Insights from the 4C-T Study suggest increased cardiovascular burden in girls with end stage kidney disease before and after kidney transplantation.

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Insights from the 4C-T Study suggest increased cardiovascular burden in girls with end stage kidney disease before and after kidney transplantation.

**HYPOTHESIS**
Arterial stiffness differs between sexes in children with chronic kidney disease (CKD) and transplantation (Tx).

**STUDY DESIGN**
4C-T STUDY
Cardiovascular Comorbidity in Children with Chronic Kidney Disease

Prospective multicenter study
704 children with CKD

4C-T cohort:
235 children with CKD and transplantation
n=80
n=155
- Annual CV assessments
- Total visits n=1368

Endpoint: PWV z-score

**AIMS**
Longitudinal assessment of PWV, a measure of vascular stiffness and predictor cardiovascular (CV) mortality, to determine sex differences and potential contributing factors.

**RESULTS**
Factors impacting faster PWV increase in girls:
- eGFR decline
- waiting time

PWV increase
Higher PWV in girls persists after Tx.

**CONCLUSION**
- Girls are more susceptible towards the development of arterial stiffening.
- Susceptibility in girls is associated with magnitude and duration of impaired kidney function.
- This finding might contribute to the higher mortality risk shown for girls with CKD.

Sugianto, 2021
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Abstract

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 4ml/min/1.73m² 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/1.73m² 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.
Introduction

Overall childhood mortality rates are declining\(^1\). In the general population, boys show higher mortality in most regions of the world\(^2,3\) largely due to more accidents\(^1\), prematurity, respiratory distress during infancy\(^2,4\) and sepsis occurring post-puberty\(^3\). 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 (ESKD) is more than 30 times higher than the general population\(^5\). Data from USRDS on 14,024 children on kidney replacement therapy (KRT) suggest a higher mortality risk in girls (HR: 1.36, 95% CI: 1.25-1.50) due to their greater risk for cardiovascular death\(^6\). Despite declining overall mortality rates in children with functioning grafts, the proportion of cardiovascular mortality remains unchanged and is about 20% higher in girls\(^7\).

Cardiovascular events as the most common causes of death in children with ESKD 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 greater than that related to non-functioning 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 CKD\(^12\). Aortic PWV can be measured non-invasively and reproducibly in children\(^13,14\). Higher PWV was demonstrated in children with CKD even after transplantation compared to their healthy peers\(^15-18\).
Findings in adults indicate that the global survival advantage of females is lost in ESKD\textsuperscript{19}, a phenomenon that is not sufficiently explained by disparities of access to transplantation due to higher levels of panel reactive antibodies in women\textsuperscript{20} and pregnancy-induced-incompatibility\textsuperscript{21}. In the pediatric population, girls are less likely to undergo pre-emptive transplantation\textsuperscript{6, 22} and show poorer graft survival than boys, the latter being partly explained by receiving male donor organs\textsuperscript{23, 24}. Our own data indicated a greater susceptibility of girls for cyclosporin A-associated hypertension\textsuperscript{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 ESKD who underwent transplantation either pre-emptively or after prior dialysis, to uncover potential sex differences.

**Methods**

**Study design, setting, participants**

The 4C-T (Cardiovascular Comorbidity in Children with Chronic Kidney Disease - Transplantation) sub-study is part of the 4C study\textsuperscript{26}, a prospective observational study. Seven-hundreds-four pediatric CKD patients (age 6-17 years) with an eGFR below 60ml/min/1.73m\textsuperscript{2} not yet receiving KRT were enrolled between 2009 and 2011. Ethical aspects and details of the data acquisition were described previously\textsuperscript{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, Würzburg, Germany) as previously described\textsuperscript{13, 14}. Every 6 months blood and urine samples, anthropometrics, casual blood pressure (BP), medical history updates were
obtained per standardized protocol. Laboratory measurements were performed centrally. eGFR was calculated using the Schwartz formula\textsuperscript{27}.

**Variables**

Sex-and height-adjusted standardized scores (z-scores) were calculated for PWV\textsuperscript{13} as the primary endpoint.

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

As ambulatory blood pressure measurements (ABPM) were only available in a subgroup of patients, we provide data for the correlation between the BP and ABPM in the Supplemental Table S2.

**Time Variable**

Time (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≤30 to transplantation and time on dialysis (see Supplemental Figure S1 for more details).

**Healthy Control Cohort**

Longitudinal PWV measurements in 307 (girls n=145) healthy children from 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\textsuperscript{30} with two repetitive PWV measurements with an interval of 12.7±3.3 months between 2017 and 2018.

**Analyses Steps**

The analyses for PWV z-score (PWVz) were performed in three analysis steps: (1) All data comprising the whole observation time. Then divided into two separate analyses according to transplantation: (2) “pre-KRT” and (3) “post-transplantation” (Figure 1).

**Step 1: All Data**

We included patients with at least one 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 following: (1) adjusted for time since inclusion and kidney disease category to understand the development of PWVz over time; (2) adjusted for sex, time since inclusion and kidney disease category to understand the development of PWVz over time depending on sex; (3) adjusted for the 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 the KRT start was included to study the development of PWVz during CKD progression. Patients with at least one visit pre-KRT was 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 prior to KRT, we screened covariates which are assumed relevant for PWVz using the above pre-defined basic model. We included patients with at least two 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 highly correlated to each other (BP values, lipid levels) were grouped. If two or more covariates from the same group were eligible, the one with the better model fit (lower Akaike Information Criterion, AIC) was selected.

To assess CKD progression eGFR decline was calculated. Delta eGFR (ΔeGFR) for each patient i at visit v, was calculated as the difference between eGFR at visit (v) and the previous visit (v-1) divided by the time (T) interval (years) between both visits ($\Delta \text{eGFR} = \frac{\text{eGFR}_i(v) - \text{eGFR}_i(v-1)}{T_i(v) - T_i(v-1)}$) (Supplemental Figure S2). In case of a missing eGFR value between two visits, ΔeGFR was interpolated.

**Step 3: Post-transplantation Data**

Patients with at least one 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 eGFR slope pre-KRT (n=195, Figure 2). Covariates were then screened using the above pre-defined basic model to identify 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 two or more covariates were eligible but highly correlated to each other, the one with the lower AIC 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, B) of the fixed effect of time pre-KRT (years) for each patient \(i\) from linear regression of eGFR. \[ eGFR_{i} = \text{intercept}_i + B_i(\text{time pre-KRT in years}) \] (Supplemental Figure S2). eGFR slopes were defined as 0 in 20 cases undergoing dialysis and 17 with pre-emptive transplantation. In all cases final CKD stages were reached and KRT was initiated before a second measurement could be performed. Three children with only one 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. In order 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 ≤30mL/min/1.73m² (pre-emptive) 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 one visit were included (n=235). Two mixed models for PWVz were performed. (1) Mixed model adjusted for the interaction term |time since inclusion*kidney disease category| to understand whether the PWVz development differs between CAKUT and non-CAKUT patients. Corrected means and the 95% confidence intervals of PWVz adjusted for the respective models were calculated for CAKUT and non-CAKUT groups. (2) Mixed model adjusted for the interaction term |time since inclusion*sex and kidney disease category (girls-CAKUT, girls-non-CAKUT, boys-CAKUT, boys-non-CAKUT)| to understand how sex influences the PWVz course in each kidney disease category.

**General statistical analysis**

Data are given as median and IQR, 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. Supplemental 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 Supplemental Tables S3a and S3b. 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 repeated effect to model within-subject variation\textsuperscript{31}. Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA). This manuscript was written according to the STROBE guidelines\textsuperscript{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 (pre-emptive n=150, with prior dialysis n=85) were included. Of those 196 had observations prior to and after transplantation, 36 only before 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 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 HDL than boys. Table 1a-e describes patient characteristics at study inclusion, pre-KRT, at transplantation and one 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) compared to boys.

We compared our study population to a cohort of healthy children with comparable height. Our cohort of healthy children demonstrated considerably lower PWVz (median: -0.28, IQR: -0.84 to 0.39) at study inclusion (Suppl. Table S4). PWVz in healthy children did not increase with time (PWVz -0.048/year, 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 for PWVz adjusted for an 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, i.e. “pre-KRT” and “post-transplantation”. A separate analysis for dialysis was not possible due to the low number of observations (only 25 of 62 patients had two or more 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 compared to boys (p=0.039; Table 2, a: basic model). One-hundred-fifty-eight patients were included in the covariate screening for final model (Suppl. Table S5).

The final model revealed that ∆eGFR was a strong predictor for PWVz in girls. An eGFR decline of -4ml/min/1.73m² per year pre-KRT was associated with a higher PWVz of 0.16 in girls (p=0.017) compared to boys. A Higher diastolic BP z-score and higher LDL were associated with a higher PWVz in both sexes (Table 2, b: final model). Supplemental 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 (Suppl. Table S6).

**The effect of sex on PWV post-transplantation**

We analyzed 199 patients. PWVz for girls was 0.44 higher when compared to 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, a: basic model; Suppl. Table S7 shows the basic model separated by the transplantation type i.e. pre-emptive and after prior dialysis).

We further analyzed 195 patients and screened for potential covariates using the basic model (Suppl. 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 of 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, b: pre-final model).

The previously observed effect of the eGFR decline on PWVz prior to KRT in girls was further elucidated by introducing two interaction terms: |sex*eGFR slope| and |sex*time to transplantation| (Table 3, c: final model). While 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/1.73m² or after dialysis start) revealed significant associations with higher PWVz in girls. An eGFR decline, for example, of -4ml/min/1.73m²/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, c: final model). The associations of other contributing factors with PWVz persisted (Table 3, model b and c). A sensitivity analysis for PWVz post-transplantation model including individual eGFR slopes computed from single mixed model is provided in the Supplemental Table S9 showing similar findings. A description of PWVz and absolute PWVz at different time points is given in the Supplemental Table S10.

**Sex differences are independent of underlying disease**
As expected the distribution of underlying kidney disease differed between the sexes with a higher proportion of CAKUT in boys (63%) and non-CAKUT in girls (59%). We explored potential effect of the difference in diseases distribution. An additional model showed that the 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, CI=0.20-0.58) and non-CAKUT (mean=0.40, CI=0.19-0.60) (Suppl. 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, 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 four categories (Suppl. 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 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 prior to transplantation reflecting a greater vulnerability of girls’ vascular system towards the magnitude as well as the duration of the exposure to an impaired renal 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 greater susceptibility of females with CKD to develop arterial stiffness in conjunction with renal disease progression has not been described to date. Studies
in adults so far have shown more severe arterial stiffness in women compared to men\textsuperscript{33} and an association between arterial stiffness and eGFR decline without sex differences\textsuperscript{34}. A tendency towards faster decline in renal function in girls compared to boys has been demonstrated especially prior to puberty\textsuperscript{22}. While in the general population and in 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\textsuperscript{35-38}. This could be explained by the absence of estrogens’ nephro-protective effect\textsuperscript{39, 40}. In light of lower levels of sex hormones during puberty in children with CKD contributing to their well-known delayed puberty\textsuperscript{41}, one could speculate that girls in our cohort are less protected by estrogen and renal function decline should be similar between the sexes. However, this is not the case, suggesting sex hormones alone do not explain the differences seen.

While 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 prior to transplantation and was associated with 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 (PTH, vitamin D and calcium, phosphorus and their product itself)\textsuperscript{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 PTH 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 PTH level. Another important factor
in mineral metabolism is FGF23\textsuperscript{45}, which was measured only at baseline in our cohort. Postmenopausal women without estrogen substitution show higher FGF23 levels than women with estrogen substitution or men\textsuperscript{46}. It is conceivable that the FGF23 pathway is more active in pre-pubertal girls or girls with an altered estrogen metabolism due to their uremic state. Similarly, osteoprotegerin (OPG), a cytokine that regulates bone resorption, is associated with cardiovascular events in CKD and hemodialysis patients\textsuperscript{47-49}. In the CRIC study higher OPG levels were associated with an increased PWV and females had about 10 percent higher OPG levels than male CKD patients\textsuperscript{50},

Cholesterol and its subclasses LDL and HDL are known to influence PWV and predict cardiovascular risk \textsuperscript{51, 52}. In the general population, LDL is associated with an increased risk and HDL with decreased risk. In children with CKD \textsuperscript{53, 54}, increased HDL promotes endothelial dysfunction and is associated with vascular damage possibly due to a uremia-associated altered HDL functionality \textsuperscript{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 progression of CKD and PWV could explain why the introduction of HDL together with the interaction term “\text{ΔeGFR/year} \times \text{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 since in adults with CKD and on dialysis, LDL lowering with atorvastatin and ezetimibe was successful in reducing atherosclerotic events\textsuperscript{55}.
PWV increased further after transplantation. In previous studies PWV tended to stabilize or slightly decrease during the first year after kidney transplantation\textsuperscript{18, 56-58}, but longer observation in adults revealed an increase of PWV\textsuperscript{59}. The increase of PWV post-transplantation with time likely reflects an ongoing damage even after transplantation in addition to the “pre-existing” 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 highlights the clinical importance of an even faster access for girls to transplantation, especially in light of studies showing a slower access to pre-emptive transplantation\textsuperscript{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 to dialysis\textsuperscript{18}. The observed association between higher BP and higher PWV in both sexes is in accordance with previous findings in the general pediatric population\textsuperscript{60, 61}, in various patient groups\textsuperscript{15, 17, 62} and after kidney transplantation\textsuperscript{18, 63}. Uncontrolled or untreated hypertension is present in 30-40\% of pediatric kidney allograft recipients\textsuperscript{25, 63}. Notably, we previously showed that girls are more susceptible to cyclosporine A-associated hypertension than boys\textsuperscript{25}.

Patients were allocated to dialysis or preemptive transplantation based on 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 was available for the final model due to the timing of examinations, there was a potential selection bias. However, as there were no differences in PWV or sex distribution between patients that were or were not included in the final models (pre-KRT: inclusion n=156, non-inclusion n=74; post-transplantation: inclusion n=187, non-inclusion 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\textsuperscript{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**

None.

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**Supplementary Material**

1. Supplemental Figure S1. Assessment of time variables.
2. Supplemental Figure S2. Assessment of changes in eGFR during pre-KRT.
3. Supplemental Figure S3. Number of observations over time since inclusion, differentiated by the modality of kidney replacement therapy at each visit since the inclusion.
4. Supplemental Figure S4. Effect estimates and 95% confidence interval for factors associated with PWVz during pre-KRT.
5. Supplemental Figure S5. Additional mixed model for PWVz adjusted for kidney underlying disease category.
6. Supplemental Table S1. Sub-classifications of primary kidney diseases.
7. Supplemental Table S2. Correlation between Casual BP and ABPM.
8. Supplemental Table S3. Matrix of missing data for each final model of pre-KRT (a) and post-transplantation (b).
9. Supplemental Table S4. Comparison of baseline characteristics between the study population (at first pre-KRT visit pre-KRT) and healthy children cohort (at study inclusion).
10. Supplemental Table S5. Covariates screening based on basic model for PWVz “pre-KRT”.
11. Supplemental Table S6. Final models for PWVz “pre-KRT” separated by sex.
12. Supplemental Table S7. Basic mixed models for PWVz “post-transplantation” separated by transplantation type, i.e. pre-emptive transplantation and transplantation after prior dialysis.
13. Supplemental Table S8. Covariates screening based on basic model for PWVz “post-transplantation”.
14. Supplemental Table S9. Sensitivity analysis for the effect of the pre-KRT eGFR slopes calculated from single mixed model on post-transplantation PWVz.
15. Supplemental Table S10. PWVz and PWV (m/s) at different time points for all patients and differentiated by sex.
Table 1. Patients’ characteristics at inclusion, at last visit pre-KRT and at one year post-transplantation.

| 1a: Disease and transplant modality | Girls (n=80) | Boys (n=155) |
|----------------------------------|-------------|--------------|
| Underlying disease               |             |              |
| CAKUT, n (%)                     | 33 (41%)    | 98 (63%)     |
| Non-CAKUT, n (%)                 | 47 (59%)    | 57 (37%)     |
| Transplantation                  |             |              |
| Pre-emptive, n (%)               |             |              |
| With prior dialysis, n (%)       |             |              |

| 1b: At inclusion | Girls (n=78) | Boys (n=152) |
|-----------------|-------------|--------------|
| N               | Median (IQR) | N            | Median (IQR) |
| Age             | 78          | 12.2 (9.24, 14.3) | 152          | 11.6 (8.98, 14.2) |
| Height (cm)     | 78          | 139 (126, 151) | 152          | 138 (124, 155)     |
| Height z-score  | 78          | -1.45 (-2.09,-0.32) | 152         | -1.16 (-2.03,-0.19) |
| BMI (kg/m2)     | 78          | 16.6 (15.3, 18.4) | 152          | 17.5 (15.7, 19.9)   |
| BMI z-score     | 78          | -0.62 (-1.08,-0.18) | 152         | -0.08 (-0.79, 0.84) |
| Systolic BP (mmHg)* | 78          | 110 (100, 114) | 152          | 114 (106, 123)    |
| Systolic BP z-score* | 78          | 0.4 (-0.08, 1.29) | 152        | 1.09 (0.38, 1.74)   |
| Diastolic BP (mmHg) | 78          | 65.5 (60.0, 78.0) | 152         | 69.0 (62.0, 78.0)  |
| Diastolic BP z-score* | 78          | 0.41 (-0.16, 1.30) | 152       | 0.72 (0.18, 1.31) |
| eGFR (mL/min/1.73m²) | 78          | 21.5 (15.4, 30.7) | 152         | 19.3 (14.6, 28.0) |

| 1c: At last visit pre-KRT | Girls (n=78) | Boys (n=152) |
|--------------------------|-------------|--------------|
| N                        | Median (IQR) | N            | Median (IQR) |
| Age                      | 78          | 13.5 (11.1, 15.8) | 152          | 13.7 (11.9, 15.7) |
| Time since inclusion (years) | 78          | 1.2 (0, 3.1) | 152          | 1.9 (0, 3.1) |
| Time from last visit during CKD to KRT start (years) | 78 | 0.5 (0.3, 0.8) | 152 | 0.6 (0.2, 0.8) |
| Height (cm)*             | 78          | 149 (135, 157) | 152          | 152 (137, 165) |
| Height z-score           | 78          | -1.23 (-2.12,-0.43) | 152         | -0.96 (-2.14,-0.22) |
| BMI (kg/m2)              | 78          | 18.0 (16.1, 19.1) | 152          | 18.1 (16.6, 20.5) |
| BMI z-score              | 78          | -0.57 (-1.21, 0.28) | 152         | -0.25 (-0.99, 0.60) |
| Systolic BP (mmHg)*      | 78          | 112 (105, 119) | 152          | 120 (109, 128)    |
| Systolic BP z-score*     | 78          | 0.55 (-0.12, 1.39) | 152        | 1.09 (0.26, 1.90)   |
| Diastolic BP (mmHg)      | 78          | 70.0 (63.0, 80.0) | 152         | 70.0 (65.0, 80.0)  |
| Diastolic BP z-score*    | 78          | 0.46 (-0.01, 1.48) | 152       | 0.81 (0.13, 1.43) |
| eGFR (mL/min/1.73m²)     | 78          | 13.9 (11.5, 16.4) | 148         | 13.3 (10.9, 17.0) |
| Cholesterol (mg/dL)      | 77          | 185 (165, 219) | 150          | 169 (142, 200)    |
| HDL (mg/dL)*             | 77          | 54.0 (44.0, 64.0) | 150          | 43.9 (36.5, 53.0) |
| LDL (mg/dL)              | 77          | 99.0 (81.0, 123) | 150          | 88.6 (69.5, 122.3) |
| Hemoglobin (g/dL)*       | 77          | 10.6 (9.80, 11.8) | 145         | 11.2 (10.3, 12.1) |
| Ferritin (µg/L)          | 72          | 99.8 (52.3, 181) | 134         | 101.5 (51.0, 204) |
| Sodium (mmol/L)*         | 77          | 140 (136, 142) | 149          | 140 (137, 142)    |
Table 1. /continued

|                   | Girls (n=78) | Boys (n=152) |
|-------------------|-------------|--------------|
| **1c: At last visit pre-KRT** |             |              |
| Potassium (mmol/L) | 4.40 (4.00, 4.80) | 4.50 (4.10, 4.98) |
| Calcium (mmol/L)*  | 2.30 (2.15, 2.48) | 2.38 (2.20, 2.52) |
| Phosphorus (mmol/L)| 1.61 (2.41, 2.92) | 1.67 (1.51, 1.97) |
| Bicarbonate (mmol/L)| 21.0 (19.0, 23.0) | 21.0 (19.0, 23.0) |
| Parathyroid hormone (pmol/L) | 16.3 (7.50, 38.9) | 24.9 (13.5, 40.9) |
| Uric acid (mg/dL)* | 6.12 (5.30, 7.31) | 7.17 (6.18, 8.31) |
| Urea (mg/dL)*      | 60.7 (43.2, 79.5) | 73.0 (56.5, 116)  |
| Antihypertensive use, n (%) | 54 of 78 (69%) | 104 of 152 (68%) |
| RAAS antagonists, n (%) | 37 (47%) | 54 (36%) |
| CCB, n (%)          | 29 (37%) | 57 (38%) |
| β blockers, n (%)   | 8 (10%) | 28 (18%) |
| Peripheral α blockers, n (%) | 5 (6%) | 8 (5%) |
| Central α-blockers, n (%) | 0 | 1 (1%) |
| Loop diuretics, n (%) | 6 (8%) | 13 (9%) |
| Thiazide diuretics, n (%) | 4 (5%) | 2 (1%) |

**1d: Transplantation**

|                   | Girls (n=71) | Boys (n=127) |
|-------------------|-------------|--------------|
| Age at transplantation | 15.2 (12.3, 17.0) | 14.7 (12.6, 16.3) |
| Time from eGFR≤30 to tx (years) | 2.3 (1.5, 3.8) | 2.5 (1.3, 4.0) |
| Time since inclusion to tx (years) | 2.4 (1.4, 4.4) | 2.6 (1.4, 4.1) |
| Time on dialysis (years) | 1.1 (0.7, 2.1) | 1.2 (0.9, 1.6) |
| Transplanted>1 year after eGFR≤30 or after dialysis start, n (%) | 42 (59%) | 82 (65%) |
| Transplanted≤1 year after eGFR≤30 or after dialysis start, n (%) | 29 (41%) | 45 (35%) |

**1e: At 1 year post-transplantation**

|                   | Girls (n=53) | Boys (n=103) |
|-------------------|-------------|--------------|
| Age               | 15.7 (13.3, 18.2) | 15.3 (13.1, 17.3) |
| Time since inclusion (years) | 3.1 (2.2, 5.1) | 3.2 (2.1, 5.0) |
| Time post-transplantation (years) | 0.9 (0.7, 1.1) | 0.9 (0.8, 1.2) |
| Height (cm)*      | 153 (143, 160) | 160 (147, 168) |
| Height z-score    | -1.00 (-1.98, -0.03) | -1.02 (-2.13, -0.37) |
| BMI (kg/m2)       | 20.3 (17.6, 22.5) | 21.1 (18.0, 23.5) |
| BMI z-score       | -0.12 (-0.93, 0.95) | 0.37 (-0.50, 1.11) |
| Systolic BP (mmHg)* | 115 (107, 121) | 120 (112, 130) |
| Systolic BP z-score | 0.73 (-0.02, 1.51) | 1.04 (0.21, 1.76) |
| Diastolic BP (mmHg) | 71 (63.0, 77) | 72 (65, 79) |
| Diastolic BP z-score | 0.54 (-0.14, 1.14) | 0.71 (0.13, 1.33) |
| eGFR (mL/min/1.73m²)* | 68.4 (50.3, 82.3) | 59.4 (46.7, 74.0) |
| Tacrolimus trough level (µg/L) | 5.00 (4.00, 7.00) | 6.00 (5.00, 8.00) |
| Cyclosporin A trough level (µg/L) | 100 (67.0, 129) | 104 (85.5, 124) |
Table 1. /continued

|                  | Girls (n=53) | Boys (n=103) |
|------------------|--------------|--------------|
|                  | N            | Median (IQR) | N            | Median (IQR) |
| Steroid dosage (mg/day) | 46           | 5.00 (3.00, 5.00) | 79           | 5.00 (4.00, 7.50) |
| Antihypertensive use, n (%) | 32 of 53 (60%) | 64 of 103 (62%) |
| RAAS-antagonists, n (%) | 9 (17%)       | 16 (15%)     |
| CCB, n (%)        | 25 (47%)     | 47 (46%)     |
| β-blockers, n (%) | 7 (13%)       | 29 (28%)     |
| Peripheral α-blockers, n (%) | 0          | 4 (4%)       |
| Central α-blockers, n (%) | 1 (2%)      | 1 (1%)       |
| Loop diuretics, n (%) | 1 (2%)      | 6 (6%)       |
| Thiazide diuretics, n (%) | 0           | 4 (4%)       |
| Immunosuppresive use, n (%) | 53 of 53 (100%) | 103 of 103 (100%) |
| Steroid, n (%)    | 46 (87%)     | 79 (77%)     |
| CNI, n (%)        | 52 (98%)     | 101 (98%)    |
| MMF, n (%)        | 47 (87%)     | 82 (80%)     |
| mTOR, n (%)       | 6 (11%)      | 13 (13%)     |

*, p-value of <0.05.

**Abbreviations:** BMI, body mass index; BP, blood pressure; CAKUT, congenital anomalies of kidney and urinary tract; CCB, Calcium Channel Blocker; 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.
Table 2. Mixed models for PWVz “pre-KRT”: (a) basic model adjusted for pre-KRT time, interaction term |pre-KRT time*sex|, sex and kidney disease category; (b) final model adjusted for the covariates included in the basic model, delta eGFR, interaction term |delta eGFR*sex|, diastolic BP z-score and LDL.

| Variables                           | a: Basic model (AIC:2221) | b: Final model (AIC:1340) |
|-------------------------------------|----------------------------|---------------------------|
|                                     | 230 patients, 650 observations | 156 patients, 410 observations |
| Intercept                           | β: 0.14, SE: 0.14, p: 0.33 | β: -0.89, SE: 0.25, p: 0.0005 |
| Pre-KRT time (years)                | β: 0.027, SE: 0.039, p: 0.48 | β: 0.0003, SE: 0.048, p: 0.99 |
| Pre-KRT time*girls                  | β: 0.15, SE: 0.072, p: 0.039 | β: 0.13, SE: 0.093, p: 0.18 |
| Pre-KRT time*boys                   | Ref.                       | Ref.                      |
| Girls (ref: boys)                   | β: -0.018, SE: 0.22, p: 0.94 | β: 0.028, SE: 0.28, p: 0.92 |
| Non-CAKUT (ref: CAKUT)              | β: 0.042, SE: 0.20, p: 0.83 | β: -0.11, SE: 0.20, p: 0.59 |
| Delta eGFR (ml/min/1.73m²/year)     | β: - Ref.                  | β: 0.002, SE: 0.012, p: 0.89 |
| Delta eGFR/year*girls               | β: - Ref.                  | β: -0.040<sup>a</sup>, SE: 0.017, p: 0.017 |
| Delta eGFR / year *boys             | β: - Ref.                  | β: Ref.                   |
| Diastolic BP z-score                | β: 0.47, SE: 0.064, p: <0.0001 | β: 0.007, SE: 0.002, p: 0.0001 |
| LDL (mg/dL)                         | β: - Ref.                  | β: Ref.                   |

<sup>a</sup>, explanatory comment: a delta eGFR (a decline of eGFR) of -1 ml/min/1.73m²/year was associated with an increase of 0.04 PWVz in girls compared to boys.

Abbreviations: 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; p, p-value ; SE, standard error.
Table 3. Mixed models for PWVz “post-transplantation”: (a) basic model adjusted for post-transplantation time, sex, kidney disease category and time on dialysis; (b) pre-final model adjusted for covariates included in basic model, PWVz at last visit during pre-KRT, diastolic BP z-score and cholesterol and (c) final model adjusted for covariates included in pre-final model, eGFR slope, interaction term |eGFR slope*sex| and interaction term |time to transplantation*sex|.

| Variables | a: Basic model (AIC:1900) | b: Pre-final model (AIC: 1620) | c: Final model (AIC:1606.4) |
|-----------|--------------------------|--------------------------------|-----------------------------|
|           | Intercept                 | Post-transplantation time (years) |                                |                                |                                |
|           | β  SE p                   | β  SE p                        | β  SE p                      |                                |                                |
| Intercept | 0.0001 0.14 0.99         | 0.12 0.04 0.003                | 0.13 0.03 <0.0001            | 0.14 0.033 <0.0001             |
| 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 (years) | 0.25 0.09 0.006 | 0.19 0.09 0.032 | 0.19 0.085 0.024 |
| PWVz at 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 (mg/dL) | - | 0.003 0.001 0.059 | 0.002 0.001 0.10 |
| eGFR slope pre-KRT (ml/min/1.73m2/year) | - | - | 0.012 0.014 0.42 |
| eGFR slope pre-KRT*girls | - | - | -0.054a 0.026 0.039 |
| eGFR slope pre-KRT*boys | - | - | Ref. |
| Girls-longer time to transplantationb | - | - | 0.57 0.24 0.017 |
| Girls-shorter time to transplantationb | - | - | Ref. |
| Boys-longer time to transplantationb | - | - | 0.29 0.18 0.10 |
| Boys-shorter time to transplantationb | - | - | Ref. |

a, explanatory comment: an eGFR slope of -1 ml/min/1.73m2/year (a declining slope) at pre-KRT was associated with a higher PWVz of 0.054 in girls after transplantation compared to boys.
b, transplanted >12 months (longer time to transplantation) or ≤12 months (shorter time to transplantation) after eGFR≤30 or after dialysis start.

Abbreviations: 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; p, p-value; PWVz, pulse wave velocity z-score; SE, standard error.
**Figure Legends**

**Figure 1.** Process flow of the analyses for PWVz. Step 1, as shown in box, describes the analyses for the complete observation period including pre-KRT, dialysis and post-transplantation. Step 2, as marked in the light grey area, describes the analyses flow for PWVz during pre-KRT. Step 3, as marked in the dark grey area, describes the analyses flow for PWVz post-transplantation.

*Abbreviations: CAKUT, congenital anomalies of kidney and urinary tract; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; KRT, kidney replacement therapy; PWVz, pulse wave velocity z-score.*

**Figure 2.** Inclusion flow chart of the study population.

*Abbreviations: CKD, chronic kidney disease; KRT, kidney replacement therapy; PWV, pulse wave velocity; Tx, transplantation.*

**Figure 3.** Analyses of 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 at lower panel). (b) Spline regression fit and 95% confidence interval differentiated by sex showing red line for girls and blue line for boys (upper panel) and mixed model for PWVz adjusted for time since inclusion, kidney disease and sex (the table at lower panel).

*Abbreviations: β, regression coefficient; CAKUT, congenital anomalies of kidney and urinary tract; KRT, kidney replacement therapy; PWVz, pulse wave velocity z-score; SE, standard error; p, p-value.*
Figure 4. The regression fit of PWVz and 95% confidence interval in the cohort of healthy children differentiated by sex showing red line for girls and blue line for boys adjusted for the mixed model for PWVz as given in the table below the graph.

Abbreviations: $\beta$, regression coefficient; PWVz, pulse wave velocity z-score; SE, standard error; $p$, p-value.

Figure 5. Mixed model for PWVz adjusted for interaction term $|\text{time since inclusion} \times \text{sex} \times \text{KRT modality (pre-KRT/dialysis/transplantation)}|$. Red shaded rows highlight the effect of time pre-KRT and dialysis in girls. Blue shaded rows highlight the effect of time pre-KRT and dialysis in boys (the table at upper panel) and the different slopes of estimates according to the given linear mixed model differentiated by sex and KRT modality. Yellow area shows the time pre-transplantation (including pre-KRT and dialysis) and the green area shows the time post-transplantation. Pink lines show the PWVz slopes for girls, blue lines for boys.

Abbreviations: $\beta$, regression coefficient; CAKUT, congenital anomalies of kidney and urinary tract; KRT, kidney replacement therapy; PWVz, pulse wave velocity z-score; SE, standard error; $p$, p-value; Tx, transplantation.
Reference List

1. Viner RM, Coffey C, Mathers C, et al. 50-year mortality trends in children and young people: a study of 50 low-income, middle-income, and high-income countries. *Lancet* 2011; **377**: 1162-1174.

2. Sawyer CC. Child mortality estimation: estimating sex differences in childhood mortality since the 1970s. *PLoS Med* 2012; **9**: e1001287.

3. Ghuman AK, Newth CJ, Khemani RG. Impact of gender on sepsis mortality and severity of illness for prepubertal and postpubertal children. *J Pediatr* 2013; **163**: 835-840 e831.

4. Bhaumik U, Aitken I, Kawachi I, et al. Narrowing of sex differences in infant mortality in Massachusetts. *J Perinatol* 2004; **24**: 94-99.

5. Groothoff JW, Gruppen MP, Offringa M, et al. Mortality and causes of death of end-stage renal disease in children: a Dutch cohort study. *Kidney Int* 2002; **61**: 621-629.

6. Ahearn P, Johansen KL, McCulloch CE, et al. Sex Disparities in Risk of Mortality Among Children With ESRD. *Am J Kidney Dis* 2019; **73**: 156-162.

7. Laskin BL, Mitsnefes MM, Dahhou M, et al. The mortality risk with graft function has decreased among children receiving a first kidney transplant in the United States. *Kidney Int* 2015; **87**: 575-583.

8. Mitsnefes MM. Cardiovascular disease in children with chronic kidney disease. *J Am Soc Nephrol* 2012; **23**: 578-585.

9. Francis A, Johnson DW, Melk A, et al. Survival after Kidney Transplantation during Childhood and Adolescence. *Clin J Am Soc Nephrol* 2020; **15**: 392-400.

10. Harambat J, van Stralen KJ, Kim JJ, et al. Epidemiology of chronic kidney disease in children. *Pediatr Nephrol* 2012; **27**: 363-373.

11. Ferreira JP, Girerd N, Pannier B, et al. High Pulse-Wave Velocity Defines a Very High Cardiovascular Risk Cohort of Dialysis Patients under Age 60. *Am J Nephrol* 2017; **45**: 72-81.

12. Safar ME, Plante GE, Mimran A. Arterial stiffness, pulse pressure, and the kidney. *Am J Hypertens* 2015; **28**: 561-569.

13. Thurn D, Doyon A, Sozeri B, et al. Aortic Pulse Wave Velocity in Healthy Children and Adolescents: Reference Values for the Vicorder Device and Modifying Factors. *Am J Hypertens* 2015; **28**: 1480-1488.

14. Kracht D, Shroff R, Baig S, et al. Validating a new oscillometric device for aortic pulse wave velocity measurements in children and adolescents. *Am J Hypertens* 2011; **24**: 1294-1299.
15. Kis E, Cseprekal O, Horvath Z, et al. Pulse wave velocity in end-stage renal disease: influence of age and body dimensions. *Pediatr Res* 2008; 63: 95-98.

16. Covic A, Mardare N, Gusbeth-Tatomir P, et al. Arterial wave reflections and mortality in haemodialysis patients—only relevant in elderly, cardiovascularly compromised? *Nephrol Dial Transplant* 2006; 21: 2859-2866.

17. Schaefer F, Doyon A, Azukaitis K, et al. Cardiovascular Phenotypes in Children with CKD: The 4C Study. *Clin J Am Soc Nephrol* 2017; 12: 19-28.

18. Schmidt BMW, Sugianto RI, Thurn D, et al. Early Effects of Renal Replacement Therapy on Cardiovascular Comorbidity in Children With End-Stage Kidney Disease: Findings From the 4C-T Study. *Transplantation* 2018; 102: 484-492.

19. Carrero JJ, de Mutsert R, Axelsson J, et al. Sex differences in the impact of diabetes on mortality in chronic dialysis patients. *Nephrol Dial Transplant* 2011; 26: 270-276.

20. Wolfe RA, Ashby VB, Milford EL, et al. Differences in access to cadaveric renal transplantation in the United States. *Am J Kidney Dis* 2000; 36: 1025-1033.

21. Bromberger B, Spragan D, Hashmi S, et al. Pregnancy-Induced Sensitization Promotes Sex Disparity in Living Donor Kidney Transplantation. *J Am Soc Nephrol* 2017; 28: 3025-3033.

22. Hogan J, Couchoud C, Bonthuis M, et al. Gender Disparities in Access to Pediatric Renal Transplantation in Europe: Data From the ESPN/ERA-EDTA Registry. *Am J Transplant* 2016; 16: 2097-2105.

23. Bobanga ID, Vogt BA, Woodside KJ, et al. Outcome differences between young children and adolescents undergoing kidney transplantation. *J Pediatr Surg* 2015; 50: 996-999.

24. Lepeytre F, Dahhou M, Zhang X, et al. Association of Sex with Risk of Kidney Graft Failure Differs by Age. *J Am Soc Nephrol* 2017; 28: 3014-3023.

25. Sugianto RI, Schmidt BMW, Memaran N, et al. Sex and age as determinants for high blood pressure in pediatric renal transplant recipients: a longitudinal analysis of the CERTAIN Registry. *Pediatr Nephrol* 2020; 35: 415-426.

26. Querfeld U, Anarat A, Bayazit AK, et al. The Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) study: objectives, design, and methodology. *Clin J Am Soc Nephrol* 2010; 5: 1642-1648.

27. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009; 20: 629-637.

28. Adolescent. NHBPEPWGoHBPiCa. The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. *Pediatrics* 2004; 114: 555-576.
29. de Onis M, al e. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva, 2006.

30. Memaran N, Schwalba M, Borchert-Mörlins B, et al. Gesundheit und Fitness von deutschen Schulkindern. Monatsschrift Kinderheilkunde 2020; 168: 597-607.

31. Liu C, Cao D, Chen P, et al. Random and repeated statements–how to use them to model the covariance structure in proc mixed. MWSUG 2007 Conference Proceedings. October 28-30, 2007. Iowa.

32. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med 2007; 4: e296.

33. Russo C, Jin Z, Palmieri V, et al. Arterial stiffness and wave reflection: sex differences and relationship with left ventricular diastolic function. Hypertension 2012; 60: 362-368.

34. Chen SC, Chang JM, Liu WC, et al. Brachial-ankle pulse wave velocity and rate of renal function decline and mortality in chronic kidney disease. Clin J Am Soc Nephrol 2011; 6: 724-732.

35. Erikssen BO, Ingebretsen OC. The progression of chronic kidney disease: a 10-year population-based study of the effects of gender and age. Kidney Int 2006; 69: 375-382.

36. Halbesma N, Brantsma AH, Bakker SJ, et al. Gender differences in predictors of the decline of renal function in the general population. Kidney Int 2008; 74: 505-512.

37. Ricardo AC, Yang W, Sha D, et al. Sex-Related Disparities in CKD Progression. J Am Soc Nephrol 2019; 30: 137-146.

38. Jafar TH, Schmid CH, Stark PC, et al. The rate of progression of renal disease may not be slower in women compared with men: a patient-level meta-analysis. Nephrol Dial Transplant 2003; 18: 2047-2053.

39. Valdivielso JM, Jacobs-Cacha C, Soler MJ. Sex hormones and their influence on chronic kidney disease. Curr Opin Nephrol Hypertens 2019; 28: 1-9.

40. Melamed ML, Blackwell T, Neugarten J, et al.Raloxifene, a selective estrogen receptor modulator, is renoprotective: a post-hoc analysis. Kidney Int 2011; 79: 241-249.

41. Schaefer F, Veldhuis JD, Robertson WR, et al. Immunoreactive and bioactive luteinizing hormone in pubertal patients with chronic renal failure. Cooperative Study Group on Pubertal Development in Chronic Renal Failure. Kidney Int 1994; 45: 1465-1476.
42. Ix JH, De Boer IH, Peralta CA, et al. Serum phosphorus concentrations and arterial stiffness among individuals with normal kidney function to moderate kidney disease in MESA. *Clin J Am Soc Nephrol* 2009; 4: 609-615.

43. Pirro M, Manfredelli MR, Helou RS, et al. Association of parathyroid hormone and 25-OH-vitamin D levels with arterial stiffness in postmenopausal women with vitamin D insufficiency. *J Atheroscler Thromb* 2012; 19: 924-931.

44. Mellor-Pita S, Tutor-Ureta P, Rosado S, et al. Calcium and vitamin D supplement intake may increase arterial stiffness in systemic lupus erythematosus patients. *Clin Rheumatol* 2019; 38: 1177-1186.

45. Cannata-Andia JB, Martin KJ. The challenge of controlling phosphorus in chronic kidney disease. *Nephrol Dial Transplant* 2016; 31: 541-547.

46. Ix JH, Chonchol M, Laughlin GA, et al. Relation of sex and estrogen therapy to serum fibroblast growth factor 23, serum phosphorus, and urine phosphorus: the Heart and Soul Study. *Am J Kidney Dis* 2011; 58: 737-745.

47. Nakashima A, Carrero JJ, Qureshi AR, et al. Plasma osteoprotegerin, arterial stiffness, and mortality in normoalbuminemic Japanese hemodialysis patients. *Osteoporos Int* 2011; 22: 1695-1701.

48. Morena M, Terrier N, Jaussent I, et al. Plasma osteoprotegerin is associated with mortality in hemodialysis patients. *J Am Soc Nephrol* 2006; 17: 262-270.

49. Sigrist MK, Levin A, Er L, et al. Elevated osteoprotegerin is associated with all-cause mortality in CKD stage 4 and 5 patients in addition to vascular calcification. *Nephrol Dial Transplant* 2009; 24: 3157-3162.

50. Scialla JJ, Leonard MB, Townsend RR, et al. Correlates of osteoprotegerin and association with aortic pulse wave velocity in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2011; 6: 2612-2619.

51. Riggio S, Mandraffino G, Sardo MA, et al. Pulse wave velocity and augmentation index, but not intima-media thickness, are early indicators of vascular damage in hypercholesterolemic children. *Eur J Clin Invest* 2010; 40: 250-257.

52. Correia-Costa A, Correia-Costa L, Caldas Afonso A, et al. Determinants of carotid-femoral pulse wave velocity in prepubertal children. *Int J Cardiol* 2016; 218: 37-42.

53. Kaseda R, Jabs K, Hunley TE, et al. Dysfunctional high-density lipoproteins in children with chronic kidney disease. *Metabolism* 2015; 64: 263-273.

54. Shroff R, Speer T, Colin S, et al. HDL in children with CKD promotes endothelial dysfunction and an abnormal vascular phenotype. *J Am Soc Nephrol* 2014; 25: 2658-2668.

55. Baigent C, Landray MJ, Reith C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart
and Renal Protection): a randomised placebo-controlled trial. *Lancet* 2011; **377**: 2181-2192.

56. Buus NH, Carlsen RK, Hughes AD, *et al.* Influence of Renal Transplantation and Living Kidney Donation on Large Artery Stiffness and Peripheral Vascular Resistance. *Am J Hypertens* 2020; **33**: 234-242.

57. Aoun B, Lorton F, Wannous H, *et al.* Aortic stiffness in ESRD children before and after renal transplantation. *Pediatr Nephrol* 2010; **25**: 1331-1336.

58. Sidibe A, Fortier C, Desjardins MP, *et al.* Reduction of Arterial Stiffness After Kidney Transplantation: A Systematic Review and Meta-Analysis. *J Am Heart Assoc* 2017; **6**.

59. Desjardins MP, Sidibe A, Fortier C, *et al.* Impact of kidney transplantation on aortic stiffness and aortic stiffness index beta0. *J Hypertens* 2019; **37**: 1521-1528.

60. Fischer DC, Schreiver C, Heimhalt M, *et al.* Pediatric reference values of carotid-femoral pulse wave velocity determined with an oscillometric device. *J Hypertens* 2012; **30**: 2159-2167.

61. Reusz GS, Cseprekal O, Temmar M, *et al.* Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension* 2010; **56**: 217-224.

62. Klinge A, Allen J, Murray A, *et al.* Increased pulse wave velocity and blood pressure in children who have undergone cardiac transplantation. *J Heart Lung Transplant* 2009; **28**: 21-25.

63. Borchert-Morlins B, Thurn D, Schmidt BMW, *et al.* Factors associated with cardiovascular target organ damage in children after renal transplantation. *Pediatr Nephrol* 2017; **32**: 2143-2154.
STEP 1

Analysis of PWVz including pre-KRT, dialysis and post-transplantation
1. Mixed model adjusted for time since inclusion and kidney disease category (CAKUT/non-CAKUT).
2. Mixed model adjusted for sex, time since inclusion and kidney disease category (CAKUT/non-CAKUT).
3. Mixed model adjusted for interaction of [sex*KRT modality*time since inclusion] and kidney disease category (CAKUT/non-CAKUT).

STEP 2

Analysis of PWVz pre-KRT

Basic Model
Adjusted for pre-KRT time, sex, |sex*pre-KRT time| and kidney disease category.

Reason for using delta eGFR in this analysis step as a covariate: to account for different changes at each visit pre-KRT.

Covariate screening

Final model

Final model girls  Final model boys

STEP 3

Analysis of PWVz post-transplantation

Basic Model
Adjusted for post-transplantation time, sex, |sex*post-transplantation time|, kidney disease category and time on dialysis.

Reason for using eGFR slope in this analysis step as a covariate: to reflect the overall decline during the CKD progression (time pre-KRT).

Covariate screening

Pre-final model

Final model
704 patients at CKD stage II-IV

338 received KRT

Included:
235 received transplantation (Tx)
(150 pre-emptive & 85 with prior dialysis)

Excluded:
- 4 had no PWV measurements
- 99 received dialysis only

196 had observations prior to and after transplantation:
- 195 with visits pre-KRT & post-Tx
- 1 with visit at dialysis & post-Tx

36 had observations prior to Tx only:
- 24 pre-KRT
- 11 pre-KRT & dialysis
- 1 only at dialysis

3 had observations after Tx only
### 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 (years) | 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 (years) | 0.094  | 0.016 | <0.0001 |
| Girls (ref: boys)          | 0.295  | 0.15 | 0.045 |
| Non-CAKUT (ref: CAKUT)     | 0.098  | 0.14 | 0.97  |
Mixed model for PWVz in healthy cohort
(subjects n=307, observations n=614)

|                      | $\beta$ | SE  | p     |
|----------------------|---------|-----|-------|
| Intercept            | -0.26   | 0.070 | <0.001 |
| Time since inclusion (years) | -0.048  | 0.043 | 0.27  |
| Girls (ref.: boys)   | 0.050   | 0.097 | 0.60  |
Mixed model for PWVz with interaction term between KRT modality, sex and time since inclusion (years)
(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 (years)              | 0.17  | 0.057 | 0.004   |
| Girls on dialysis (years)          | 0.20  | 0.073 | 0.006   |
| Girls post-Tx (years)              | 0.13  | 0.024 | <0.0001 |
| Boys pre-KRT (years)               | 0.022 | 0.038 | 0.56    |
| Boys on dialysis (years)           | 0.084 | 0.053 | 0.11    |
| Boys post-Tx (years)               | 0.075 | 0.019 | <0.0001 |
| Non-CAKUT (ref: CAKUT)             | 0.015 | 0.14  | 0.92    |