Quantitative Assessment of Blood Flow in Pediatric Recipients of Renal Transplants

Judith Ash, Margaret DeSouza, Michael Peters, Dan Wilmot, Doris Hausen, and David Gilday

The Hospital for Sick Children, Toronto, Canada and Hammersmith Hospital, London, England

We applied a renal blood flow (RBF) quantification technique to pediatric data, which depends minimally on bolus shape, uses a conventional radiopharmaceutical ^{99m}Tc-DTPA, and generates a value for RBF as a percentage of cardiac output (RBF/CO). Mean RBF was 16.9% (±4.8) for normal transplants, 13.1% (±2.9) for transplants undergoing mild-to-moderate chronic rejection, 7.9% (± 1.3) for those with mild acute rejection and 3.3% (±1.4) for those with moderate-to-severe acute rejection. Very low blood flow values within 24 hr following transplantation may have prognostic significance. Patients who required transplant-nephrectomy had significantly lower RBF/CO values than children who retained their allograft.

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Several methods have been developed to aid in the detection of acute renal transplant rejection (*1–8*), using a variety of radiotracers (*3–5*), and, employing computerized methods to quantify changes in renal blood flow (RBF) (*1,2,9*). However, the bolus fragmentation seen in children as a result of the small-vessel caliber, and the lower administered radiotracer dose, combine to produce poor quality curves with ill-defined peaks, when these techniques are employed.

This paper describes a computerized method for quantifying RBF, easily adapted to clinical use, using a conventional radionuclide, standard computer software, and simple mathematical principles. It uses the entire kidney as one region of interest (ROI), accounts for soft-tissue attenuation, and is less dependent on bolus width. The method has the additional advantage of being independent of the time interval between the arterial and organ time-activity curves; other techniques require these to be simultaneous (*1,10,11*).

The theoretical basis of this relatively noninvasive method for measuring organ blood flow has been described previously (*12,13,14*). Briefly, the technique is based on the principle of fractionation of cardiac output (*15*). It effectively determines the count rate that would be recorded over an organ if the tracer behaved like radiolabeled microspheres and was completely trapped in the organ's vascular bed on first-pass. The estimated first-pass activity plateau, following correction for organ depth, is equal to the organ's fraction of the cardiac output (CO).

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For reprints contact: Dr. Judith M. Ash, Department of Radiology, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada M5G 1X8.

MATERIALS AND METHODS

Patients

Twenty-three pediatric patients, examined consecutively, who underwent renal transplantation, were studied prospectively over a one-year period. There were 13 males and 10 females, whose ages ranged from 1 to 19 yr (mean age 11.9 yr ± 4.7). Twenty-two patients received a cadaveric kidney, and one a living donor kidney. The evaluated data related to the first kidney transplant in 14 patients, the second in six, and the third in three. Five patients had had a transplant-nephrectomy by the end of the study; four in the first-transplant patients and one in a second-transplant patient.

All patients received quadruple therapy: prednisone, imuran, Minnesota antilymphocyte globulin (ALG), and cyclosporin. The ALG was administered for 2 wk following transplantation. Treatment with cyclosporin was begun two days before ALG was discontinued, using a divided dose of 5 mg/kg/day, and was discontinued if serum levels measured >100 μg/l by radioimmunoassay. None of the patients developed cyclosporin toxicity.

All patients underwent a comprehensive diagnostic evaluation including history, physical examination, serial hematologic and biochemical analyses, urine culture and urinalysis, chest radiography, and, when necessary, renal sonography. The clinical status of the transplant patients was evaluated without knowledge of the calculated RBF values, both during the course of the study, and retrospectively where necessary. Sixteen of the 23 patients had a renal biopsy. Biopsies were done within 24 hr of the technetium-99m-diethylenetriamepentaacetic acid (^{99m}Tc-DTPA) study in 13 of the patients, within 72 hr for two patients, and within 6 days for one stable normal patient.

A baseline study with ^{99m}Tc-DTPA was performed within 48 hr of transplantation, in all patients. Follow-up investigations were acquired as indicated over the course of a year, with a total of 119 studies obtained in the 23 patients. Five studies were not completed for various technical reasons, and computer quantification was not done in nine other studies because of extreme bolus fragmentation. As a result, there were 105 valid examinations available for analysis.

Ethical considerations preclude a study design in which a biopsy is performed after each radionuclide study. Therefore, RBF values derived during the course of this study, without corresponding pathologic examination, were categorized separately on the basis of relevant clinical and biochemical results.

Patients were classified as normal if they had normal clinical status and biochemical results at the time of the study. Acute tubular necrosis (ATN) was considered to be present when oligura from other causes was ruled out, and when the clinical course was appropriate to the diagnosis. The diagnosis of rejection was confirmed histologically in all cases by needle biopsy, or at the time of transplant-nephrectomy by pathologic examination of the allograft.

Suspected renal artery stenosis was investigated by means of Doppler sonography and contrast angiography. Three cases of renal vein thrombosis were confirmed using contrast venography. Infection, either focally in the transplanted kidney, or systemically, was diagnosed by microbiologic techniques. One patient was classified as having nephropathy of unknown etiology. Eight studies were classified as transitional in patients who were recovering from either ATN, infection, or obstruction at the time of their renal scans.

Radionuclide Studies

The patients were given an i.v. bolus injection of up to 370 MBq (according to weight) of ^{99m}Tc-DTPA, usually administered through a large antecubital vein. A gamma camera with an all-purpose parallel-hole collimator was used for data acquisition. Flow images were collected at 1 frame/sec for 64 sec, followed by renogram images at 1 frame/min for 15 min in a 64 × 64 byte matrix. A 1-min static image of the kidney and an image of the injection site were acquired at 30 min into the study.

A 5-sec image of the injection apparatus was acquired prior to, and after, administration of the radiotracer dose, using lucite bars between the syringe and the camera, for attenuation of the very high counts encountered.

A depth-correction factor was calculated by measuring the distance between an anterior abdominal wall marker, and the center of the transplanted kidney. To do this, an image was acquired as a supine lateral view, for 1 min, in a 64 × 64 matrix with the marker in place for 10 sec.

Data Analysis

Regions of interest were applied to the data from the flow study. The first ROI was an outline drawn manually around a summed image of the renal transplant (Fig. 1). Although in some cases the transplanted kidney overlaid the iliac artery, it has been shown that the error generated by including the iliac artery within the transplant-ROI is negligible (*13*). A series of regions, each 2–3 pixels long, was drawn anywhere along the course of the abdominal aorta (Fig. 1); a longer region tends to produce an erroneous blood flow value, since the theory assumes that the arterial radioactivity is sampled as if by a point detector (*12,13*).

Renal and aortic time-activity curves were generated from approximately the first 35 frames of the flow study (Fig. 2A). From the series of aortic curves, the aortic curve with the optimum shape was fitted with a gamma function (Fig. 2B), integrated, and the curve plateau obtained (Fig. 2C). The maximum upslope of the integrated gamma function was found using a linear fit (Fig. 3A). The maximum upslope of the raw-data renal curve was also fitted (Fig. 3B). The ratio of the maximum upslope of the renal curve to the maximum upslope of the integrated gamma function aortic curve was calculated. As a quality control procedure, the integrated aortic curve was then multiplied by this ratio. If this ratio is accurate, the resultant curve will parallel the renal curve (Fig. 3C), and will effectively be a prediction of the organ curve that would be obtained if the tracer were trapped in the kidney on first-pass, as would be the case with microspheres (*13*). If parallelism is not achieved, the original or another aortic curve is fitted to generate a new maximum upslope, and the ratios are again calculated to test parallelism.

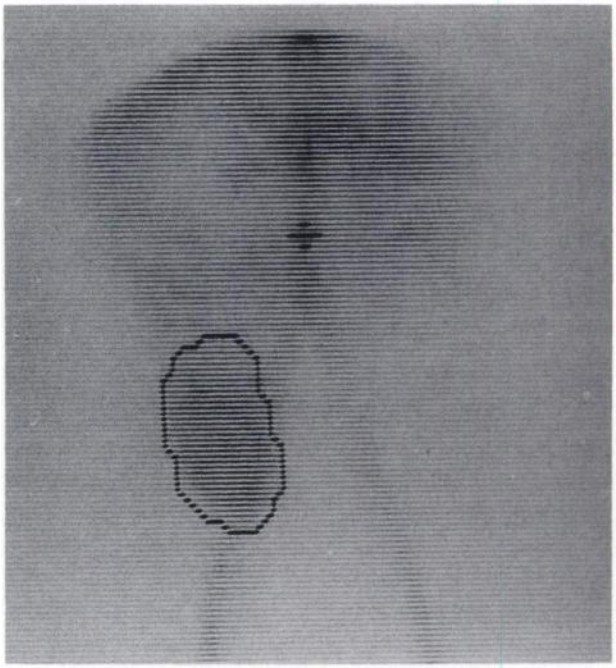

FIGURE 1
Regions of interest over kidney and aorta. Note kidney region includes underlying iliac artery. Aortic region is only 3 pixels long.

Regions of interest were drawn on the frames acquired by counting the syringe and injection apparatus, pre- and post-injection. The decay-corrected postdose counts were subtracted from the predose counts. In order to give the net patient dose in counts/second, the result was multiplied by 2.05 (attenuation for 6 cm of lucite) and divided by 5 (5-sec acquisition).

To obtain the kidney depth correction factor, the lateral kidney image with the marker was first spatially calibrated, and then the distance from the center of the kidney to the marker was obtained in pixels/cm. The depth correction factor was calculated by multiplying a soft-tissue attenuation coefficient of 0.125 cm^{-1}, by the distance between the abdominal wall marker and the center of the kidney. In order to eliminate variations in depth measurement, the initially calculated depth was used on all subsequent studies of the same patient, unless there had been an obvious change in position of the kidney.

RBF/CO was calculated as follows:

$$\frac{\text{slope of kidney}}{\text{slope of aorta}} \times \frac{\text{area (plateau) aorta}}{\text{dose}} \times \text{DCF} \times 100,$$

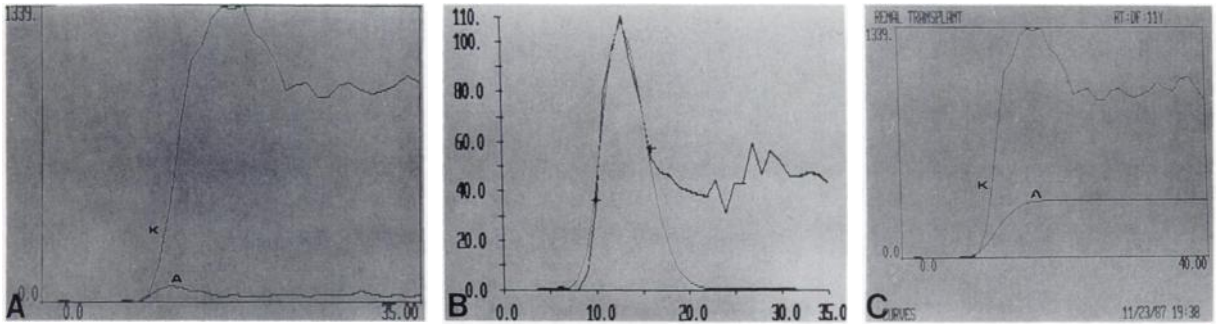

FIGURE 2
(A) Raw-data renal and aortic curves, K and A, respectively. (B) Gamma variate fit to aortic curve. (C) Curve A is the integrated gamma variate to the aortic curve showing the plateau (curve K is the raw-data renal curve).

where RBF/CO = RBF as % of CO; slope of kidney = maximum upslope of renal curve; slope of aorta = maximum upslope of integrated aortic curve; area (plateau) aorta = plateau height of integrated aortic gamma function in cts/sec; dose = net patient dose in cts/sec; DCF = depth correction factor.

Two radiologists, unaware of the clinical status of the patients and of the RBF/CO values, evaluated each renal scan for both transplant perfusion and overall function. Where there was disagreement in rating between the observers, an average of the two ratings was calculated.

Statistical Analysis

A Kruskal-Wallis one-way analysis of variance (*16*) was done on the RBF/CO values for the 16 patients who had a biopsy. Day-1 RBF/CO values were also compared using a one-way analysis of variance. Correlations were computed by standard techniques.

RESULTS

Table 1 indicates that there was a statistically significant difference ($p < 0.01$) among RBF/CO values in the 16 patients, who were normal, or had mild/moderate chronic rejection, or had mild or moderate/severe acute rejection on biopsy. Patients with normal biopsies had higher RBF/CO values (mean = 16.9%) than those with mild/moderate chronic rejection (mean = 13.1%), who in turn had values higher than patients with mild (mean = 7.9%) or moderate/severe (mean = 3.3%) acute rejection ($p < 0.01$). No biopsy results were available for patients with ATN, since the clinical status of these patients did not warrant biopsy.

Table 2 reviews RBF/CO values for the remaining 89 scans, which were categorized on the basis of clinical and biochemical status. When an RBF/CO value of 6.1% was used as the limit below which rejection was diagnosed, a sensitivity of 100% was achieved, in that all transplanted kidneys clinically categorized as having moderate-to-severe rejection were identified (6.1% was the value 2 s.d.s above the mean RBF/CO for patients in the biopsy-proven moderate/severe acute rejection group). When this boundary was used, specificity for the diagnosis of severe rejection, in the group having this diagnosis clinically, was 83.3%.

Although the mean for ATN was higher, there was some overlap between the RBF/CO values for patients with acute mild rejection and for those with ATN. On the basis of a single value alone, these two conditions could not always be differentiated, but the clinical course of the two disease processes was usually different.

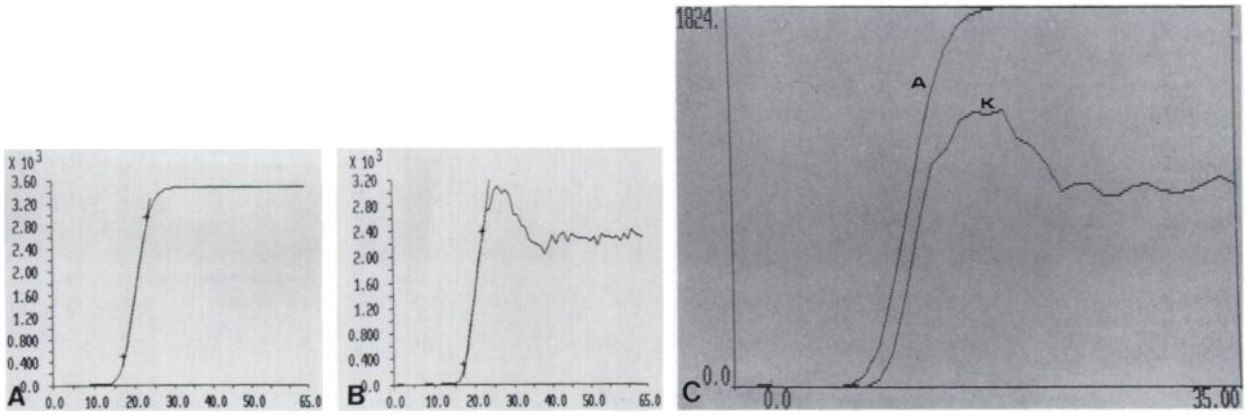

FIGURE 3
Linear fit to maximum upslopes of (A) integrated gamma variate of aortic curve and (B) raw-data renal curve. (C) The gamma function in (A) is multiplied by the slope in (B) with the slope in (A) to produce a new aortic curve, A, with a slope parallel to the renal curve, K. This confirms that the slopes derived in (A) and (B) were accurate.

TABLE 1
RBF/CO Values in Biopsy-Proven Normal and Rejecting Transplants

		Rejection		
		Chronic	Acute	
Biopsy diagnosis	Normal	Mild/Mod	Mild	Mod/Severe
N	3	6	3	4
X	16.9%	13.1%	7.9%	3.3%*
s.d.	4.8	2.9	1.3	1.4
s.e.m.	2.8	1.2	0.8	0.7

N = number of studies; X = mean, s.d. = standard deviation, and s.e.m. = standard error of the mean.
* = $p < 0.01$.

Vascular disorders, such as renal vein thrombosis or renal artery stenosis, could be distinguished from ATN, and from chronic rejection, but not from severe acute rejection. Despite the inability of the technique to differentiate some of these conditions, the studies were useful to assess the severity of renal impairment.

Fourteen of the 23 patients had RBF/CO values calculated on Day 1. Nine of the fourteen patients retained their renal transplant. Five patients had a transplant-nephrectomy despite aggressive treatment for rejection; one of these patients did not receive the standard protocol of immunosuppression because of staphylococcal infection on Day 1, and was not included in the following analysis.

Three patients had initial values in the mild acute rejection range, with a mean of 7.52% and lost their transplanted kidneys within 3 mo of transplantation as a result of rejection. The fourth had a day-1 value of 1.62%, which was in the severe acute rejection range, and underwent nephrectomy because of rejection, 2 wk later. The mean day-1 RBF/CO value of 6.05% (±1.5 s.e.m.) for the group who underwent nephrectomy, was significantly lower ($p < 0.05$) than the mean value of 13.4% (±2.1 s.e.m.) for those children who did not require nephrectomy, for management of rejection. All four patients showed a rise in RBF/CO percentages subsequent to the initial low value (mean 85.8% = ±21.7 s.e.m.) and then a fall prior to nephrectomy. Those patients with the higher Day 1 RBF/CO values had retained their transplanted kidneys for an average of 9.8 mo (±1.2 s.e.m.), by the end of the study. This result suggests that day-1 RBF values may be indicative of ultimate transplant outcome.

Serial renal studies were used to examine the magnitude of change in renal perfusion in stable normal patients. In patients with ATN, the percentage increase at recovery was calculated, since there were no pre-existing stable values prior to ATN, which was present at Day 1. In the case of the remaining patients, a percentage decrease in the RBF/CO was calculated for a change from stable graft function. No stable values for comparison were found for patients with renal vein thrombosis.

The percentage change in values between studies of stable normal patients was moderate (mean change 16.5% ± 3.0 s.e.m.), while a large decrease was noted in patients with moderate/severe acute rejection (mean 58.9% ± 8.2 s.e.m.) (Table 3). Although the percentage changes in the patients affected by other pathologic processes showed some overlap with each other, the mean absolute values (see Table 2), and the clinical settings, were different.

The technique had good reproducibility; the mean difference for intraobserver measurements was 1.42 (± 0.32, s.e.m.) and for interobserver measurements was 1.67 (±0.35 s.e.m.). A correlation of 0.61 was obtained between the radiologists' ratings of perfusion and RBF/CO values, and 0.54 between overall impression of the transplant function and RBF/CO values. This supports previous reports that visual estimates are insensitive to the presence of flow abnormalities (*1,2*)

DISCUSSION

Early diagnosis and treatment of renal transplant rejection is important in order to limit damage to the allograft. Many factors are weighed when making a diagnosis of acute rejection. Blood flow to the trans-

TABLE 2
RBF/CO Values in Transplant Recipients Based on Clinical and Biochemical Diagnosis

		Rejection								
Clin. Dx.	Normal	Chronic	Acute mild	Acute mod/ severe	RAS hypertension	Renal vein thrombosis	ATN	Transition	Infection	Nephropathy
N	25	7	12	9	13	3	4	8	10	2
X	18.6%	12.9%	7.2%	3.8%	10.0%	4.1%	11.6%	9.4%	8.5%	5.0%
s.d.	5.4	2.8	0.7	1.2	2.3	1.1	4.3	2.4	2.7	0.07
s.e.m.	1.1	1.1	0.2	0.5	0.6	0.6	2.1	0.9	0.9	0.05

N = number of studies; X = mean of RBF; s.d. = standard deviation; s.e.m. = standard error of the mean; Clin Dx = clinical diagnosis; RAS = renal artery stenosis; and ATN = acute tubular necrosis.

TABLE 3
Percentage Change (%) in RBF/CO Values (mean ± s.e.m.) Over Previous or Subsequent Renal Scan

	Rejection					
Normal	Chronic	Acute mild	Acute mod/ severe	RAS hypertension	ATN	Infection
16.5 ±3.0	27.5 ±1.4	35.7 ±3.5	58.9 ±8.2	41.0 ±12.9	35.7 ±16.5	41.9 ±5.5

RAS = renal artery stenosis; ATN = acute tubular necrosis; and s.e.m. = standard error of the mean.

planted kidney is one variable shown to be a significant predictor of rejection (17,18). Objective methods for calculating blood flow to the allograft have been seen as a desirable adjunct to the subjective comparison of renal scans acquired during the course of a patient's treatment (*1,2*).

Hilson (*1*) noted that for a technique to be suitable for routine use in renal transplant investigation, it must be safe, rapid, sensitive, and capable of being repeated on alternate days. The results should be available on the same day as the study, and the method ought to be able to separate the various pathophysiologic processes that affect transplants. Our method, using a conventional radiopharmaceutical, is safe, easily repeated, and requires ~35 min to acquire and analyze, and provides clinical information within ~1 hr. Peters et al. (*19*) have validated the principle of the technique in a comparative study with intraventricular radiolabeled microspheres in dogs. It has a number of advantages over other methods of blood flow evaluation (*12*), and has very good intraobserver and interobserver reproducibility estimates.

This technique is comparable to other methods based on first-pass organ and arterial time-activity curves (*10, 11*), but is independent of the time difference between them (*1,10,11*); because of this, the placement of the arterial region is not critical, and the region may be drawn anywhere along the abdominal aorta, to derive a curve that fits a gamma function. The method does not require a compact bolus for successful quantification, a particularly important element when dealing with pediatric patients. Delivery of a compact bolus is made especially difficult by the following factors: patient motion at the time of injection, crying, veins that are small and used repeatedly over the course of treatment. In this study, only 7% of the scans could not be analyzed because of a fragmented bolus. Another advantage of this method is that RBF is calculated in meaningful physiologic units expressed as a percentage of cardiac output.

The present study data indicate that the method is sensitive for detecting severe acute rejection, and provides ranges that separate important pathophysiologic processes. All studies in patients clinically diagnosed as having moderate/severe acute rejection, had blood flow values that fell within the range identified as being associated with moderate/severe acute rejection as determined by biopsy: sensitivity 100%, specificity 83.3%. Although the actual RBF/CO values indicated normal function or rejection, serial comparison of a patient's results also provided very useful clinical information (*1,2*). In this study, a large drop in blood flow in a stable patient correlated with a change in clinical status. The main value of sequential studies was to determine whether a significant change in renal status had occurred, since clinical parameters could be misleading. Hattner (*18*) has emphasized that the major role of nuclear medicine is to determine whether allograft function has changed, rather than to determine absolute values for various clinical conditions. Our experience has been that both the absolute RBF/CO value and a comparison of RBF/CO data over time, can be aids to renal allograft evaluation for a particular patient. Visual comparisons were insensitive to changes between scans because of their subjectivity, and because of the influence of differing imaging techniques.

One promising finding of this investigation was that the day-1 RBF/CO value seemed to be predictive of allograft survival. As a group, patients who had a transplant-nephrectomy had statistically lower day-1 values than children who retained their kidney until the end of the study. Assessment of RBF on the first day following transplantation clearly merits further study.

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