GOOD LONG-TERM RENAL GRAFT SURVIVAL AND LOW INCIDENCE OF CARDIAC PATHOLOGY IN ADULTS AFTER SHORT DIALYSIS PERIOD AND RENAL TRANSPLANTATION IN EARLY CHILDHOOD

- A cohort study

Running Title

Long-term RTX outcome and cardiac pathology

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TH, KR and HJ designed the research. TH, JT, OR, MT and MH were involved in data collection. TH analyzed the data. TH and DG wrote the manuscript draft. All authors contributed to and approved the final submitted version of the manuscript.

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Conflict of interest

The authors have no conflict of interest to disclose.

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SUMMARY

Over the past 30-years, there has been an improvement in both patient and graft survival after pediatric renal transplantation (RTX). Despite this success, these patients still carry an elevated risk for untimely death, partly through premature aging of the vasculature. The aim of this study was thus to investigate the long-term outcome of individuals with RTX in childhood, as well as to explore the cardiovascular health of these adults more than a decade later.

We studied 131 individuals who had undergone a RTX between the years 1979 and 2005. Furthermore, left ventricular hypertrophy (LVH), coronary artery calcifications (CAC) and related metabolic factors were investigated in a cross-sectional study including 52 individuals as part of the initial cohort. The mortality rate (n=131) was 12.2%. The median estimated graft survival was 17.5 years (95% CI 13.6–21.3), being significantly better in children transplanted below the age of 5 years (18.6 vs. 14.3 years, $p<0.01$) compared to older ones. CAC were found in 9.8% and LVH in 13% of the patients. Those with cardiac calcifications had longer dialysis vintage and higher values of parathyroid hormone (PTH) during dialysis. Left ventricular mass correlated positively with systolic blood pressure, PTH and phosphate measured at the time of the study.

Introduction

Over a period of more than 30 years, both patient and graft survival have improved after pediatric renal transplantation (RTX), especially thanks to improved management of early complications. During 1970–2010, ten- and 20-year graft survival (GS) rates improved in patients with pediatric RTX from 52 to 85% and 30 to 38%, whereas 10- and 20-year patient survival rates improved from 79 to 94% and 66 to 82%, respectively [1-10]. The patients included in these studies were 10–15 years old at the time of RTX, and their cumulative dialysis time was 2–5 years [2-6,10-14]. The inferior GS times of small children (<2–5 years at RTX) compared to older children have disappeared; today, infants show the best survival rates [6,15,16].

The mortality of young adults after pediatric RTX has varied between 7% and 28%, being largely due to cardiovascular disease (CVD; 25–50%) [4-8,10,11,14]. Premature cardiovascular calcifications have been detected in 10–92% of young adults with a history of renal replacement therapy (RRT), and the calcifications have been related to hyperparathyroidism and its treatment rather than traditional CVD risk factors [12-14,17-19].

We decided to study the long-term graft survival, mortality and general outcome in pediatric RTX patients in Finland. The median ages of children initiating RRT (2.3 years) and at the time of RTX (4.4 years) are lower in Finland than in most other countries, because of the high number of patients with congenital nephrotic syndrome of the Finnish type (CNF) [20]. The unfavorable lipid profile during the nephrosis as well as subsequent early dialysis commencement in our CNF patients might increase the risk for cardiac complications in our patient population. Therefore, we decided to investigate further in a cross-sectional study the incidence of coronary artery calcification (CAC) and left ventricular hypertrophy (LVH) as well as possible risk factors, especially the treatment of hyperparathyroidism (cumulative intake of active vitamin D and calcium carbonate [$CaCO_3$]) associated with them.

Patients and methods

Patients

A total of 131 pediatric patients, who received their first RTX in Finland during 1979 – 2005 and had been treated in the national pediatric transplantation center, Children's Hospital, Helsinki University Central Hospital, were included in this study. Fifty-two patients participated further in the cross-sectional study.

The study protocol was in accordance with the Declaration of Helsinki as revised in 2000 and approved by local Ethics Committee. A written informed consent was obtained from each participant.

Study design

Age at first RTX, patient and median GS at 10 and 15 years, as well as causes for death were drawn for the 131 patients from the Finnish Renal Transplantation Registry. Completeness of this registry has previously been demonstrated (Report of the Finnish Registry for Kidney Diseases 2014). Patient and graft survival were calculated at the time of graft loss, patient death, or end of the observation period, which ever came first.

Patient records since the commencement of RRT were reviewed for the 52 patients participating in the cross-sectional study. The following data were collected: cumulative dialysis time, laboratory values possibly related to LVH and/or CAC, pharmacological treatment for hypertension, hyperparathyroidism, and growth failure. Values were used for analysis at 3-month intervals during dialysis and 12-month intervals post RTX. Cross-sectional evaluations were performed at the Children's Hospital in Helsinki. Parathyroid hormone values (PTH) were indexed because of changes in laboratory methods and reference values during the observation period. Uric acid was also indexed to allow comparison of values between different ages and genders. Indexes were calculated as the actual value divided by the upper limit of normal. Estimated glomerular filtration rate (eGFR) was calculated using the 2012 CKD-EPI creatinine-cystatin C formula [21]. Overweight was defined as BMI 25–30 kg/m^2 and obesity as BMI >30 kg/m^2 and as waist

circumference-to-height ratio (WHtR) ≥0.539 based on NHANES III data, which is independent of gender [22].

Renal replacement therapy

Dialysis was started or RTX performed when the child reached severe uremia, showed failure to thrive under conservative therapy, or was nephrectomized due to CNF. The basic immunosuppressive protocol after RTX consisted of triple medication including cyclosporine A, azathioprine, and methyl prednisolone as previously described [23-25]. Basiliximab induction was used since the year 2000. Mycophenolate acid and/or tacrolimus were used if calcineurin inhibitor toxicity or recurrent rejection episodes were detected. The rejection episodes were verified by core needle or fine needle biopsies [23].

Coronary CT image acquisition and analysis

Computer tomography (CT) scans were performed using a 64-row spiral scanner (Siemens Somatom AS+, Erlangen, Germany) and interpreted by a pediatric radiologist. All patients underwent non-contrast prospective electrocardiography (ECG) gated sequential coronary imaging to measure coronary artery calcium score (CACS). The CT imaging parameters used were 120 kV tube voltage, effective tube current with 250 mAs, collimation 64 × 0.6 mm, and gantry rotation time 330 ms.

Images were analyzed with a commercially available software package and delicate workstation (Siemens Calcium Score, Siemens, Erlangen, Germany). The total calcium burden in the coronary arteries was quantified by the scoring algorithm proposed by Agatston et al. for adults with and without coronary artery disease (a cutoff value of 130 Hounsfield Units on an area density), and pre-defined calcium score was classified as normal (0), mild (1–100), moderate (101–400), or severe (>400) [26, 27]. Calcium in the aorta, aortic valve (AV), mitral annulus or valve (MV), pericardium, myocardium, atrium or bronchial tree was excluded from the CACS and reported independently.

Cardiac investigations

Echocardiographic studies were performed using GE Vivid 7 Pro (General Electric Company, NYSE: GE, UK) or Philips IE33 (Philips Medical Systems, Andover, Massachusetts, USA) echocardiographic devices. Left ventricular (LV) dimensions were measured from the parasternal long-axis M-mode and expressed as Z scores with the use of body surface area (BSA)-related normal limits [28]. Left ventricular mass (LVM) by echocardiography was derived from the 2-D measurements of intraventricular septal thickness, posterior wall thickness and LV internal dimensions in diastole as recommended by the American Society of Echocardiography [29]. LVM was divided by BSA to achieve indexed LVM (LVMI) [28]. LVH was defined as LVMI >95 g/m^2 in females and >115 g/m^2 in males [29].

Statistical analysis

All analyses were done with SPSS version 22 software (SPSS, Chicago, IL, USA). Normally distributed data was expressed as mean ± SD and non-normally distributed data as median with interquartile ranges (IQR). Mann-Whitney U test was used to compare differences between two groups for continuous variables. Pearson Correlation analysis was used for normally distributed values and Spearman Correlation analysis for nonparametric values. The Kaplan-Meier method was used for calculation of graft survival and log rank test to test for differences in survival between groups. Logistic regression analysis was used to identify predictors for increased cardiac calcifications and multiple linear regression analysis to find explanatory factors for increase in LVMI. P-values <0.05 were considered significant.

Results

All RTX patients

Median age at the time of the RTX for all 131 eligible patients was 8.8 (2.7–14.2) years. The 10-year GS was 77% and median estimated graft survival 17.5 (95% CI 13.6–21.3) years, being significantly better for children transplanted after year 1990

(21.4 vs 11.8 years, p=0.002) and for those transplanted below the age of 5 years (18.6 vs 14.3 years, p=0.003) compared to those having RTX before 1990 or older than 5 years, respectively (Figure 1). Sixteen patients (12.2%) had died at the median age of 24.7 (15.3 – 30.9) years within a median follow-up time of 16.8 (11.9–20.14) years. The causes of death were as follows: myocardial infarction (n=4), cerebral hemorrhage (n=3), aortic aneurysm (n=1), infection (n=4), head injury (n=1), digestive tract hemorrhage (n=1), lymphoma (n=1) and under unknown circumstances (n=1). Only three deaths occurred in individuals with a working first renal graft.

The basic demographics of patients participating in the cross-sectional study did not differ from the whole cohort of RTX patients (Table 1).

Cross-sectional study

Demographics and laboratory findings

The median age in the cross-sectional study was 23.5 (19.5–27.8) years. The final heights were 170.7±7.0 and 157.0±5.5 cm in males and females, respectively. More than half of the patients (65.4%) were treated with growth hormone after diagnosis of end-stage renal disease. Systolic blood pressure (SBP) was 125±13 mmHg and diastolic blood pressure (DBP) 71±9 mmHg. SBP exceeded 130 mmHg in 34.6% and DBP 80 mmHg in 28.8%, despite the fact that 55.7% were on antihypertensive medication. Mean WHtR was 0.50±0.06; 25% of the patients were classified as obese. Based on BMI, only 3.8% were categorized as obese and 19.2% as overweight. Despite the difference in the prevalence of obesity defined as WHtR or BMI, a significant correlation (R=0.86, p<0.01) was found between these two variables. The median cumulative alphacalcidol dose was 225 µg (31–502) and CaCO3 dose 66 g (38–181). Laboratory findings at the time of the cross-sectional study are compiled in Table 2 separately for those with a working graft and those on dialysis. Brain natriuretic peptide (proBNP) correlated with eGFR (R= -0.44, p=0.003) and BMI with blood glucose (R=0.28, p= 0.047), while no correlation was found between proBNP and SBP or DBP, or between lipids and BMI or WHtR, or between WHtR and glucose.

Renal replacement therapy

Peritoneal dialysis was commenced at the median age of 6.8 (1.2–12.5) years. The median time on first-ever dialysis was 0.65 (0.44–1.27) years and the cumulative dialysis time was 0.93 (0.51–1.92) years. One patient received his first renal transplant pre-emptively.

First RTX was performed at the median age of 8.8 (2.7–14.2) years, and 37% of the patients were under 5 years of age at that time. Of the first renal grafts, 67% were from deceased donors (DD) whereas 33% were from living donors (LD). Ninety-two percent of LD grafts, compared to 65% of DD grafts, functioned after 10 years, compared to 92% vs. 48% after 15 years, respectively. At the time of the cross-sectional study, 34 of the first renal grafts (65.4%) were working after a median follow-up of 15.8 (9.0–17.1) years, 10 patients had a working re-transplant, and 8 were on dialysis (6 patients were waiting for 2nd and two for 3rd graft). Eighteen of the first transplants were lost mostly due to acute or chronic rejection episodes. Median eGFR for patients with working first graft was 55 (42–69) mL/min/1.73m^2.

Echocardiography

All patients had normal anatomy of the heart. One patient had undergone pulmonary valvuloplasty as a newborn, and another patient an atrium septum defect closure by catheter intervention at 21 years of age. Physiological valve insufficiency (grade I/III) was found in 54% of the patients. One patient had grade II/III insufficiency in the aortic valve caused by valve calcifications (the finding was confirmed by cardiac CT). LVH was diagnosed in seven male patients (13% of all patients), but in none of the female patients. LVMI correlated (Spearman Correlation analysis) significantly with SBP, PTH index as well as phosphate (Table 3). SBP, cumulative alphacalcidol dose and phosphate at the time of the cross-sectional study predicted a 21% increase in LVMI in a multiple linear regression analysis, (β [Adjusted R Square] =0.59, 95% CI 0.08–1.103, p=0.03), (β=0.01, 95% CI 0.00–0.02, p=0.06), (β=17.95, 95% CI -1.27–37.17, p=0.07), respectively.

Cardiac CT

Cardiac CT was examined in 51 patients. CACs were found in five patients (9.8%), other cardiac calcifications in two (3.9%), and extra cardiac calcifications in one (2.0%) individual. The overall cardiac calcification rate was thus 13.7% (Table 3). CAC was categorized as mild in three patients, as moderate in one, and as severe in one patient. The CACS varied between 15.4 and 1122.4. The patient with the highest CACS also expressed calcifications in the left side of the heart (left atrium [LA], MV and AV). The individual with the third highest CACS showed calcifications in the bronchi, aortic arch and left side of the heart (AV, MV). Five years before study, a subcutaneous inflamed calcified infiltration was removed from the left groin of a patient who expressed bronchial and AV calcifications but no CAC.

Clinical differences in patients with and without calcifications in cardiac CT are summarized in Table 4. The follow-up time since the first dialysis did not differ between those with calcifications and those without, but patients with calcifications had longer cumulative dialysis time, higher PTH-index during dialysis, and higher DBP at the time of the investigation. In the logistic regression analysis, calcifications found in the CT were significantly associated with cumulative dialysis time (OR 0.41, 95% CI 0.19–0.89, p=0.02), but not with mean PTH index during dialysis (OR 0.78, 0.56–1.10, p=0.14). Calcium and phosphate balance was comparable between the groups (Table 4).

Discussion

We found good long-term outcome in a nationally representative cohort of 131 young adults who received their first renal transplant in childhood, being 8.8 years old, between the years 1979 and 2005. The 10-year GS was 77%. Of the first transplants, 65% were working 15.8 years after the transplantation in a sub-cohort of 52 (eGFR 55 mL/min/1.73m^2) not differing from the whole population. The prevalence of CAC (9.8%) and LVH (13%) was surprisingly low. Patients with cardiac calcifications had longer duration of dialysis and higher PTH at dialysis.

We observed a better 10-year GS compared to most other cohorts (50–65%) [2,3,6,9]. One of the previous studies was registry-based [2], whereas the others were single center studies like ours [3,6,9]. Two reports (a French cohort and a registry study from the Nordic countries) have indicated similar 10-year GS (72–76%) as in this study [4,15]. The 10-year GS were better for LD kidneys than for DD kidneys, in line with previous studies [9, 30-34].

Data on overweight in kidney transplant patients and its association with cardiovascular complications are scarce. Obesity as well as underweight have been proposed to increase mortality in RTX patients [35]. The prevalence of obesity was in line with 70 adult RTX patients (21.1%) reported by Malgorzewicz et al., even though they defined obesity differently (as BMI >30 kg/m^2) [36]. Despite good correlation between BMI and WHtR, BMI as such was found to be a less sensitive marker for obesity in our cohort. Only one out of 52 individuals had a BMI of >30 kg/m^2 (2%), whereas 11 patients (21%) had a WHtR of >0.539, equally defining obesity [22]. WHtR has been suggested to be a more sensitive marker to identify cardiovascular disease risk than BMI, since it is proposed to correlate more strongly with harmful visceral fat, lipids and glucose [22]. WHtR has been found to be a significant contributor to post-transplant LVH in children [37]. However, we were not able to find any correlation between WHtR and lipids, glucose or LVMI, nor did WHtR differ between those with and without CAC.

The scarce data on cardiac CT imagining in young adults receiving RTX in childhood have indicated higher CAC prevalence (35%–92%) compared to our finding [14,18,19]. The CAC severity and prevalence of 9.8% in our patients nearly equals the 5% in 60 healthy controls aged 20–30 years in the study by Goodman et al. [18]. In previous studies, CACS has been positively correlated with treatment of secondary hyperparathyroidism (intake of active vitamin D and calcium) as well as higher age and longer duration of dialysis [12-14,18,19,38]. We also found a correlation between cardiac calcifications and longer dialysis vintage, as well as higher PTH-index during dialysis, but not between cardiac calcifications and active vitamin D intake, even though autopsy-based data on children exist to support the role of active vitamin D in the development of vascular calcifications [39]. Like Shroff

et al., we were not able to support the association between CAC prevalence and cumulative phosphate binder (CaCO3) dosage [38]. The median PTH index during dialysis was just less than twice the upper limit of normal (ULN) in patients without calcifications in adult age. Shroff et al. reported PTH greater than twice the ULN to increase the prevalence of cardiac calcifications as early as in school age in children on dialysis, which supports our finding [38]. Treatment of hyperparathyroidism with diet as well as CaCO3 as phosphate binder may have been favorable in our patient cohort, possibly leading to low prevalence of calcifications. Successful treatment of hyperparathyroidism with diet and oral phosphate binders (CaCO3) might also explain the weak correlation between the dose of active vitamin D and cardiac calcifications.

The prevalence of LVH was lower in the present study compared to previous reports (43–62%), even considering the lower cut-off for LVMI to define LVH compared to Gruppen et al. [12,13]. In these two studies, the LVMIs were clearly higher: 142–150 g/m^2 for males and 110–119 g/m^2 for females. Gruppen et al. concluded that LVH was strongly related to pressure load measured at the time of the investigation [12]. In concordance with the Dutch study, SBP was the only significant independent predictor for LVH in our patients. Briese et al. also identified an association between LVMI and PTH, as did we [13]. They reported further that the intake of active vitamin D and calcium independently predicted LVMI [13]. However, we could not confirm this relationship. The individuals in the study of Briese et al. were older at the time of RTX and had a longer duration of dialysis which may have affected the results [13].

This study is not without limitations. The baseline data were collected retrospectively in order to find correlations with cardiovascular pathology during follow-up. Another limitation is the absence of reference values for CACS for young adults, resulting in the use of adult reference values in the present study. Despite our study being population based, the median age at the time of RRT entry and at the first RTX were above the national median. This age difference can be explained by the fact that the active RTX program started in the mid-80s and was expanded to include infants a decade later. Thus, we needed to exclude many of the youngest individuals from the study. Despite patients being older than estimated, the strength of our study is the younger age at the time of entering both RRT and having a first-ever RTX, as well as

shorter cumulative dialysis time. Further, a major strength is that the study population represents almost half of the eligible national target cohort, the population in this study being thus nationally representative. Compared to earlier contributions, our study included a relatively large number of subjects, and the patients were treated according to a uniform RRT protocol in their childhood. Prospective and large studies are certainly warranted to explore the role of obesity/altered body dimension, oral phosphate binders and active vitamin D in the development of cardiovascular pathology.

In summary, shorter dialysis vintage and well-controlled parathyroid function associated with fewer long-term pathological cardiac changes 15.8 years after RTX performed in early childhood. Our study further supports the theory that good dietary phosphorus control and therapeutic doses of active vitamin D, adequate to suppress parathyroid hormone levels without inducing systemic hypercalcemia and hyperphosphatemia, do not induce vascular calcification as much as higher dosages might do [38,4041]. Larger prospective studies are needed to explore the role of other factors involved in the development of vascular calcifications.

REFERENCES

1. Holmberg C, Jalanko H.Long-term effects of paediatric kidney transplantation. *Nat Rev Nephrol*. 2016; 12:301-311. doi: 10.1038/nrneph.2015.197.

2. Van Arendonk KJ, Boyarsky BJ, Orandi BJ et al. National trends over 25 years in pediatric kidney transplant outcomes. *Pediatrics.* 2014; 133:594-601. doi: 10.1542/peds.2013-2775.

3. Yamada A, Tashiro A, Hiraiwa T, Komatsu T, Kinukawa T, Ueda N. Long-term outcome of pediatric renal transplantation: a single center study in Japan. *Pediatr Transplantation.* 2014; 18:453-462. doi: 10.1111/petr.12299.

4. Harambat J, Ranchin B, Bertholet-Thomas A et al. Long-term critical issues in pediatric renal transplant recipients: a single-center experience. *Transpl Int.* 2013; 26:154-61. doi: 10.1111/tri.12014.

5. Foster BJ, Dahhou M, Zhang X, Platt RW, Hanley JA. Change in mortality risk over time in young kidney transplant recipients. *Am J Transplant.* 201; 11:2432-2442. doi: 10.1111/j.1600-6143.2011.03691.x.

6. Rees L, Shroff R, Hutchinson C, Fernando ON, Trompeter RS. Long-term outcome of paediatric renal transplantation: follow-up of 300 children from 1973 to 2000. *Nephron Clin Pract.* 2007; 105:68-76.

7. McDonald SP, Craig JC; Australian and New Zealand Paediatric Nephrology Association. Long-term survival of children with end-stage renal disease. *N Engl J Med.* 2004; 350:2654-2662. doi: 10.1056/NEJMoa031643.

8. Englund M, Berg U, Tydén G. A longitudinal study of children who received renal transplants 10-20 years ago. *Transplantation* 2003; 76:311-318. doi: 10.1097/01.TP.0000076472.45979.65.

9. Vats A, Gillingham K, Matas A, Chavers B. Improved late graft survival and half-lives in pediatric kidney transplantation: a single center experience. *Am J Transplant.* 2002; 2:939-945.

10. Offner G, Latta K, Hoyer PF et al. Kidney transplanted children come of age. *Kidney Int.* 1999; 55:1509-1517. doi: 10.1046/j.1523-1755.1999.00356.x.

11. 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. doi: 10.1046/j.1523-1755.2002.00156.x

12. Gruppen MP, Groothoff JW, Prins M et al. Cardiac disease in young adult patients with end-stage renal disease since childhood: a Dutch cohort study. *Kidney Int.* 2003; 63:1058-1065. doi: 10.1046/j.1523-1755.2002.00156.x.
13. Briese S, Wiesner S, Will JC et al. Arterial and cardiac disease in young adults with childhood-onset end-stage renal disease-impact of calcium and vitamin D therapy. *Nephrol Dial Transplant.* 2006; 21:1906-1914. doi: 10.1093/ndt/gfl098
14. Oh J, Wunsch R, Turzer M et al. Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. *Circulation* 2002; 106:100-105.
15. Jahnukainen T, Bjerre A, Larsson M et al. The second report of the Nordic Pediatric Renal Transplantation Registry 1997-2012: More infant recipients and improved graft survivals. *Pediatr Transplant.* 2016; 20:364-371. doi: 10.1111/petr.12686.
16. Jalanko H, Mattila I, Holmberg C. Renal transplantation in infants. *Pediatr Nephrol.* 2016; 31:725-735. doi: 10.1007/s00467-015-3144-0.
17. Groothoff JW, Lilien MR, van de Kar NC, Wolff ED, Davin JC. Cardiovascular disease as a late complication of end-stage renal disease in children. *Pediatr Nephrol.* 2005; 20:374-379. doi: 10.1007/s00467-004-1624-8.
18. Goodman WG, Goldin J, Kuizon BD et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med.* 2000; 342:1478-1483. doi: 10.1056/NEJM200005183422003
19. Eifinger F, Wahn F, Querfeld U et al. Coronary artery calcifications in children and young adults treated with renal replacement therapy. *Nephrol Dial Transplant.* 2000; 15:1892-1894.
20. Jalanko H. Congenital nephrotic syndrome. *Pediatr Nephrol.* 2009; 24:2121-2128. doi: 10.1007/s00467-007-0633-9.
21. Inker LA, Schmid CH, Tighiouart H et al. AS; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* 2012; 367:20-29. doi: 10.1056/NEJMoa1114248.
22. Kahn HS, Imperatore G, Cheng YJ. A population-based comparison of BMI percentiles and waist-to-height ratio for identifying cardiovascular risk in youth. *J Pediatr.* 2005; 146:482-488. doi: 10.1016/j.jpeds.2004.12.028.

23. Seikku P, Krogerus L, Jalanko H, Holmberg C. Better renal function with enhanced immunosuppression and protocol biopsies after kidney transplantation in children. *Pediatric Transplant.* 2005; 9:754-762. doi: 10.1111/j.1399-3046.2005.00374.x.

24. Helenius I, Remes V, Salminen S et al. Incidence and predictors of fractures in children after solid organ transplantation: a 5-year prospective, population-based study. *J Bone Miner Res.* 2006; 21:380-387. doi: 10.1359/JBMR.051107

25. Valta H, Mäkitie O, Rönnholm K, Jalanko H. Bone health in children and adolescents after renal transplantation. *J Bone Miner Res.* 2009; 24:1699-1708. doi: 10.1359/jbmr.090407.

26. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990; 15:827-832.

27. Arad Y, Goodman KJ, Roth M, Newstein D, Guerci AD. Coronary calcification, coronary disease risk factors, C-reactive protein, and atherosclerotic cardiovascular disease events: The St. Francis Heart Study. *J Am Coll Cardiol.* 2005; 46:158-165.

28. Kampmann C, Wiethoff CM, Wenzel A et al. Normal values of M mode echocardiographic measurements of more than 2000 healthy infants and children in central Europe. *Heart* 2000; 83:667-672.

29. Lang RM, Bierig M, Devereux RB et al.; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005; 18:1440-1463.

30. Smith JM, Martz K, Blydt-Hansen TD. Pediatric kidney transplant practice patterns and outcome benchmarks, 1987-2010: a report of the North American Pediatric Renal Trials and Collaborative Studies. *Pediatr Transplant.* 2013; 17:149-157. doi: 10.1111/petr.12034.

31. Dale-Shall AW, Smith JM, McBride MA, Hingorani SR, McDonald RA. The relationship of donor source and age on short- and long-term allograft survival in

pediatric renal transplantation. *Pediatr Transplant.* 2009; 13:711-718. doi: 10.1111/j.1399-3046.2008.01054.x.
32. Shapiro R. Living donor kidney transplantation in pediatric recipients. *Pediatr Transplant.* 2006; 10:844-850. doi: 10.1111/j.1399-3046.2006.00557.x.
33. Tangeraas T, Bjerre A, Lien B et al. Long-term outcome of pediatric renal transplantation: the Norwegian experience in three eras 1970-2006. *Pediatr Transplant.* 2008; 12:762-768. doi: 10.1111/j.1399-3046.2007.00896.x.
34. Papachristou F, Stabouli S, Printza N et al. Long-term outcome of pediatric kidney transplantation: A single-center experience from Greece. *Pediatr Transplant.* 2016; 20:500-506. doi: 10.1111/petr.12700.
35. Ahmadi SF, Zahmatkesh G, Streja E el.al. Body mass index and mortality in kidney transplant recipients: a systematic review and meta-analysis. Am J Nephrol. 2014; 40(4):315-24. doi: 10.1159/000367812.
36. Małgorzewicz S, Wołoszyk P, Chamienia A, Jankowska M, Dębska-Ślizień A. Obesity Risk Factors in Patients After Kidney Transplantation. *Transplant Proc.* 2018; 50(6):1786-1789. doi: 10.1016/j.transproceed.2018.02.099.
37. Sgambat K, Clauss S, Lei K.Y. et al. Effects of obesity and metabolic syndrome on cardiovascular outcomes in pediatric kidney transplant recipients: a longitudinal study. *Pediatr Nephrol* 2018; 33:1419-1428. doi: 10.1007/s00467-018-3913-7.
38. Shroff RC, Donald AE, Hiorns MP et al. Mineral metabolism and vascular damage in children on dialysis. *J Am Soc Nephrol.* 2007; 18:2996-3003. doi: 10.1681/ASN.2006121397.
39. Milliner DS, Zinsmeister AR, Lieberman E, Landing B. Soft tissue calcification in pediatric patients with end-stage renal disease. *Kidney Int* 1990; 38:931-936.
40. Shroff R, Long DA, Shanahan C. Mechanistic insights into vascular calcification in CKD. *J Am Soc Nephrol.* 2013; 24:179-189. doi: 10.1681/ASN.2011121191.
41. Mathew S, Tustison KS, Sugatani T, Chaudhary LR, Rifas L, Hruska KA. The mechanism of phosphorus as a cardiovascular risk factor in CKD. *J Am Soc Nephrol.* 2008; 19: 1092-1105. doi: 10.1681/ASN.2007070760.

Figure legends

Figure 1 Graft survival (GS) for the first renal transplants (n=123). A) Median GS for patients transplanted after 1990 is 21.4 years and before 1990 11.8 years, p=0.002.

B) Median GS for patients transplanted in age <5 years is 18.6 years and in age >5 years 14.3 years, p=0.003.

Table 1. Basic demographics of all RTX patients and the patients participating in the cross-sectional study.

Variable	All RTX patients n=131	Cross-sectional study n=52
Male patients (%)	91 (69.5%)	36 (69.2%)
Age at first RTX	8.8 (2.7-14.2)	8.9 (2.4-14.3)
0-1 years	22 (16.8%)	11 (21.1%)
2-5 years	26 (19.8%)	8 (15.4%)
6-12 years	40 (30.5%)	15 (28.9%)
13-18 years	43 (32.8%)	18 (34.6%)
Age at study	23.7 (20.5-27.7)	23.5 (19.5-27.8)
Primary disease		
Hereditary renal	53 (40.5%)	23 (44.2%)
CAKUT	47 (35.9%)	16 (30.8%)
Glomerular	19 (14.5%)	9 (17.3%)
Other	12 (9.1%)	4 (7.7%)
Transplantation		
1 renal graft	103 (78.6%)	40 (76.9%)
2 renal grafts	22 (16.8%)	9 (17.3%)
3 renal grafts	5 (3.8%)	2 (3.9%)
4 renal grafts	1 (0.8%)	1 (1.9%)
Cumulative alphacalcidol intake, µg	NA	225.7 (30.7-501.7)
Cumulative calcium intake, g/kg	NA	66.3 (38.3-181.4)
Use of growth hormone	NA	34 (65.4%)
Use of bisphosphonates	NA	10 (19.2%)
Lipid lowering medication	NA	9 (17.3%)

CAKUT, congenital anomalies of kidney and urinary tract. NA, not assessed/applicable.

Table 2. Comparison of laboratory characteristics between patients with working renal graft and those on dialysis.

Value (Reference value)	RTX n=44	Dialysis n=8
p-Hb (g/L)	123 (114-136)	116 (93-123)
s-hsCRP (≤2.5 mg/L)	1.38 (0.56-2.59)	0.88 (0.33-9.10)
p-IL-6 (≤5.9 ng/L)	4.0 (2.5-6.9)	4.5 (2.2-15.0)
p-Calcium (≤2.51 mmol/L)	2.39 (2.32-2.51)	2.38 (2.13-2.44)
p-Phosphate (≤1.53 mmol/L)	1.00 (0.88-1.15)	1.65 (1.17-2.02)
p-Ca x p-P product	2.45 (2.10-2.75)	3.57 (2.63-5.11)
p-PTH-index[a]	1.03 (0.50-1.76)	5.47 (2.87-7.50)
p-Magnesium (≤0.94 mmol/L)	0.74 (0.67-0.82)	0.91 (0.85-1.01)
fp-Zinc (11-22 μmol/L)	12.0 (10.0-13.0)	13.5 (10.5-15.7)
p-Homocystein (≤15 μmol/L)	19.6 (14.9-26.4)	18.4 (15.4-34.2)
fs-Folic acid (5.3-40 nmol/L)	9.8 (7.2-19.4)	26.0 (9.0-30.1)
s-Vitamin B12 (140-540 pmol/L)	438 (346-616)	525 (420-724)
p-Uric acid index[b]	1.15 (1.04-1.25)	0.87 (0.58-1.16)
p-Creatinine (≤100 μmol/L)	125 (106-152)	692 (468-734)
p-Albumin (36-48 g/L)	41.6 (38.1-44.6)	37.6 (33.0-43.5)
p-proBNP (<84 ng/L)	89 (64-243)	1875 (561-4215)
fp-Cholesterol (<5.0 mmol/L)	4.1 (3.5-4.7)	4.1 (3.0-4.5)
fp-ApoA-1 (>1.2 g/L)	1.39 (1.24-1.58)	1.30 (1.03-1.53)
fp-ApoB (<1.1 g/L)	0.69 (0.56-0.78)	0.66 (0.57-0.81)
eGFR (mL/min/1.73m^2)	55 (42-69)	

[a] Calculated as actual PTH divided by upper reference value, [b] Calculated as actual Uric Acid divided by upper reference value. p-P, plasma phosphate. ApoA-1, apolipoprotein A-1. ApoB, apolipoprotein B-100. eGFR, estimated glomerular filtration rate.

Table 3. Cardiac CT and echocardiography.

Variable	Study Patients n=51	P-value
Cardiac CT		
Coronary artery calcification score	90.0 (52.4-682.7)	
Location of the calcification:		
Coronary	5 (9.8%)	
Cardiac, non-coronary:	2 (3.9%)	
AV, AA		
LA, MV		
Extracardial:		
Bronchial	1 (2.0%)	
Cardiac echocardiography		
LVMI (females / males), g/m^2	66.4±14.0 / 85.1±25.6	
LVMI correlation[a]		
PTH index[b]	R=0.30	0.03
Phosphate	R=0.30	0.03
SBP	R=0.38	<0.01
DBP	R=0.14	0.34
Cumul. alphacalcidol intake	R=0.24	0.09
Homocystein	R=0.28	0.06
proBNP	R=0.12	0.40
WHtR	R=-0.17	0.66

[a] Spearman Correlation analysis

[b] Calculated as actual PTH divided with upper reference value

AV, aortic valve; AA, aortic arch; LA, left atrium; MV, mitral valve; LVMI, left ventricle mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BNP, brain natriuretic peptide. WHtR, waist-to-height ratio

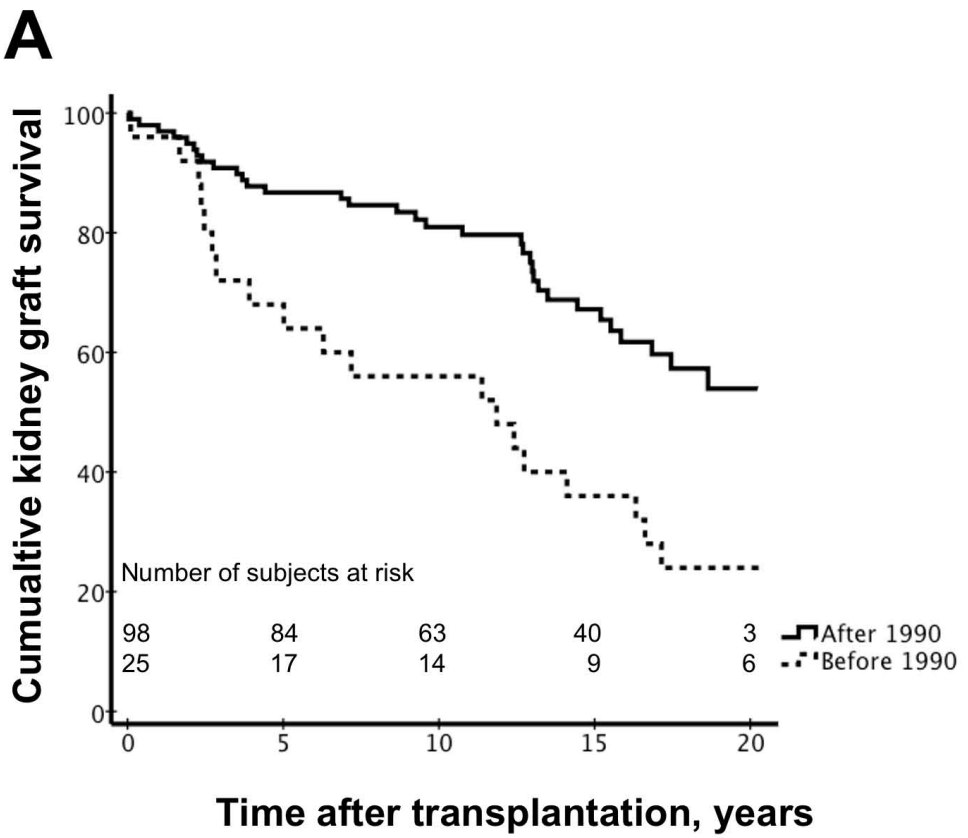
Table 4. Patients with and without calcifications in cardiac CT. Differences were calculated with Mann-Whitney U test.

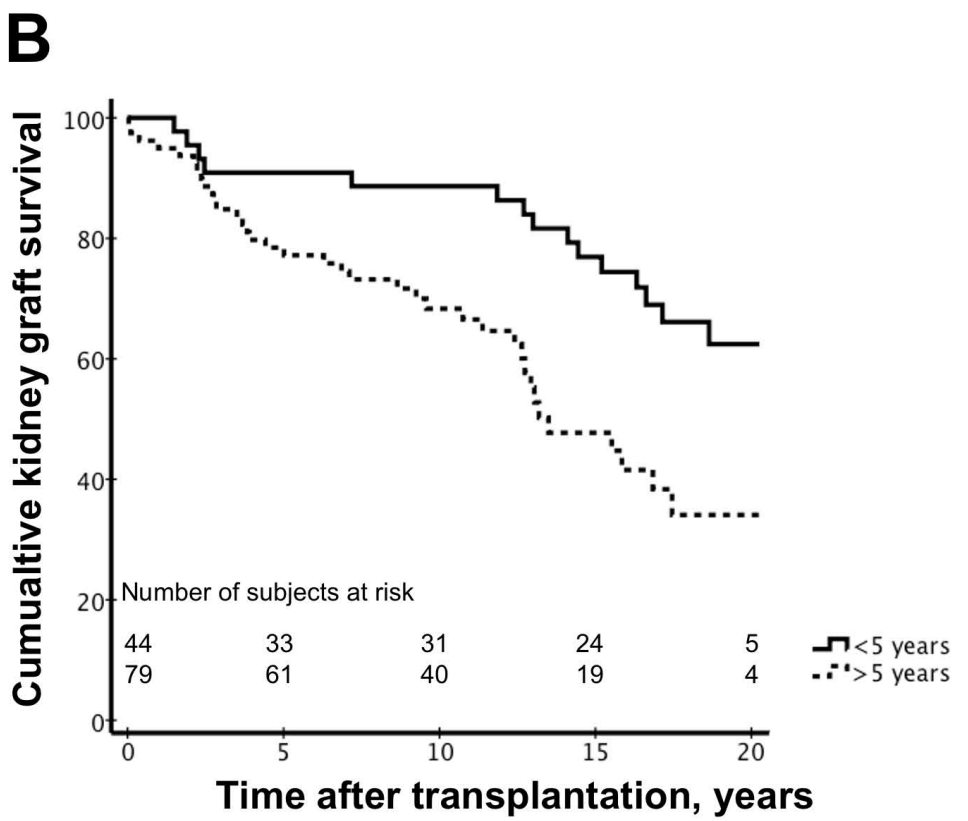
	Calcifications n=8	No calcifications n=43	P value / Corr[a]
Age at first dialysis, years	11.2 (2.6-14.1)	6.7 (1.2-12.5)	0.407 / 1.0
Age at first RTX, years	12.0 (3.1-15.4)	7.3 (2.4-13.6)	0.550 / 1.0
Age at study, years	28.9 (21.9-31.2)	23.2 (19.1-27.5)	0.101 / 1.0
Time first dialysis to study, years	17.2 (12.4-22.2)	17.5 (10.9-21.3)	0.694 / 1
Cumulative dialysis time, years	2.3 (0.9-8.1)	0.8 (0.5-1.6)	0.016 / 0.24
SBP at the time of study, mmHg	135 (121-137)	122 (115-132)	0.059 / 0.88
DBP at the time of study, mmHg	86 (75-92)	72 (65-79)	0.021 / 0.31
Cumulative alphacalcidol dose, µg	448 (220-2136)	180 (22-484)	0.063 / 0.94
Cumulative CaCO3 dose, g/kg	110 (20-344)	66 (38-180)	0.584 / 1.0
Mean s-Ca during dialysis, mmol/L	2.4 (2.3-2.5)	2.4 (82.3-2.5)	0.571 / 1.0
Mean s-P during dialysis, mmol/L	1.97 (1.67-2.13)	1.82 (1.65-2.04)	0.615 / 1.0
Mean s-ca x s-P during dialysis	4.4 (4.0-5.1)	4.5 (3.9-5.0)	0.851 / 1.0
Mean PTH index[b] during dialysis	3.6 (2.6-5.6)	1.9 (1.0-3.2)	0.019 / 0.28
LVMI, g/m^2	91 (75-94)	77 (64-100)	0.464 / 1.0
WHtR	0.52 (0.48-0.55)	0.49 (0.45-0.54)	0.889 / 1.0

[a] P value after Bonferroni multiplicity correction

[b]PTH-index was calculated as actual PTH divided by upper reference limit

RTX, renal transplantation. SBP, systolic blood pressure. DBP, diastolic blood pressure. Ca, calcium. P, phosphate. LVMI, left ventricular mass index. WHtR, waist-to-height ratio


A
Cumulative kidney graft survival
100
80
60
40
20
0
Number of subjects at risk
98 84 63 40 3
25 17 14 9 6
After 1990
Before 1990
0 5 10 15 20
Time after transplantation, years


B
Cumulative kidney graft survival
100
80
60
40
20
0
Number of subjects at risk
44 33 31 24 5
79 61 40 19 4
<5 years
>5 years
0 5 10 15 20
Time after transplantation, years