
Early Diagnosis of Acute Postoperative Renal Transplant Rejection by Indium-111-Labeled Platelet Scintigraphy

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A prospective evaluation of ¹¹¹In-labeled platelet scintigraphy (IPS) for the early diagnosis of acute postoperative renal transplant rejection (TR) was undertaken. The results of IPS were compared with in vitro biochemical tests, the clinical finding of graft tenderness, and combined [^{99m}Tc]DTPA and [¹³¹I]orthoiodohippurate scintigraphy. With a sensitivity of 0.93 and a specificity of 0.95, IPS provided otherwise unavailable diagnostic information. Furthermore, postoperative IPS was a good predictor of long-term allograft survival.

J Nucl Med 27:1266-1272, 1986

The early postoperative diagnosis of renal transplant rejection (TR) is difficult but imperative in order that early therapy may be instituted and damage to the allograft minimized. Unfortunately, neither clinical impression nor routine in vitro biochemic tests are reliable in establishing the early diagnosis of TR. To meet this diagnostic need, a variety of scintigraphic techniques employing iodine-131 (¹³¹I) and iodine-125 (¹²⁵I) fibrinogen, technetium-99m diethylenetriamine-pentaacetic acid ([^{99m}Tc]DTPA), ¹³¹I and iodine-123 orthoiodohippurate, gallium-67 citrate, [^{99m}Tc]sulfur colloid, and indium-111 (¹¹¹In) labeled leukocytes have been employed (1-11). However, distinguishing postoperative TR from acute tubular necrosis (ATN), vascular insufficiency, ureteral obstruction, infection, and drug-induced nephrotoxicity remains difficult (12,13). Indium-111-labeled platelet scintigraphy (IPS) has recently been suggested for the early diagnosis of TR (14-25). To evaluate the additional diagnostic utility of IPS above and beyond the information provided by routine biochemical tests, the clinical finding of graft tenderness, and a combined [^{99m}Tc]DTPA and [¹³¹I]orthoiodohippurate scintigraphic examination, a prospective study of recent renal transplant recipients was undertaken.

MATERIALS AND METHODS

Between June of 1984 and April of 1985 a prospective study using IPS to assess all recent transplant recipients for the early postoperative diagnosis of TR was undertaken. Thirty-six patients (24 males, 12 females) ranging in age from 17 to 60 yr (mean 41 yr) were studied 4 to 13 days following surgery. All but two patients underwent IPS between postoperative days 5 and 11. Twenty-seven patients received cadaveric transplants, while nine received a kidney from a living related donor. All patients received routine postoperative maintenance immunosuppression (methylprednisolone and azathioprine: 12 patients; methylprednisolone and cyclosporine: 8; methylprednisolone, azathioprine, and antithymocyte globulin: 12; and methylprednisolone, azathioprine, and cyclosporine: 4). Of the 12 patients receiving cyclosporine, all but one were on a low dose regimen (8.5 mg/kg daily). In addition, once clinicians—who were aware of the results of IPS—had established the diagnosis of TR, patients were treated aggressively with methylprednisolone (100 mg/kg daily for 3 consecutive days). Eight patients were undergoing this aggressive antirejection treatment at the time of IPS, and an additional six patients were treated for rejection after completion of IPS.

Patients were injected intravenously with 500 μ Ci of autologous ¹¹¹In platelets prepared using a previously described technique (26). Anterior view 5-min analog images and digital data were collected over the pelvis at

Received Aug. 29, 1985; revision accepted Jan. 21, 1986.

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24, 48 and, occasionally, 72 hr postinjection using a large field-of-view gamma camera fitted with a medium-energy collimator and linked to a dedicated computer system. Both the 173 and 247 keV photopeaks of ^{111}In were used with a 20% symmetrical energy window at each level.

For analysis of the digital data, three regions of interest (ROIs) were defined: (a) an ROI surrounding the transplant but excluding ipsilateral iliac vessels, (b) a mirror image background ROI over the opposite side of the pelvis, and (c) an iliac vascular ROI over the iliac vessels on the contralateral side. After correcting for the relative size of the different ROIs, transplant/background (T/B) and transplant/ilial (T/I) ratios were calculated as:

$$T/B = (\text{transplant ROI counts}) / (\text{background ROI counts})$$

$$T/I = ((\text{transplant ROI counts}) - (\text{background ROI counts})) / ((\text{iliac ROI counts}) - (\text{background ROI counts}))$$

T/I ratios > 1.0 were considered indicative of rejection. T/B ratios were investigated for the cutoff value that best separated TR from other renal transplant states.

Thirty-two of the 36 renal transplant recipients underwent baseline [^{99m}Tc]DTPA and [^{131}I]orthiodohippurate scintigraphy soon after transplantation and before IPS. For these patients, adjustments in the above calculations were made for possible Compton scatter from ^{131}I decay contributing counts to the ^{111}In energy windows. By using data obtained immediately prior to the injection of ^{111}In -labeled platelets, the Compton scatter fraction (SF) was calculated as the ratio of counts in the transplant ROI at the ^{111}In windows divided by counts in the same ROI at the ^{131}I window (364 keV, 20% energy window). Data from the ^{131}I windows of the transplant ROI were acquired at 24, 48 and, occasionally, 72 hr. Corrections for Compton scatter were made as follows:

$$\text{Corrected } ^{111}\text{In counts/pixel} = (^{111}\text{In counts/pixel}) - (^{131}\text{I counts/pixel}) \times (\text{SF})$$

In a separate phantom study using scattering media, energy windows, count densities, and instrumentation which mimic the clinical imaging situation, it was determined that the scatter fraction into the ^{111}In windows from ^{131}I was 0.70 to 0.75. Our clinical studies were in agreement with this experimental result.

For visual analysis of the analog images, activity in the graft was compared to activity in the iliac vessels. The iliac vascular activity was assumed to represent the maximum amount that could be expected in the graft solely due to circulating ^{111}In -labeled platelets. Therefore, if the intensity of activity in the graft exceeded the

intensity of activity in the iliac vessels, the IPS study was considered positive for TR. If residual ^{131}I activity was present, a study was considered positive for TR only if the intensity of activity from the allograft in the ^{111}In windows exceeded both the ^{111}In activity over the iliac vessels and the ^{131}I activity from the allograft.

To simplify the analytic methods and data presentation, only analog images and digital data collected at 48 hr following injection are presented in this paper. Background activity generally was greater at 24 than at 48 hr. Therefore, to demonstrate the best possible results for IPS, the 48-hr data is presented. However, separate calculations of diagnostic efficacy based only on 24-hr data identified all patients with TR but included two instances when the 24-hr exam was falsely positive for TR. Furthermore, changes in transplant platelet uptake between 24 and 48 hr were no more valuable than the 48-hr evaluations for predicting TR.

Immediately after completing IPS, each patient also was evaluated for transplant rejection with [^{99m}Tc]DTPA and [^{131}I]orthiodohippurate scintigraphy using previously described techniques and interpretive criteria (12). When compared with the prior baseline study, any interval decrease in allograft perfusion on the [^{99m}Tc]DTPA exam or allograft function on the [^{131}I]orthiodohippurate exam was considered indicative of TR.

In vitro biochemical tests and the clinical finding of allograft tenderness [previously described as being indicative of TR (27)] were tabulated and compared with the results of IPS. Rather than enter numerical biochemical data into the statistical analysis, interpretive criteria routinely used by transplant surgeons at our institution were tabulated as a series of 13 parameters that might be either present (+) or absent (-). The 13 biochemical and one clinical parameters were evaluated on a day-to-day basis and were considered (+) if any of the following conditions were met.

1. Increase in serum creatinine of at least 0.3 mg/100 ml.
2. Decrease in creatinine clearance of at least 20%.
3. Decrease in creatinine clearance of at least 20% and increase in serum creatinine of at least 0.3 mg/100 ml.
4. Increase in blood urea nitrogen of at least 20%.
5. Fever greater than 100° Fahrenheit.
6. Leukocytosis $> 10,600$ cells per mm^3 .
7. Diastolic blood pressure > 100 mm of Hg.
8. Increase in diastolic blood pressure of at least 5 mm of Hg.
9. Increase in weight of at least 0.5 kg.
10. Decrease in urine volume of at least 25%.
11. Increase in weight of at least 0.5 kg and decrease in urine volume of at least 25%.
12. Proteinuria of at least 1 g in a 24-hr urine collection.

13. Increase in proteinuria of at least 0.5 g since previous day.

14. Allograft tenderness.

For purposes of data analysis, all patients treated for rejection either before, during, or within 7 days after IPS were considered to have sustained TR. The discharge diagnosis of TR, ATN, or other complications was made by the transplant surgeons and was considered authoritative. There were 14 cases of TR, eight of ATN without TR, 11 with normal postoperative allograft function, two with significant urinary tract infections, and one with renal artery thrombosis. Prior reports indicate that a retrospective review of the postoperative course is adequate to determine the presence of TR, ATN, and/or other causes for decreased renal allograft function in the crucial period following transplantation (28-32). Furthermore, while it has been reported that the histologic diagnosis of early postoperative TR may be misleading and that biopsy may show changes of TR in clinically normal recipients (33), we note that the available histologic data for six of the 14 patients classified as having TR showed histologic evidence of rejection in all six instances. In addition, retrospective analysis of the postoperative course for these 36 renal transplant recipients uncovered no convincing evidence of cyclosporine nephrotoxicity (34).

RESULTS

While for the T/I ratio 1.0 was the cutoff value that best distinguished between the presence and absence of TR, a cutoff value of 1.6 was found to be optimal for the T/B ratio (Fig. 1). This result is in agreement with previous reports that found 1.5 to be the optimal cutoff value for similarly defined T/B ratios (14,21). Further analysis of our data showed that normal renal transplant function could not reliably be distinguished from ATN or other causes for decreased allograft function using either T/I or T/B ratios.

The quantitative T/I ratio yielded the best diagnostic results with a sensitivity of 0.93 and specificity of 0.95 (Table 1). These results for the T/I ratio are similar to those obtained with visual interpretation and appear to be better than results for the T/B ratio. However, these differences are not statistically significant. The two patients with TR correctly diagnosed by T/I ratio but falsely classified as normal by visual interpretation of analog images were both undergoing active treatment for TR at the time of IPS injection.

The clinical finding of allograft tenderness and biochemical parameters suggestive of TR are analyzed in Table 2. None of these diagnostic measures has sensitivities and specificities comparable to IPS (chi-square, $p < 0.01$). In addition, stepwise discriminant analysis (35) showed that the information provided by IPS still

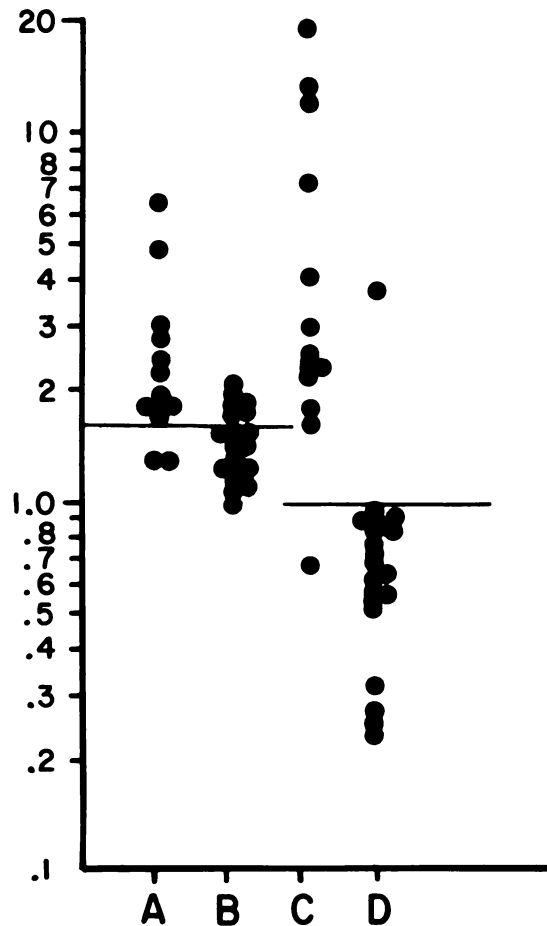


FIGURE 1

Quantitative IPS analysis. A: Transplant-to-background ratios of patients with TR. B: Transplant-to-background ratios of patients without TR. (Note that cutoff at 1.6-to-1 provides best discrimination between presence and absence of TR.) C: Transplant-to-iliac ratios of patients with TR. D: Transplant-to-iliac ratios of patients without TR

yielded a statistically significant improvement in diagnostic efficacy when it was added to the best linear combination of the biochemical parameters (i.e., results of IPS added to best linear combination of parameters 1, 10, 12) ($p < 0.05$).

Retrospective analysis indicates that for six of the 14 patients eventually proven to have TR, IPS was positive before there was clinical evidence of rejection with a decision to begin aggressive immunosuppressive therapy. All six of these cases occurred early in this prospective study. Given the ethical issues involved, it was not possible to withhold the results of IPS so as to achieve an unbiased comparison of IPS with other tests in identifying TR at the earliest possible time.

To date, 11 patients have lost their renal allografts while the remaining 25 patients are still being followed 3 to 12 mo post-transplantation. Only six of 14 allografts (43%) who had positive IPS show long-term survival while 19 of 22 patients with negative IPS show allograft survival (86%). Using the Wilcoxon-Gehan

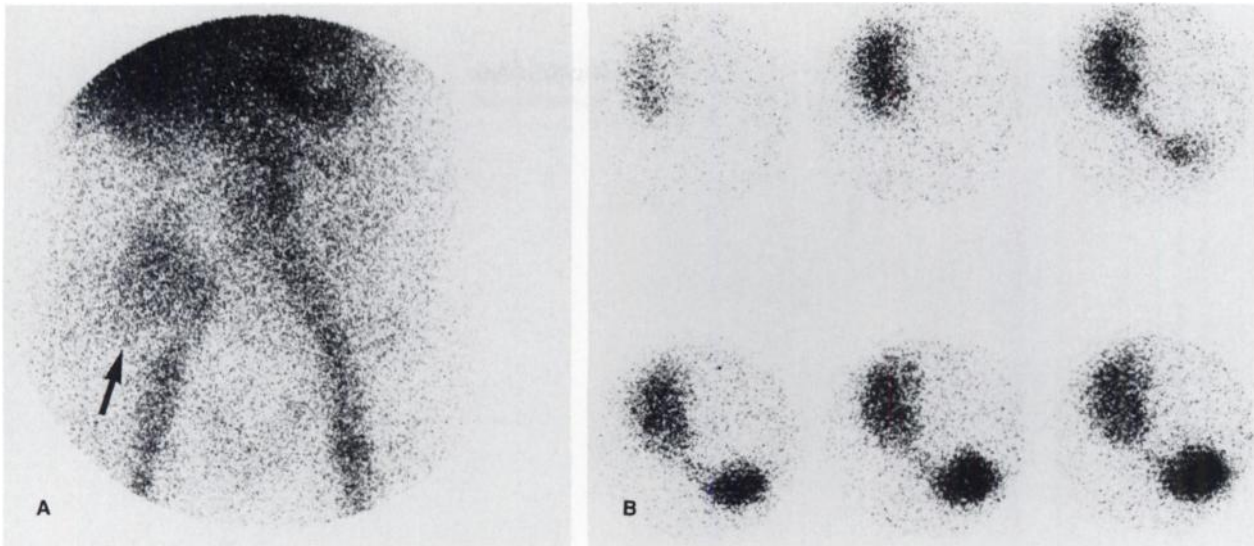


FIGURE 2

Normal IPS study. Analog IPS image (A) shows that for renal transplant in right iliac fossa (arrow) activity is slightly less intense than iliac vessels on left side of pelvis. Two-minute interval [¹³¹I]orthoiodohippurate scintigrams (B) obtained immediately following IPS study show rapid uptake and excretion indicating normal renal transplant function

procedure to compare survival distributions (36), this result is statistically significant ($p < 0.01$).

Thirty-two of the 36 patients in this series had at least one combined [^{99m}Tc]DTPA and [¹³¹I]orthoiodohippurate scintigraphic examination as a baseline study before IPS. This same dual isotope study was obtained for all patients at the completion of IPS as part of this research protocol. When the 32 available baseline and

follow-up [^{99m}Tc]DTPA and [¹³¹I]orthoiodohippurate studies were examined visually for decreased allograft perfusion and function, using previously described criteria (12), a sensitivity of 0.64 and a specificity of 1.00 for TR was obtained. Any interval decrease in allograft perfusion on the [^{99m}Tc]DTPA exam or allograft function on the [¹³¹I]orthoiodohippurate exam was considered indicative of TR.

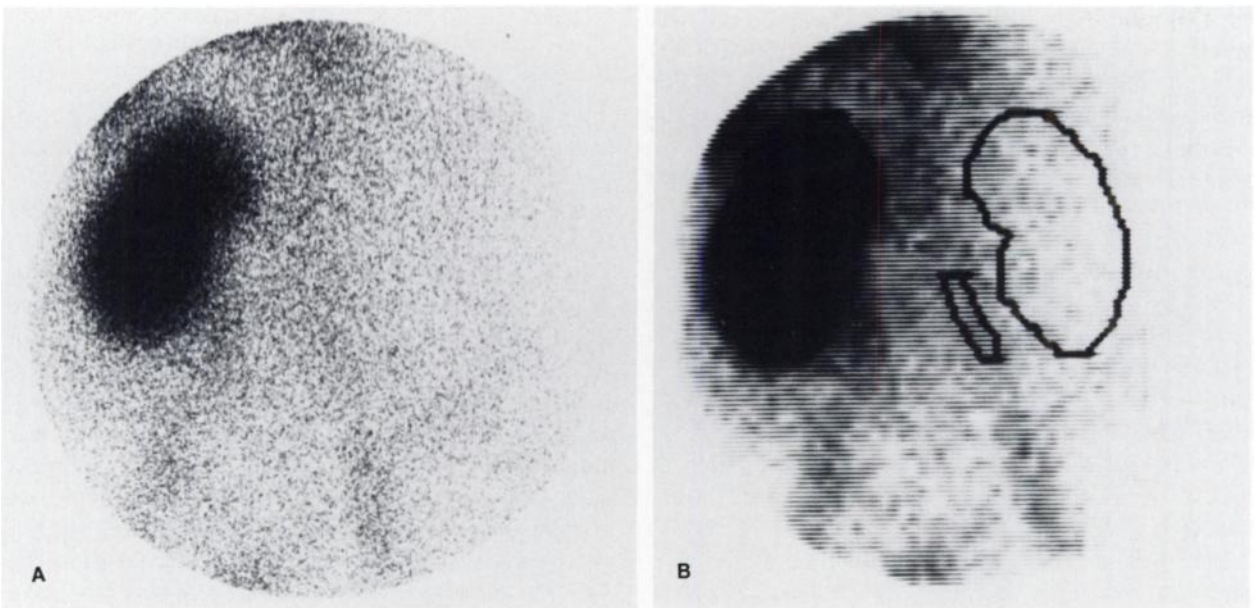


FIGURE 3

Renal transplant rejection confirmed in subsequent nephrectomy specimen. Analog IPS image (A) shows intense uptake in renal transplant far exceeding activity seen in iliac vessels. Digital IPS image (B) with ROIs drawn for quantitative analysis. Transplant-to-iliac ratio of 11.7:1 is above normal cutoff value of 1.0

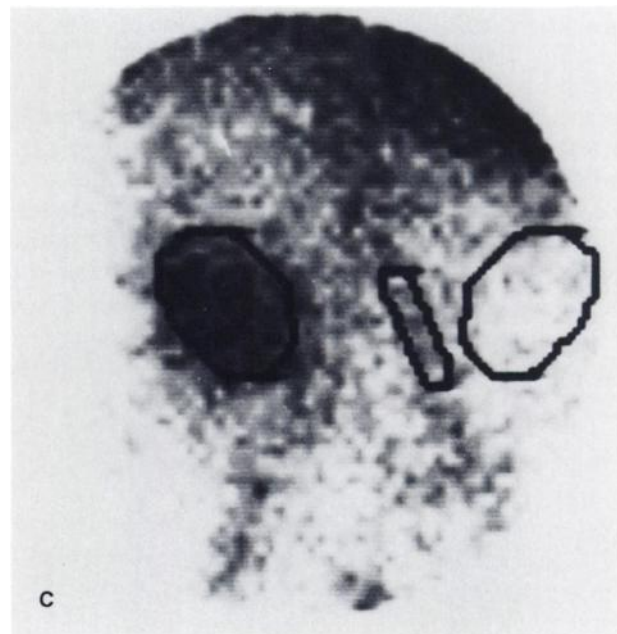
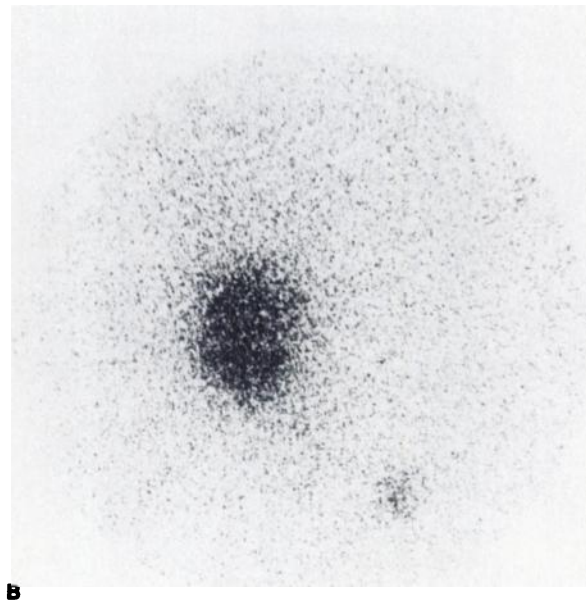
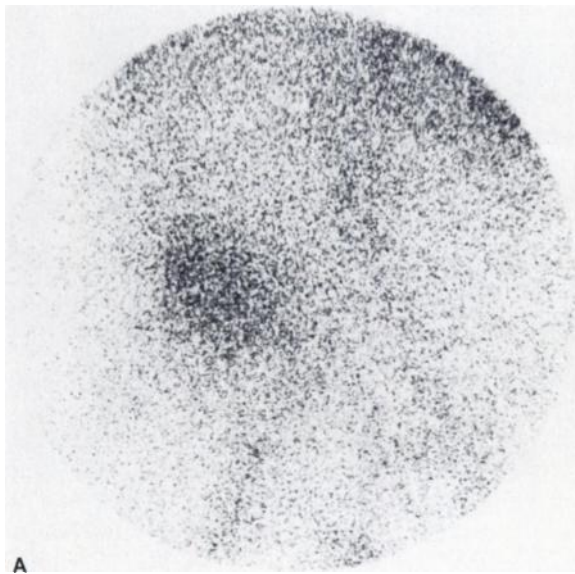


FIGURE 4
Postoperative ATN with subsequent recovery of renal function. IPS image (A) shows that activity over renal transplant is more intense than activity over iliac vessels. However, intensity of activity in ^{131}I window (B) over renal transplant is even more pronounced and, therefore, visual criteria for rejection are not met. Quantitative analysis (C) using ^{111}In counts which have been corrected for Compton scatter from ^{131}I shows transplant-to-iliac ratio of 0.3

DISCUSSION

This prospective study of postoperative renal transplant recipients has shown that IPS provides diagnostic information not available by clinical examination, in vitro biochemical testing, or combined [$^{99\text{m}}\text{Tc}$]DTPA and [^{131}I]orthoiodohippurate scintigraphy. This sug-

gests that IPS is of value for confirming the clinical diagnosis of TR. Furthermore, preliminary results—which are limited by the ethical constraints of human investigations—suggest that IPS frequently will identify incipient TR before clinicians suspect the condition. Finally, we note that IPS when performed 4 to 13 days following transplantation is a remarkably good predictor of long-term allograft survival.

The application of IPS to the postoperative diagnosis of TR may be limited if one always waits 48 hr after injection before interpreting the examination. However, successful IPS imaging at 4 hr has been reported (17, 18), and when early confirmation of TR is urgently requested, preliminary day-of-injection readings can be rendered. Furthermore, the persistence of ^{111}In -labeled

TABLE 1
Diagnostic Sensitivity and Specificity of Different IPS Techniques

Technique	Sensitivity	Specificity
Visual reading	0.79 (11/14)	0.91 (20/22)
T/I Ratio	0.93 (13/14)	0.95 (21/22)
T/B Ratio	0.86 (12/14)	0.77 (17/22)

TABLE 2
Diagnostic Sensitivity and Specificity of Clinical and Biochemical Parameters

Parameter	Sensitivity	Specificity
1	0.64	0.73
2	0.50	0.82
3	0.36	0.91
4	0.71	0.68
5	0.29	0.91
6	0.86	0.45
7	0.50	0.32
8	0.36	0.59
9	0.64	0.59
10	0.57	0.77
11	0.31	0.95
12	0.79	0.59
13	0.27	0.86
14	0.21	0.82

platelets in circulation for 3 or more days following injection creates the opportunity for sequential daily imaging to monitor for TR during the critical period beginning one week following transplantation. Finally, it has been shown that perinephric hematomas (15,18), acute cyclosporine nephrotoxicity (34), or IPS exams begun immediately following surgery (22) may cause ¹¹¹In platelets to concentrate in a renal allograft. Therefore, further investigations involving a larger series of patients undergoing IPS at various times following transplantation are needed to establish the specificity of this examination.

ACKNOWLEDGMENT

The authors thank Drs. Lionel Lieberman and David W. Palmer for their suggestions and encouragement.

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