatric population,[60,61] in various patient groups,[15,17,62] and after kidney transplantation.[18,63] Uncontrolled or untreated hypertension is present in 30% to 40% of pediatric kidney allograft recipients.[25,63] Notably, we previously showed that girls are more susceptible to cyclosporine A–associated hypertension than boys.[25]

Patients were allocated to dialysis or preemptive transplantation on the basis of clinical decisions. Bias by indication was overcome by adjusting for all factors that potentially influence the treatment decision (e.g., kidney disease, center, time, and kidney function parameter). As not all patients' data were available for the final model because of the timing of examinations, there was a potential selection bias. However, as there were no differences in PWV or sex distribution between patients who were or were not included in the final models (pre-KRT: inclusion, $n = 156$; noninclusion, $n = 74$ and post-transplantation: inclusion, $n = 187$; noninclusion, $n = 43$), this should have not influenced the results of the comparison between sexes. The majority of our study population is Caucasian (88%) and so was the population from which PWV reference values were calculated.[13] This might limit the generalizability of our finding.

Conclusion

The observed higher susceptibility of girls for cardiovascular organ damage in conjunction with kidney disease progression highlights the importance of a closer attention to cardiovascular and kidney function parameters early in the disease course in female patients. Importantly, girls are more vulnerable toward eGFR decline and when exposed to a longer waiting time to transplantation. Early interventions and a faster access of girls to transplantation are crucial to tackle the sex differences in cardiovascular and mortality risk. Strict BP control and management of dyslipidemia are of importance for both sexes.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Assessment of time variables.
Figure S2. Assessment of changes in estimated glomerular filtration rate (eGFR) during pre–kidney replacement therapy (KRT).
Figure S3. Number of observations over time since inclusion, differentiated by the modality of kidney replacement therapy at each visit since the inclusion.
Figure S4. Effect estimates and 95% confidence interval for factors associated with PWV *z* scores (PWVz) during pre–kidney replacement therapy (KRT).
Figure S5. Additional mixed model for with PWV *z* scores (PWVz) adjusted for kidney underlying disease category.
Table S1. Sub-classifications of primary kidney diseases.
Table S2. Correlation between Casual blood pressure (BP) and ambulatory BP measurement (ABPM).
Table S3. Matrix of missing data for each final model of pre–kidney replacement therapy (KRT; **A**) and post-transplantation (**B**).
Table S4. Comparison of baseline characteristics between the study population (at first visit pre–kidney replacement therapy [KRT]) and healthy children cohort (at study inclusion).
Table S5. Covariates screening based on basic model for pulse wave velocity *z* scores (PWVz) "pre–kidney replacement therapy (KRT)".
Table S6. Final models for pulse wave velocity *z* scores (PWVz) "pre–kidney replacement therapy (KRT)" separated by sex.
Table S7. Basic mixed models for pulse wave velocity *z* scores (PWVz) "post-transplantation" separated by transplantation type, i.e., preemptive transplantation and transplantation after prior dialysis.
Table S8. Covariates screening based on basic model for pulse wave velocity *z* scores (PWVz) "post-transplantation".
Table S9. Sensitivity analysis for the effect of the pre–kidney replacement therapy (KRT) estimated glomerular filtration rate (eGFR) slopes calculated from single mixed model on post-transplantation pulse wave velocity *z* scores (PWVz).
Table S10. Pulse wave velocity *z* scores (PWVz) and pulse wave velocity (PWV; m/s) at different time points for all patients and differentiated by sex.

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