Renal Transplantation in Children Managed With Lymphocyte Depleting Agents and Low-Dose Maintenance Tacrolimus Monotherapy

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Objective. Describe the safety and efficacy of antithymocyte globulin or alemtuzumab preconditioning, steroid avoidance and reduced calcineurin inhibitor (CNI) immunosuppression in 34 children undergoing renal transplantation.

Methods. ATG (n=8) or alemtuzumab (n=26) were infused at the time of transplantation. This was followed by low-dose twice a day tacrolimus monotherapy with consolidation to once daily dosing by 6 months and once every other day dosing by 12 months. Follow-up ranged from 0.5–2.9 years (mean 1.33 years), with a minimum of 6 months.

Results. Both ATG and alemtuzumab were well tolerated. Lymphopenia occurred routinely and resolved after 3–6 months. Acute cellular rejection occurred in 9%; it was related to medical nonadherence in two patients and resulted in one graft loss at 1.5 years. Important adverse events included transient neutropenia in 10 children (none with serious infection), and autoimmune hemolytic anemia in two (resolved with a steroid course in both and conversion to sirolimus in one). Estimated glomerular filtration rate (e-GFR) was stable and averaged 88 mL/min/1.73 m^2 at latest follow-up. Fifteen preadolescents had a greater increase in height Z-score at 1 year (1.3 vs. 0.5, $P=0.001$), and a higher e-GFR (94.8±21 vs. 76.6±20 ml/min/1.73 m^2, $P<0.05$), when compared to case-matched historical controls who were weaned off steroids by 6 months after transplantation and received twice daily tacrolimus monotherapy.

Conclusion. This simple regimen appears safe, has a low risk for acute cellular rejection or other adverse effects, and is associated with excellent growth and renal function. Such a regimen may also improve compliance and limit CNI nephrotoxicity.

Keywords: Kidney transplantation, Children, Antithymocyte globulin, Alemtuzumab, Tacrolimus, Steroid avoidance.

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There has been little improvement in renal allograft survival during the years between 1995 and 2000, mainly because of an inability to prevent chronic allograft nephropathy (CAN) (*1–4*). Major contributors to CAN are acute and chronic rejection, and nephrotoxicity associated with the long-term use of calcineurin inhibitors (CNI) (*5*). Recent studies have demonstrated that preconditioning with antithymocyte globulin (ATG) or alemtuzumab (Campath-1H, an anti-CD52 antibody), to aggressively deplete T-cell clones prior to transplantation, together with maintenance CNI, effectively suppresses acute cellular rejection (ACR) without the need of corticosteroids (*6–12*). This nonmyeloablative regimen, which does not destroy bone marrow stem cells (*13*), may facilitate immunologic engagement between the recipient and donor leukocyte subpopulations, possibly by modifying the function of dendritic cells, thereby enhancing long-term engraftment or "tolerance" (*14, 15*). It has also been further theorized that minimizing maintenance tacrolimus immunosuppression may enable more controlled immunologic interactions between donor and recipient immune systems, and may be more "tolerogenic" while limiting chronic CNI nephrotoxicity (*16, 17*). Such simple tacrolimus monotherapy regimen may be especially advantageous to the pediatric renal transplant population as it may obviate the stunted growth, obesity, hypertension, and body disfigurement and associated drug noncompliance which continues to plague current multidrug immunosuppressive regimens that include steroids. Also, a low rate of ACR together with lower chronic tacrolimus exposure may limit CNI nephrotoxicity and may potentially permit longer allograft survival.

Experience with preconditioning agents in pediatric renal recipients is limited. In one study, good allograft survival was obtained in 17 children managed with short-term Thymoglobulin induction followed by a multidrug maintenance immunosuppression regimen (*18*). In another report, Campath induction followed by variable maintenance immunosuppressive regimens was associated with ACR in three of four children (*19*). A protocol was recently developed at our center utilizing a preconditioning agent at transplantation, followed by steroid avoidance and weaning of tacrolimus, and was very effective in adults undergoing renal transplantation (*10, 16*). This paradigm was then adopted in children and resulted in very encouraging preliminary results (*20*). The current report extends our experience to 34 pediatric renal transplant recipients and provides a comprehensive evaluation of the benefits and risks of the current protocol over a longer follow-up time.

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PATIENTS AND METHODS

Lymphocyte depletion, with either rabbit antithymocyte globulin (ATG, Thymoglobulin; Sangstat Medical Corp., Fremont, CA) or with alemtuzumab (Campath-1H; Millennium Pharmaceuticals, Cambridge, MA), followed by tacrolimus (Prograf; Fujisawa, Deerfield, IL) monotherapy, has been the standard of care in renal transplant recipients at Children's Hospital of Pittsburgh from May 2003 to the present. Consent and assent forms were obtained after approval by the Innovative Practices and Pharmacy and Therapeutic Committees for off-label use of Campath. The current study had no funding source except for the current retrospective data analysis (Astellas Pharmaceutical Grant).

Thirty four consecutive children ranging in age from 1–18 years who met the minimum criterion of 6 months of follow-up by December 2005 were included in the current analysis. The diagnosis and other pertinent clinical data of the recipients are shown in Table 1. The surgical procedure and fluid management were standard (*21*) except for the placement of double-J ureteral stents for 3–4 weeks in the majority of the children. ATG was utilized in the initial eight children. Because Campath has relatively fewer infusion reactions, greater potency (*6*, *7*), and longer effect, possibly due to its half-life of 2–3 weeks (*22*), it was utilized in the subsequent 26 recipients.

ATG (5 mg/kg body weight) or Campath (0.4–0.5 mg/kg body weight) were given intravenously over a period of 3–6 hr starting at transplantation with completion before the cross clamp release. Intravenous methylprednisolone (10–15 mg/kg body weight) was given just prior to the antibody infusion for prevention of cytokine release syndrome, and this dosage was repeated during the arterial anastomosis. Tacrolimus was begun on the day after transplantation either orally (0.2 mg/kg body weight divided in two dosages per day) or intravenously (0.1 mg/kg body weight over 24-hours), and the dosage was adjusted to attain blood levels of 10±2 ng/mL measured by immunoassay (Abbott Diagnostics). After about 1 month, a level of 6–10 ng/mL was acceptable in most recipients without ACR and absence of panel reactive antibodies. After 4–6 months, recipients who had not experienced acute rejection received the total daily tacrolimus dose as a single dose (consolidation), and after 10–12 months this same dosage was given on alternate days (spaced weaning) without regard to target blood levels.

All children received valganciclovir (Valcyte, Roche, Nutley, NJ) at an oral dosage of 450 mg/1.73 m^2 once daily for a period of 6 months. Valganciclovir prophylaxis was extended to 1-year in all seronegative recipients of Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV) seropositive donor grafts who also received a single prophylactic intravenous dose of cytomegalovirus immune globulin (100 mg/kg body weight; CytoGam, MedImmune, Gaithersburg, MD) on the day after transplantation. Other routine prophylactic medications included Mycostatin 300,000–500,000 units given by the swish and swallow method four times daily for 4 months; trimethoprim-sulfamethoxazole (Bactrim, 2 mg/kg/day, or maximum of 40 mg/day, based on trimethoprim content), daily for 1 month and then three times per week indefinitely; and aspirin 81 mg/day for 2–4 weeks. Gastroprotective agents were given intraoperatively and for 2–6 weeks subsequently, as needed. Supplemental magnesium or Bicitra were utilized selectively.

TABLE 1. Selected clinical data in 34 children receiving Thymoglobulin or Campath preconditioning at the time of transplantation

Characteristic	Data
Age, mean±SEM	10.1±1.1
Range (years)	1–18
Indications for transplantation	
Congenital uropathy (n=14)	
Hypodysplasia	5
Posterior urethral valves	4
Other	5
Acquired glomerulopathy (n=9)	
Focal glomerulosclerosis	3
Other	6
Hereditary/familial (n=10)	
Nephronophthisis	3
Congenital nephrotic syndrome	2
Other	5
Male	19
Preadolescent	22
Retransplantation	3
Other transplants	2[a]
Dialysis/pre-emptive	22/12[b]
Living donor/cadaveric	23/11[c]
Cold ischemia time (mean hours)	24.5
Average length of stay (days)	12.2
One-year allograft survival (%)	100
PRA >20%	2
ABDR HLA mismatch (mean±SD)	3.3±1.3
Follow-up	
6-month (n)	34
≥1-year (n)	25
Mean±SEM, years	1.84±0.12
Overall years (mean±SD)	1.33±0.75
Blood tacrolimus levels (ng/mL, mean±SD)	
1 month	9.9±2.6
3 months	8.7±2.2
6 months	7.0±2.6
1 year	5.0±1.75
Latest follow-up	2.7±1.75

[a] Both liver, small bowel.
[b] Twelve PD, 5 HD, 5 PD and HD.
[c] Two unrelated.

Serial measurements of human leukocyte antigen class I and II antibodies were performed by enzyme-linked immunosorbent assay testing. Individuals with antibody titers exceeding 10% had further testing for donor specific antibodies (DSA; Elisa, Lambda Antigen Tray-Mixed, One Lambda) to aid in the determination of immunological risk for humoral rejection before tacrolimus weaning.

Patients were evaluated closely for hematologic and infectious complications. Automated and manual blood pres-

sures and growth parameters were recorded. Estimated glomerular filtration rate (e-GFR) was calculated by Schwartz formulas (*23*). Growth was assessed by multiple parameters as previously outlined (*24*). Because height age lags behind the chronological age in children with chronic renal failure (*23*), in the current series we used the height age to assess changes in body mass index (BMI) and weight for height index (WHI). Growth and GFR could not be assessed in three children with severe orthopedic or neurological disorders (VATER syndrome, spina bifida). Their latest serum creatinine levels were 0.7, 1.1 and 0.6 mg/dL, respectively.

Data were abstracted from the chart utilizing an "honest broker" method and patients were de-identified prior to analysis under the University of Pittsburgh institutional review board guidelines.

Statistical Methods

The ATG and Campath subgroups were clinically indistinguishable permitting analysis of the combined patient series. Descriptive data are expressed as means±standard deviation. Two independent sample *t* test analyses were preformed for non-growth data comparisons. We compared growth data from each time point to the next, and between adolescents and non-adolescents. A non-parametric Mann-Whitney test was applied to comparisons of GFR and growth data. Growth data were also analyzed by the more conservative Wilcoxon signed rank test which takes into consideration that a few children did not have growth data at each time point of evaluation. The *P* values shown below reflect these more conservative results. Statistical significance was defined by a *P* value <0.05.

Growth and GFR in 15 preadolescent children were also compared with data of 15 case-matched historical controls managed with our prior protocol of twice daily tacrolimus with steroid weaning and withdrawal by 6 months after transplantation (*24*).

RESULTS

As shown in Table 1, our unselected patients had typical pediatric renal disorders leading to renal failure. Eight children were black and 26 were white. The majority received living donor grafts. The mean follow-up time was 1.33 years. Twenty-five children were maintained on the current protocol for a period ranging from 1.0 to 2.9 years. The initial length of hospital stay was 12.2±6.8 days (mean±SD). Six infants with major preexisting gastrointestinal disorders (gastrostomy tubes and/or colostomies) had longer hospital stays; excluding such infants, the average length of hospital stay was 10 days.

As noted in Table 1, tacrolimus blood levels diminished during the first 6 months after transplantation, and fell further after 6 months with decreasing dosing frequency and longer trough time. Among the 34 children maintained on this protocol for ≥6 months, 6 were not considered eligible for once daily tacrolimus dosing consolidation because of early or late ACR, or because of fluctuating serum creatinine concentrations related to bladder dysfunction. A total of 27 of the remaining 28 children, or 96%, were successfully switched to once daily tacrolimus dosing at 5.4±1.9 months (mean±SD; range 3–10.5 months) after transplantation. Also, after excluding one child who experienced late ACR, 17 of 18 children, or 94%, with long enough follow-up to permit spaced tacrolimus monotherapy were successfully switched to every other day dosing at 11.1±2.7 months (mean±SD, range 7–18 months) and at latest follow-up their mean trough tacrolimus level is 2.7 ng/mL. However, 2 of these 17 children (12%) returned to once daily dosing at 1.5 and 2.3 years, respectively, based on the appearance of DSA. Neither child had a change in e-GFR.

Hematologic data are summarized in Table 2. Compared to pretransplantation, the white blood cell count (WBC) decreased gradually and reached a nadir at 3 months after transplantation ($P<0.001$), followed by recovery by 1 year after transplantation. The initial decrease in WBC was mainly due to the fall in lymphocytes which occurred within one week of preconditioning with ATG or Campath; WBC began to recover by 1 month, with a slower recovery subsequently, and full recovery by 1 year of transplantation. The proportion of neutrophils did not change appreciably compared to pretransplant values. However, the absolute neutrophil count (ANC) fell significantly during the first 6 months and reached a nadir of 2,123±734/mm^3 at 3 months ($P<0.001$, for all intervals between 1 and 6 months compared to baseline ANC). While the ANC improved subsequently, it remained below baseline values at both 1 year and at latest follow-up but did not differ significantly from baseline ANC (P=NS).

TABLE 2. Hematologic data in 34 children with renal allografts after Thymoglobulin or Campath preconditioning

	WBC	Lymphocytes	Neutrophils (ANC)	Platelets	Hemoglobin (g/dL)
Pretransplant	8.33±3.76	36.21±13.22	49.62±13.43 (4.13)	294±120	10.8±1.9
1 week	6.95±3.42	4.69±5.57	79.21±15.87 (5.46)	232±99	10.1±1.5
2 weeks	5.99±3.28	6.73±6.07	76.71±17.32 (4.57)	378±133	9.8±1.8
1 month	4.93±2.56	18.34±18.68	68.25±19.26 (3.22)[a]	329±112	10.6±2.4
6 weeks	4.19±2.19	19.60±16.35	66.36±17.13 (2.80)[a]	323±117	9.9±1.3
3 months	3.51±1.15	24.00±13.36	61.68±15.44 (2.12)[a]	269±97	10.8±1.5
6 months	4.77±1.99	27.82±14.84	58.82±15.24 (2.76)[a]	301±119	11.6±1.5
1 year	6.00±3.54	36.00±16.15	50.35±15.42 (3.02)	277±116	11.8±2.8
Latest follow-up	6.43±2.76	35.85±12.22	50.30±14.21 (3.15)	282±71	12.3±1.2

All WBC and platelet values are in the thousands/mm^3 while lymphocytes and neutrophils are expressed as percent of a total WBC. All values are means±SD.
[a] $P<0.001$ compared to baseline absolute neutrophil count (ANC) in thousands/mm^3.

Table 3 lists the complications encountered in our patients. ACR occurred in three children (9%) with cadaveric allografts. All three received Campath, and one lost their allograft at 1.5 years. ACR developed in a highly sensitized child during the first 2 weeks of transplantation, while biopsy-confirmed rejection occurred at 1 year and at 1.5 years after transplantation in two noncompliant children, including a sensitized teenager with previous allograft loss due to noncompliance.

Seven children (21%) developed bacterial infections; only one with urosepsis required hospitalization. Although 16 children were seronegative for EBV and 16 were seronegative for CMV at the time of transplantation while their donor was seropositive for these viruses, there was no instance of EBV or CMV disease, or posttransplant lymphoproliferative disease (PTLD). Asymptomatic EBV and CMV seroconversion occurred in five children (four had a rise in EBV polymerase chain reaction and one had CMV seroconversion).

Prior to transplantation, 19 of 34 (56%) children had hypertension. At 1 month after transplantation, 4 of these 19 children were normotensive while several needed fewer antihypertensive medications. Only three children developed new onset hypertension, which resolved by 3 months after transplantation. The prevalence of hypertension was 34% at 3 months and only 15% at latest follow-up. On latest follow-up, two of five (40%) required two or more antihypertensive medications compared to 14 of 19 (74%) prior to transplantation.

Neutropenia and anemia were the most important complications. When neutropenia was defined as an ANC $<1500/mm^3$, it occurred in 10 (29%) children, with onset mainly between 3 weeks and 6 months. Antineutrophilic antibodies were absent in several children tested for this disorder. One child (3%) had neutropenia prior to transplantation compared to 18–20% between 1 and 6 months after transplantation, and 10% subsequently. One asymptomatic child had an ANC of 986, which reversed spontaneously after several days. Filgrastrim (Neupogen; Amgen, Thousand Oaks, CA) was given to four children with an ANC $<1000/mm^3$. An infant with several bouts of otitis media had the longest

TABLE 3. Complications and adverse effects linked to Thymoglobulin or Campath preconditioning followed by tacrolimus monotherapy in 34 children with renal transplants

Complication/adverse effect	Number of children affected	Comment
General		
Cytokine release syndrome	None	Prevented with 2 perioperative dosages of steroids
Delayed graft function	5	All had prolonged cold ischemia time; 2 needed dialysis. All recovered within 3 weeks
Acute cellular rejection (ACR)	3	One was a highly sensitized child whose renal function improved with plasmapheresis; in 2 ACR was linked to noncompliance
Infection (sepsis, pneumonia, cellulitis, wound infection pyelonephritis, otitis media)	7	No EBV or posttransplant lymphoproliferative disease; no CMV, parvovirus, or polyoma virus encountered
Recurrent focal glomerulosclerosis	1	Successfully managed with plasmapheresis
Feeding problems or functional bowel obstruction	4	All were infants with prior feeding problems, colostomies, and gastrostomy tubes
Hypertension (new onset)	3	None of these 3 children required antihypertensive agents beyond 3 months (see text and Table 4)
Microscopic hematuria/sterile pyuria	8	Hematuria was not accompanied by bacterial or viral infection, and resolved after ureteral stent removal
Acute pancreatitis	1	Resolved spontaneously
Unexplained ascites	1	Transudate and not urinoma; resolved after one paracentesis
Hematologic disorders		
Neutropenia	10	Onset 3 weeks to 6 months; duration 0.5–7 months. Neupogen utilized in 4 of 5 children with ANC $<1000/mm^3$. Cellulitis occurred in 1; no serious infection in other 4.
Anemia, nonhemolytic	4	Responded to erythropoietin in 3 and to iron in 1. Off erythropoietin after 2 to 5 months of therapy
Anemia, hemolytic	2	Both autoimmune (see text)
Metabolic disorders		
Posttransplant diabetes (new onset)	2	Developed in one child with rapid obesity, recurred in another with prior diabetes
Hypomagnesemia (<1.3 mg/dL)	12	Medically controlled. Uncommon after 6 months
Metabolic acidosis (tCO_2 <20 mEq/L)	11	Medically controlled. Uncommon after 6 months
Hyperkalemia (>5.5 mEq/L)	6	Medically controlled. Uncommon after 6 months
Hyperlipidemia	2	Transient. Resolved without drug use
Hyperuricemia	2	One of 2 had mild allograft dysfunction

course of filgrastim administration comprising 4 months. Another infant had a Staphylococcal urinary tract infection in the first month of transplantation, while a teenager developed foot cellulitis after a track meet at 5 months; both responded to a total of two and three dosages of filgrastim each. A fourth child developed neutropenia combined with hemolytic anemia and received filgrastim for 2 months as well as erythropoietin for 7 months. In all neutropenic children valganciclovir was changed to once every other day dosing. In the remaining five children with an ANC >1000/mm^3, infections were uncommon and included conjunctivitis, transient mouth sores, gastroenteritis, upper respiratory infection, and recurrent urinary tract infection in a child with a neurogenic bladder.

Prolonged anemia occurred in a total of four children. Coombs-positive hemolytic anemia requiring hospitalization developed at 7 months in a child who received Campath, and at 12 months in a child who received ATG. In the first child, the disorder resolved after a brief course of steroids and change in tacrolimus to every other day dosing. In the second child, steroids were also given along with a reduction in tacrolimus dose followed by conversion to cyclosporine; these measures were ineffective but hemolysis did resolve after conversion to sirolimus. Erythropoietin was transiently administered to three children. Thrombocytopenia was not observed.

Among metabolic disorders, new onset diabetes occurred in only one child and recurred in another with previously resolved posttransplant diabetes. Electrolyte disturbances were usually encountered during the initial 3 months and included hypomagnesemia, metabolic acidosis, and hyperkalemia. These disturbances were managed using magnesium oxide or magnesium gluconate, bicarbonate or sodium citrate/citric acid, and fludrocortisone (Florinef). There were no associated significant muscle cramps or weakness, seizures or other symptoms. At latest follow-up, only three children required magnesium and/or alkali supplements, and one required fludrocortisone to control hyperkalemia.

Growth data are summarized in Table 4. In the group as a whole, height Z-score increased significantly at all time points compared to the time of transplantation ($P \leq 0.001$). The change in height Z-score was also statistically significant between 6 months and 1 year as well as between 6 months and at latest follow-up (both, $P=0.005$), and also between 1 year and latest follow-up ($P=0.02$). As noted in Table 4, in the preadolescent group height Z-score increased by 1.3 and by 1.7 standard deviations at 1 year and at latest follow-up, respectively, compared to the time of transplantation ($P=0.001$). By comparison, in the 15 case-matched historical controls, the change in height Z-score at 1 year was 0.5 standard deviations ($P=0.02$, current versus control cases). Accelerated or catch-up growth, defined as a change in Z-score $\geq$0.5 SD/year, occurred in 92% and 91% of preadolescents and in 33% of adolescents at 1 year and at latest follow-up, respectively. In the adolescent subgroup height Z-score also improved and catch-up growth occurred in 33% at 1-year after transplantation but these differences were not statistically significant. Height velocity was 11.75 cm/year in the preadolescent subgroup compared to 10 cm/year in the controls (P=NS).

BMI did not change after transplantation. WHI rose from 2.8 to 8.9 during the first year of transplantation ($P=0.01$) but fell to the 6-month level at latest follow-up. New onset obesity did not occur in any of the children, although it persisted in three children who were obese prior to transplantation.

Renal function and selected electrolyte values are depicted in Table 5. Transient proteinuria occurred in two of the three children managed for ACR and in another child with focal glomerulosclerosis. GFR ranged from 82–96 mL/

TABLE 4. Growth data at different time points after renal transplantation with Thymoglobulin or Campath preconditioning

	N	Age	Z-score	ΔZ score	BMI for height age	ΔBMI for height age	WHI index	ΔWHI index
Combined preadolescent and adolescent groups								
At transplant	31	10.2±6.3	−1.8±1.8	—	18.2±2.8	—	2.3±20.4	—
6 months	26	11.3±6.1	−1.4±0.6[a]	0.5±0.6	18.1±2.6	−0.2±1.9	8.9±16.1	6.7±12.6
1 year	18	10.9±6.1	−0.9±0.17[a]	0.9±0.7	17.9±2.6	−0.5±2.3	13.7±8.4	7.8±15.0
Latest follow-up	17	11.7±6.8	−0.4±1.5[a]	1.2±1.1	18.3±2.7	−0.3±2.6	7.6±20.1	4.5±17.9
Preadolescent group								
At transplant	19	6.1±4.3	−3.1±1.65	—	16.7±2.4	—	2.8±18.2	—
6 months	15	7.0±4.3	−2.4±1.8[a]	0.6±0.5	16.2±1.1	−0.5±2.4	4.0±7.8	3.4±14.5
1 year	12	7.5±4.6	−1.4±1.9[a]	1.3±0.7[a]	16.4±1.3	−0.9±2.7	8.9±13.0[b]	4.7±17.4
Latest follow-up	11	7.8±5.0	−0.7±1.7[a]	1.7±1.0[a]	16.6±1.6	−0.8±3.0	4.2±9.8[b]	−1.1±18.8[b]
Adolescent group								
At transplant	12	16.8±1.4	−0.4±0.9	—	20.4±1.4	—	1.5±24.3	—
6 months	11	17.2±1.4	−0.2±1.0	0.2±0.3	20.7±1.3	0.2±0.6	15.7±21.8	11.2±8.2
1 year	6	17.5±1.3	0.0±1.0	0.3±0.2	20.9±1.6	0.2±1.1	23.2±24.9	14.1±5.5
Latest follow-up	6	18.9±1.5	0.1±0.9	0.3±0.3	21.4±0.8	0.7±1.1	13.8±32.2	14.7±11.1

All values are means±SD.
[a] $P<0.05$ by paired t test and by Wilcoxon signed rank t test.
[b] $P<0.01$ by paired t test and by Wilcoxon signed rank t test.

TABLE 5. Renal function and selected electrolytes in 31 children receiving renal allografts with Thymoglobulin or Campath preconditioning

Follow-up	N	Blood urea Nitrogen (mg/dL)	Creatinine (mg/dL)	e-GFR (mL/min/1.73 m^2)	Change in e-GFR (mL/min/1.73 m^2)	tCO_2 (mmol/L)	Potassium (mmol/L)	Magnesium (mg/dL)	Uric acid (mg/dL)
2 weeks	34	22.1±10.6	1.00±0.53	84.1±32.9	—	22.7±2.6	4.6±0.86	1.44±0.31	5.5±1.8
1 month	31	21.9±13.8	0.87±0.43	82.4±.37.8	6.6±15.8	22.3±2.2	4.7±0.74	1.39±0.27	5.3±1.4
3 months	29	19.0±5.80	0.93±0.51	87.2±27.8	6.3±29.1	22.2±2.8	4.7±0.72	1.39±0.28	5.5±1.5
6 months	31	20.5±5.90	0.93±0.50	95.6±35.3	10.6±35.0	22.9±3.0	4.7±0.38	1.34±0.25	5.2±1.5
1 year	20	18.4±5.25	0.89±0.39	90.7±20.8	4.9±33.5	24.4±2.8	4.6±0.49	1.51±0.43	5.6±1.3
Latest follow up	20	19.0±5.30	1.03±0.49	88.0±21.8	3.2±40.1	23.9±2.9	4.4±0.47	1.5±0.25	5.2±1.5

e-GFR was calculated in 31/34 patients due to lack of height data in three patients.

min/1.73 m^2 with a mean value of 88 mL/min/1.73 m^2 at latest follow-up. Also, blood urea nitrogen and serum creatinine, tCO_2, potassium, magnesium, and uric acid levels remained constant and within the normal range (albeit after medical intervention in selected cases as noted above). Notably, at 1-year follow-up preadolescent children in the current study had a GFR which was 18 ml/min/1.73 m^2 higher than the historical case-matched controls ($P<0.05$).

As noted in Table 6, maintenance tacrolimus levels were significantly lower in the current preadolescents compared to controls ($P<0.001$).

DISCUSSION

The current study is characterized by a high rate of successful transition to once daily (94%) and to an every other day (96%) dosing of tacrolimus, and a low ACR rate (9%). Because previous studies have shown a high correlation between biopsy-confirmed renal allograft rejection and presence of DSA (*25–27*), we took the precaution of returning two children who developed DSA to daily tacrolimus dosing. Although neither child had any sign of rejection, both are considered failures of tacrolimus weaning.

TABLE 6. Maintenance blood trough tacrolimus levels (ng/mL) in 15 current and control preadolescent children ($P < 0.001$)*

Patient	Current	Controls
1	3.2	7.3
2	3.6	7.3
3	2.6	5.9
4	2.5	5
5	5.9	8.9
6	4.9	5.2
7	<2.5	9.2
8	5.8	7.4
9	4.9	6.8
10	<2.5	5
11	2.6	5.7
12	<2.5	9.7
13	<2.5	8.3
14	<2.5	7.5
15	<2.5	7.4
Total (means±SD)	3.43±1.28	7.11±1.52

Six children in the current series had tacrolimus levels below the assay detection limit of <2.5 ng/mL. This limit was utilized for calculation of the above results.

The regimen was well tolerated. Review of the complications and adverse effects (Table 3) indicates no instance of cytokine release syndrome when ATG or Campath were administered with perioperative steroid prophylaxis. Also, all five children with delayed graft function had a rapid and complete recovery of renal function. Such recovery may be enhanced by these preconditioning agents that effectively suppress ACR thereby permitting delayed use and lower amounts of tacrolimus, as noted previously (*28*). During a mean period of follow up of 1.33 years, we encountered three episodes of ACR two of which were associated with confirmed medical noncompliance. Despite the low blood trough tacrolimus levels, ACR was not encountered in the remaining 31 children. Moreover, this simple tacrolimus monotherapy regimen is devoid of body disfigurement and appears to be associated with excellent adherence. This contrasts with poor adherence with multidrug immunosuppressive regimens particularly in adolescents with solid organ transplants in whom the incidence of noncompliance is fourfold greater than in adults, and is a major cause of graft loss (*29, 30*).

Further review of the complications and adverse effects listed in Table 3 indicates that the rate of infection was low and the consequences of such infections were not serious. No polyoma or parvovirus infections were observed. Despite a high rate of EBV and CMV seronegativity, we did not observe EBV or CMV infections. Also, PTLD or overt malignancy did not occur during a follow-up period of up to 2.9 years. Other centers utilizing similar preconditioning agents have also noted a low incidence of opportunistic infectious, including CMV and EBV (*31–33*). Similarly, among 117 children receiving ATG for heart transplantation, only one developed PTLD (*34*). The low risk for PTLD may relate to inhibition of both T and B cells by ATG or Campath as well as the antiviral prophylaxis and low blood CNI levels in our patients.

Neutropenia and autoimmune hemolytic anemia are the most clinically relevant complications and appear to be related to use of ATG or Campath. Currently it is not known if lower dosages of these agents may be effective in limiting ACR while preventing these hematologic disturbances. Al-

though rapid lymphocyte depletion was anticipated with use of either preconditioning agent, the gradual and significant fall in neutrophil number during the first 6 months after transplantation was surprising (Table 2). In fact, the ANC remained 23% lower on latest follow-up compared to baseline. Although previous studies have examined leukocyte subpopulations affected by Campath, a high incidence of neutropenia has not been previously reported in transplanted adults or children receiving ATG or Campath induction (*8, 28, 34*). These agents may be directly linked or may uncover a genetic predisposition to neutropenia. However, the mechanism does not appear to involve antineutrophilic antibodies. While absolute neutropenia (ANC $<1500/mm^3$) occurred in 10 children, severe neutropenia (ANC $<1000/mm^3$) was observed in two infants and in three older children over a period ranging from three weeks to 6 months after transplantation. Generally, this disorder was transient and responded to filgrastim administration. Remarkably, there was no serious morbidity associated with neutropenia. Of greater concern was the late development of autoimmune hemolytic anemia in two children who required hospitalization. In addition to the administration of filgrastim, strategies employed to reverse the neutopenia include decreasing the valganciclovir dosage and lowering tacrolimus blood levels as permitted by the child's clinical course. A brief course of corticosteroids and/or switching CNI to sirolimus may aid the reversal of hemolytic anemia.

Because hypertension is a common disorder in pediatric transplant recipients, we assessed its prevalence and course at various follow-up periods. Only three children (10%) in the current study developed new onset hypertension, which was transient. This incidence of hypertension was even lower than that observed with our previous protocol (*35*). Also, among 56% of the children with hypertension that predated transplantation, the prevalence of hypertension after transplantation fell to 15% and blood pressure was more manageable using fewer antihypertensive medications.

New-onset posttransplant diabetes mellitus occurred in a single child with psychogenic polyphagia and rapid weight gain, and recurred in another child. This incidence is quite low compared with our previous tacrolimus-based immunosuppressive regimen (*24, 35*). Moreover, there was a low frequency of other metabolic disturbances, such as hyperkalemia, metabolic acidosis, hypomagnesemia, or hyperuricemia. These disorders were easily controlled and typically resolved after tacrolimus consolidation to once daily dosing by 6 months after transplantation.

Previous reports describe diminished height Z-score and reduced growth velocity especially in preadolescents with advanced renal failure; such growth improves after transplantation, but generally growth remains stunted with cyclosporine-based regimens that include steroids (*36*). In sharp contrast, in our preadolescent group (Table 4), the change in height Z-score increased by 1.3 ± 0.6 and by 1.7 ± 1.0 standard deviations at 1 year and at latest follow-up, respectively, compared to the time of transplantation (both $P=0.001$). Remarkably, accelerated or catch-up growth occurred in 92% of this subgroup during the first year after transplantation. Furthermore, this high rate of growth was sustained at latest follow-up. In fact, the change in height Z-score at 1 year in the current protocol far exceeds that of our case-matched controls who were managed with rapid steroid tapering and withdrawal at 6 months followed by tacrolimus monotherapy ($P=0.02$) (*24*). A greater, albeit statistically insignificant, height velocity was also noted in the current study compared to our case-matched controls. This apparent further acceleration in growth likely results from steroid avoidance right after transplantation rather than because of a lesser exposure to tacrolimus per se. Modest but statistically insignificant increases in height Z-score also occurred in the adolescent group. Moreover, this impressive growth was not associated with obesity which is very prevalent in children after renal transplantation, particularly if steroids are utilized for immunosuppression. As shown in Table 4, there was no change in BMI in our overall group. BMI values above the 95th percentile or WHI values exceeding 120% denoting obesity were uncommon, even when compared to healthy children in the general population (*37*).

The mean e-GFR values of 91 and 88 mL/min/1.73 m^2 at 1 year and at latest follow-up, respectively, in children managed with the current protocol (Table 5) parallel or exceed the excellent e-GFR reported previously (*24*). This is particularly evident in the preadolescent group, in which the GFR at 1 year significantly exceeded that of 15 historical case-matched controls by 18 mL/min/1.73 m^2 ($P=0.05$). This may be explained by a lower CNI exposure (Tables 1 and 6). Table 6 shows that although the range of maintenance tacrolimus levels in the current preadolescents varied considerably, the majority of patients had significantly lower levels than the controls. Also, proteinuria as a marker of transplant glomerulopathy was infrequent and transient. However, functional endpoints, such as e-GFR or proteinuria, are not suitable surrogates for serial biopsies for detecting subclinical rejection or injury. Thus, a lack of protocol biopsies is an important limitation of our study.

CONCLUSION

The simplicity, relative safety, and ability of the current immunosuppressive protocol to limit ACR and to maintain excellent renal function and promote growth over a period of up to 2.9 years merits further investigation. The potential of this protocol for better medical adherence, lesser CNI nephrotoxicity, and greater tolerogenicity may be of particular benefit to children with renal transplants. Close medical supervision is essential in these children exposed to such low blood trough tacrolimus concentrations over many years. The benefit of this regimen in reducing the risk for CAN and in prolonging allograft survival remains to be determined.

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